

The 7th Polish Congress of Genetics

WROCLAW, SEPTEMBER 21-24, 2026

VII Polski Kongres Genetyki

WROCLAW 21-24 września 2026

KSIĄŻKA STRESZCZEŃ
BOOK OF ABSTRACTS



**Polskie Towarzystwo
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PLENARY LECTURES - ABSTRACTS

Hereditary skin diseases in domestic animals – might this be relevant for humans as well?

Tosso Leeb

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The skin is the largest organ of the body, and its integrity is essential for survival. In humans and domestic animals, numerous hereditary diseases—many of them monogenic—have been identified that affect the skin and/or its appendages, including hair follicles, nails, and teeth. Nevertheless, many patients with inherited skin diseases or genodermatoses still lack a molecular diagnosis because the underlying causal genes remain unknown. This diagnostic gap can hamper disease management and limit opportunities for precision medicine.

Over the past ~15 years, the dog and other domestic animals have emerged as powerful model organisms for forward genetic studies. Purebred domestic animals are typically maintained as genetically isolated populations with varying degrees of inbreeding, facilitating the expression of recessive alleles. Their population structure is therefore particularly well suited for identifying disease-causing genetic variants through hypothesis-free positional cloning approaches. Combined with their physiological similarities to humans, this makes spontaneous domestic animal mutants with genodermatoses valuable models for comparative “One Health” research, offering unique opportunities to discover novel disease-associated variants and genes with direct relevance to human skin biology.

My presentation will summarize our work on genodermatoses in cats and dogs, with a particular focus on spontaneous canine mutants that have revealed novel genotype–phenotype correlations. I will present examples in which the genetic analysis of dogs with genodermatoses has informed human genetics and contributed to a better understanding of homologous skin diseases in human patients. Furthermore, on a more fundamental level, studies of dogs with specific disorders of keratinization have provided new insights into the epigenetic regulation of keratinocyte differentiation.

The past, present and future of vector-based gene therapies: How technological advancements maximize clinical impact and patient benefits

Leszek Lisowski

Recombinant viral vectors derived from adeno-associated viruses (AAVs) have emerged as the leading technology for gene transfer in the field of translational virology. More recently, an unprecedented efficiency of AAV-driven genome editing without the need for endonucleases has been reported, further expanding the clinical applicability of this “simple” vector system. It also provides new hope for bridging the gap between currently available strong diagnostic powers and therapeutic benefit / improved health outcomes, especially in the underserved therapeutic area of rare and ultra-rare genetic disorder.

However, despite exciting in vivo preclinical data from multiple animal models and evidence of early clinical success, data accumulated from hundreds of AAV-based gene therapy trials convincingly demonstrate that current generation rAAV vectors remains unable to efficiently transduce human cells. This lack of clinical predictability is most probably an outcome of suboptimal AAV bioengineering mythologies applied to date as well as the use of biologically- and clinically-non predictive preclinical models in gene therapy development and validation studies. For AAV-based gene therapy to achieve its full potential and continue to fuel the healthcare revolution, novel vectors optimised for targeting human tissues are needed.

In his talk, Dr Lisowski will introduce and provide an overview of rAAV vecotorology and vector selection strategies aiming at identification of novel variants with desired (pre)clinical properties. The talk will highlight impacts of the selection process and selection system utilized on the function of identified candidates. Dr Lisowski will provide examples of how rAAV and genome editing tools can be combined to increase the efficiency and translational potential of both technologies. Finally, he will explain how the current technology can be applied to change lives of millions of children affected by rare genetic disorders, including sharing some exciting data from recently initiated AAV- CTNNB1 clinical trial in Slovenia.

The Art of Letting Go: Transcriptional Control of Stress-Response Elements in Salmonella

Grzegorz Grabe

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Salmonella enterica is an intracellular pathogen and a leading cause of diarrheal disease worldwide. Survival outside the host and within macrophages depends on rapid rewiring of gene expression under environmental stress. Toxin-antitoxin modules are stress response elements that can promote bacterial survival by transiently arresting growth. They encode a bacteriostatic toxin and a neutralizing antitoxin that also represses the operon. The complex occupies the operator when antitoxin is abundant, but excess toxin releases that repressor. How this “letting go” occurs has been unclear. This talk examines two distinct molecular mechanisms that strip the repressor from DNA and thereby control transcription of these elements in *Salmonella*.

If you build it, they will come: a decade of genomics powered by Nextflow and nf-core

Cedric Notredame

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Biology has a data problem: we generate far more of it than we can process, and the gap is widening. In this talk I will introduce Nextflow, a pipeline language we began developing over a decade ago to address the difficulties of computational biology at scale. Nextflow has since become one of the workhorses of modern genomics, supporting analysis across thousands of laboratories and some of the largest sequencing initiatives worldwide, including the Darwin Tree of Life and Genomics England. I will discuss how this evolution happened, and how nf-core, the community-driven initiative built around Nextflow, has provided a standard that lets groups around the world collaboratively build, maintain and deploy highly complex pipelines. Together they show how open software, shared conventions and a large community can turn computational methods into scientific infrastructure.

The ecosystem grew up around genomics, but nothing binds it there. Nextflow pipelines are already in use in fields as distant as economics and astronomy, while biology itself is undergoing a profound transition. Population studies now combine genomic and other molecular measurements with imaging, clinical records, wearable devices and longitudinal, quantitative phenotypes. The problem is therefore shifting from processing ever larger volumes of sequence to integrating heterogeneous forms of biological information at scale. I will conclude with some thoughts on that transition, the computational and organisational demands it creates, and why coupling omics to rich phenotypic data may be one of the defining challenges, and opportunities, for modern genetics.

Hereditary optic nerve atrophies – the new perspectives

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Until now, hereditary optic nerve atrophies were thought to be a group of untreatable, progressive disorders of the optic nerves, that are caused by mtDNA point mutations (Leber hereditary optic neuropathy, LHON) or OPA1 gene mutations (Kjer optic nerve atrophy, ADOA). Data from the last years collected from NGS studies of large groups of patients have shown that etiology of this group of disorders is much more complicated. Over a dozen of genes (besides OPA1 gene) that can cause ADOA are identified. Additionally, a new autosomal recessive form of LHON caused by mutations in the DNAJC30 gene was also recognized. This disorder is extremely important for us because it has quickly appeared to be a “Polish specialty”.

Important progress in treatment of this group of disorders is also noted. Clinical studies have shown significant, but type of mutation dependent, efficacy of LHON treatment using idebenone. It reaches a level of 80% in patients with an autosomal recessive form of LHON. Simultaneously, a gene therapy for the most frequent causative mtDNA mutation m.11778G>A in a classic form of LHON is elaborated. Currently it is under registration clinical studies. An exciting new possibility is optic nerve regeneration based on retinal ganglion cells reprogramming towards induced pluripotent stem cells and restoring their ability to divide.

Tracing age and lifestyle through epigenetics: new frontiers in forensic genetics and public health

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Epigenetic research has the potential to address questions in forensic genetics and public health that cannot be answered by genetic studies alone. One of its major applications is biological age assessment. In forensics, it enables age estimation from biological traces, narrowing the pool of potential suspects and supporting human identification. In public health, it provides a tool for monitoring population ageing and evaluating lifestyle interventions. However, current approaches still require further refinement, while the range of information obtainable from DNA methylation continues to expand to include lifestyle traits. The EPIGENOME, ForMAT and OPUS OMEGA projects aim to advance these applications. Within these projects, we developed novel, highly accurate age prediction models for multiple biological sample types, including those relevant to forensic investigations. We also developed a smoking classifier based on only four CpG sites and a 42-CpG alcohol consumption classifier, providing practical tools for behavioural inference in forensic investigations and objective alternatives to self-reported lifestyle information. By integrating genetic and epigenetic information, we developed an epigenetic BMI classifier that has the potential to improve facial prediction models and outperforms conventional BMI as an indicator of metabolic dysregulation. We further demonstrated the value of combining genetic and epigenetic information for predicting facial skin ageing traits, including wrinkle formation, and showed that the Ion AmpliSeq platform enables highly accurate, sensitive, and highly multiplexed DNA methylation analysis. In summary, DNA methylation research provides the foundation for innovative applications in forensic science and clinical diagnostics. The developed tools require only a limited number of methylation markers while maintaining high predictive accuracy and can be implemented using targeted DNA methylation sequencing technologies, facilitating their future translation into routine laboratory practice. The study received funding from the European Union's Horizon Europe Programme (no. 101225631) and from the National Science Centre in Poland (no. 2024/53/B/NZ7/02257).

How Cancer Reprograms Immunity: From Molecular Mechanisms to Cancer Immunotherapy

Ilona Kryczek

Cancer progression is shaped by continuous interactions between malignant cells and the immune system. Within the tumor microenvironment, chronic antigen exposure, immune-checkpoint signaling, metabolic stress and tumor-derived suppressive factors progressively reprogram immune cells, limiting their capacity to eliminate cancer. At the same time, these mechanisms create therapeutic vulnerabilities that can be exploited to restore durable antitumor immunity. This lecture will discuss how molecular pathways controlling immune checkpoints, cell death and cellular metabolism shape the phenotype and function of tumor-infiltrating immune cells. Particular emphasis will be placed on the functional plasticity of CD8⁺ T cells and the mechanisms that determine whether they maintain stem-like, persistent and polyfunctional effector states or progress toward terminal differentiation and exhaustion. A central focus will be the identification of a noncanonical function of SNAIL1, a transcription factor traditionally associated with epithelial–mesenchymal transition in cancer cells. In activated CD8⁺ T cells, SNAIL1 supports persistence, stem-like properties and antitumor effector activity. Its expression is induced through IL-2–STAT5 signaling and stabilized by the PI3K–AKT–GSK-3 pathway. Loss of SNAIL1 accelerates CD8⁺ T-cell terminal differentiation and impairs tumor control, whereas sustained SNAIL1 activity enhances T-cell function and improves the efficacy of immune-checkpoint blockade and CAR-T-cell therapy in preclinical models. Together with our studies of immune-regulatory and metabolic pathways in the tumor microenvironment, these findings demonstrate that cancer-associated immune dysfunction arises from interconnected molecular programs rather than from a single inhibitory mechanism. Understanding how these programs are established and maintained may reveal opportunities to therapeutically reprogram antitumor immunity, overcome treatment resistance and achieve more durable responses to cancer immunotherapy.

Carrot transposable elements

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Carrot (*Daucus carota* L.) is a diploid species with $2n=18$ chromosomes and haploid genome size of 473 Mb of which ca. 50% is repetitive DNA mostly attributed to transposable elements (TEs). Long terminal repeat (LTR) retrotransposons are the most abundant components of the repetitive fraction, but carrot also carries an exceptionally diverse set of miniature inverted repeat transposable elements (MITEs) and related Class II DNA transposons. Many TE insertions are relatively recent, as presence/absence variants (PAVs) are frequently observed. Initially, they were utilized as a source of DNA polymorphisms to develop molecular marker systems, such as Transposon Display or Intron Length Polymorphisms. More recently, a focus on polymorphic MITE insertions proximal to genes provided evidence for their involvement in rewiring gene regulatory networks by redistributing transcription factor binding sites. Also, active copies of Alex1 and Alex3, two families of LTR retrotransposons from the Ale lineage, were identified in carrot callus cultures, as they produced extrachromosomal circular DNAs (eccDNAs) – a by-product of transposition. Successful transposition events were documented by the identification of de-novo insertions. As many traits essential to the quality of carrot seem to result from TE-driven rearrangements, e.g. recessive mutations resulting in the accumulation of carotenes in the storage root and regulation of anthocyanin production, our current efforts aim at the systematic characterization of the role of TEs in carrot domestication and improvement through pan-genomics.

INVITED LECTURERS - ABSTRACTS

Publish or Perish? How to Make Your Editor Say ‘Yes!’

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Publishing in reputable scientific journals has become an integral part of academic work. However, many manuscripts are rejected not because the research itself lacks merit, but because the content fails to meet the editor's expectations. This brief lecture offers an inside look at the publishing process and poses a seemingly simple question: what makes an editor say “yes”?

Drawing on standard editorial practices and common reasons for rejection, the presentation highlights the key elements needed to transform a promising manuscript into a publishable article. Topics covered include selecting the right journal, formulating an intriguing research question, constructing a clear and compelling argument, and presenting results in a way that demonstrates both their originality and significance. Special attention will be paid to often-overlooked aspects of successful publishing: crafting an effective title and abstract, adhering to author guidelines, writing a persuasive cover letter, responding constructively to reviewer comments, and avoiding errors that might raise red flags for the editorial team.

Rather than viewing the publication process as a mysterious phenomenon or a matter of chance, this presentation frames it as a set of skills that can be mastered and consciously honed. Participants will learn strategies to increase the likelihood that their next manuscript successfully passes the initial editorial screening, engages reviewers, and ultimately secures that long-awaited “yes.”

When the karyotype is not enough: the role of microarrays in pediatric hemato-oncology.

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Keywords: SNP microarray (CytoScan HD); molecular karyotype; acute lymphoblastic leukemia; neuroblastoma; copy-number alterations

Conventional banding cytogenetics remains the reference method in pediatric oncology, yet it requires dividing cells, fails in a substantial proportion of samples and resolves only alterations above 5–10 Mb. Under the AIEOP-BFM ALL 2017 protocol, implemented in all Polish centers since October 2018, genetic diagnostics must comprehensively define numerical and structural aberrations within defined time frames — including the IKZF1plus profile by day 33 — which karyotyping alone cannot deliver.

In our department the analysis is performed on the CytoScan HD platform (2,670,000 markers: 750,000 SNP and 1,900,000 non-polymorphic copy-number probes). Aberrations are scored using defined thresholds: ≥ 25 probes over ≥ 25 kb for deletions, ≥ 50 probes over ≥ 50 kb for duplications and 3,000 kb for loss of heterozygosity, with dedicated cytoregion files covering 1,282 ALL-relevant genes.

In acute lymphoblastic leukemia, array analysis identifies the deletions defining IKZF1plus — IKZF1 together with CDKN2A/B, PAX5 or the PAR1 region, in the absence of ERG deletion — and reveals cryptic imbalances in apparently balanced translocations, masked hypodiploidy, iAMP21 and additional lesions in T-ALL. Among 220 consecutive newly diagnosed patients, IKZF1plus status was established in 176: IKZF1 deletion in 23 and the IKZF1plus profile in 14; findings were verified by MLPA and FISH.

The same technology extends naturally to pediatric solid tumors. In neuroblastoma, high-density SNP arrays define the segmental aberrations that drive risk assignment — 1p and 11q loss, 17q gain, ALK duplication, chromothripsis, whole-chromosome changes and LOH — complementing FISH assessment of MYCN amplification, resolving clonal heterogeneity and detecting unfavorable profiles in MYCN-non-amplified tumors. Microarrays therefore complement, rather than replace, karyotyping and FISH, improving diagnostic yield and risk stratification across pediatric hemato-oncology.

Application of Cell-Free DNA in the Diagnosis and Monitoring of Lymphomas

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Keywords: Cell-free DNA; circulating tumor DNA; lymphoma; molecular profiling; minimal residual disease

Lymphomas represent a heterogeneous group of lymphoid malignancies characterized by substantial genetic and clinical diversity. Current diagnostic and monitoring strategies rely mainly on tissue biopsy and imaging, including PET/CT. However, tissue-based molecular analysis is invasive and may not fully reflect tumor heterogeneity or dynamic clonal evolution. Circulating cell-free DNA (cfDNA), and particularly its tumor-derived fraction (circulating tumor DNA, ctDNA), has emerged as a promising minimally invasive source of tumor-specific genetic information.

Current evidence regarding the biological characteristics of cfDNA/ctDNA, pre-analytical requirements, molecular profiling, and clinical applications in lymphoma was reviewed, with particular emphasis on diffuse large B-cell lymphoma (DLBCL).

ctDNA reflects the molecular characteristics of lymphoma and correlates with tumor burden. Genetic profiles obtained from ctDNA show a strong concordance with tumor tissue and may identify genetic alterations not detected in an individual biopsy, potentially providing a more comprehensive assessment of tumor heterogeneity and clonal evolution. In DLBCL, baseline ctDNA levels have prognostic value and may complement established clinical and imaging-based risk parameters. Serial ctDNA analysis enables dynamic assessment of treatment response, early identification of molecular resistance, monitoring of MRD, and potentially earlier detection of relapse. Integration of ctDNA measurements with PET/CT may improve assessment of disease burden and treatment response.

The implementation of ctDNA testing remains challenging because the tumor-derived fraction may be low, requiring highly sensitive molecular methods. Pre-analytical factors, including blood collection tubes, time to plasma separation, processing procedures, and cellular DNA contamination, can significantly influence assay sensitivity and reproducibility. cfDNA, particularly ctDNA, represents a promising tool for minimally invasive molecular characterization and longitudinal monitoring of lymphoma. Its potential applications include prognostic stratification, MRD assessment, early detection of treatment resistance, and relapse prediction. Standardization and harmonization of pre-analytical and analytical procedures, together with prospective clinical validation, are essential for the integration of ctDNA-based diagnostics into routine lymphoma management.

Hereditary Alpha-Tryptasemia (HaT) — Pathomechanism, Diagnosis, and Clinical Significance

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Keywords: hereditary alpha-tryptasemia; TPSAB1; copy number variation; droplet digital PCR; basal serum tryptase

Introduction

Hereditary alpha-tryptasemia (HaT) is an autosomal dominant genetic trait caused by increased copy number of the TPSAB1 gene encoding the α -tryptase isoform. Secreted tryptases are encoded by two genes arranged in tandem at 16p13.3 — TPSB2, which carries only β alleles, and TPSAB1, which may carry either an α or a β allele. HaT is estimated to account for approximately 90% of cases of elevated basal serum tryptase (BST) in individuals of European descent. It has been linked to multisystem manifestations, although the nature of these associations remains debated; its impact on allergic and mast cell–related disorders is the best documented. Identification of HaT is of particular relevance in patients with systemic mastocytosis (SM), in whom it occurs significantly more frequently and is associated with an increased risk of severe anaphylaxis.

Methodological considerations

Routine diagnostic methods fail to detect HaT for two reasons. Exome sequencing interrogates sequence rather than gene dosage, and short reads within segmental duplications map ambiguously and are excluded from CNV calling. Methods measuring relative dosage, in turn, do not discriminate between α and β alleles, without which the result is uninterpretable: the diagnosis depends on the number of α copies relative to the number of β copies, not on the sum of α and β copies. Droplet digital PCR (ddPCR) with allele-specific probes remains the reference method. An alternative is long-read sequencing, which spans the entire tryptase cassette within a single molecule and additionally resolves how many α copies lie in cis on an individual allele.

Objective

To outline the molecular basis of HaT and to discuss available diagnostic approaches, with particular emphasis on a single-well multiplex ddPCR assay enabling simultaneous determination of α - and β -tryptase copy numbers.

Conclusions

The principal diagnostic challenge in HaT lies not in sequence variation but in structural variation and the accurate determination of gene copy number. High sequence similarity and the susceptibility of the tryptase locus to recombination render conventional sequencing approaches insufficient for reliable genotyping. A single-well multiplex ddPCR assay represents a solution readily implementable in routine molecular diagnostics. Establishing the genotype terminates prolonged diagnostic odysseys in patients with symptoms of unexplained origin and enables genetic counselling; in haemato-oncology, it refines the interpretation of the 20 ng/mL threshold as a minor criterion for systemic mastocytosis and allows the identification of myeloid neoplasms otherwise masked by HaT.

A critical role of physiotherapy in adults with Ehlers-Danlos Syndrome

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Słowa kluczowe: EDS, hEDS, physiotherapy, hypermobility, joint stability, proprioception

EDS, particularly hEDS, present not only physiotherapists with challenges that extend far beyond generalized joint hypermobility. Adults with EDS may experience recurrent joint instability, persistent musculoskeletal pain, impaired proprioception, altered movement strategies, fatigue, and progressive limitations in everyday function. Consequently, physiotherapy should not be reduced to strengthening hypermobile joints, but should form a central component of long-term, individualized management. The aim of the study is to present the critical role of physiotherapy in adults with EDS from a clinical and biomechanical perspective. Particular attention will be given to the relationship between joint instability, impaired proprioception and neuromuscular control. Altered sensory input and motor responses may contribute to inefficient movement patterns, protective muscle overactivity and recurrent symptoms, highlighting the need to assess not only joint range of motion and muscle strength, but also movement quality, sensorimotor control and functional stability. A key clinical challenge is finding the appropriate balance between protection and activity. Excessive avoidance of movement may promote deconditioning and loss of function, whereas inappropriate loading or aggressive exercise can exacerbate pain and instability. Physiotherapy should therefore focus on carefully graded loading, dynamic joint stabilization, proprioceptive and neuromuscular training, movement retraining, and strategies supporting safe participation in daily, occupational and recreational activities. The presentation will also address upper-limb rehabilitation, where instability of the shoulder, elbow, wrist and hand can substantially affect independence and quality of life. The importance of individualized orthotic support, joint protection, pacing and patient education will be discussed as complementary elements of rehabilitation. Ultimately, effective physiotherapy in EDS requires a shift from treating isolated painful structures toward understanding the patient as a complex sensorimotor system. The goal is not simply to increase strength or flexibility, but to improve control, confidence, functional capacity and long-term self-management.

CASP: Progress, Challenges, and Impact on Computational Structural Biology

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Over the past three decades, CASP has played a central role in shaping computational structural biology by providing an independent, community-wide assessment of methods for protein structure prediction. What began as a focused experiment in evaluating prediction accuracy has grown into a broader programmatic approach to some of the most persistent problems in science: rigor, reproducibility, transparency, and the limitations of traditional peer review. By organizing blind assessments around newly solved experimental structures, CASP created a framework in which methods could be tested under conditions that minimized bias, encouraged open comparison, and made progress measurable across the field.

The dramatic advances associated with deep learning, including highly accurate predictions for many protein domains, have transformed expectations of what computational methods can achieve. At the same time, CASP continues to highlight the challenges ahead, including modeling protein complexes, conformational ensembles, ligand interactions, RNA structures and protein-RNA assemblies, and the reliable estimation of prediction confidence. As computational methods become increasingly powerful and widely used, CASP's model of rigorous community assessment remains essential.

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HUMAN GENETICS

C1.0

Summary of Research Findings on Polyposis Syndromes in Poland

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Słowa kluczowe: intestinal polyposis, familial adenomatous polyposis, Peutz–Jeghers syndrome, juvenile polyposis syndrome, PTEN hamartoma tumor syndromes, colorectal cancer, genetic mutations, APC gene, multigene panel sequencing, Polish population

Intestinal polyposis comprises a group of hereditary disorders characterized by multiple polyps in the gastrointestinal tract and an increased risk of colorectal cancer. The main syndromes include familial adenomatous polyposis (FAP), attenuated FAP (AFAP), Peutz–Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), and PTEN hamartoma tumor syndromes (PHTS). Research in Poland has primarily focused on their molecular basis, clinical features, and diagnostic strategies. Over the past three decades, biological material from nearly 1000 families with different forms of colorectal polyposis has been collected and stored in the Polish DNA Bank for Polyposis, creating a unique and well-characterized resource. The mutational spectrum of key genes—APC, MUTYH, STK11, BMPR1A, SMAD4, NTHL1, and PTEN—has been extensively analyzed, with pathogenic variants identified in more than 50% of families. The mutation spectrum in the Polish population is highly heterogeneous, including point mutations, small insertions and deletions, and large genomic rearrangements.

Recurrent (founder) variants have also been identified, enabling the development of targeted diagnostic approaches. Despite extensive molecular studies, no clear genotype–phenotype correlations have been established. Clinical features such as polyp number and distribution, age at onset, and extracolonic manifestations remain important for guiding genetic testing and prioritizing gene analysis. The collected material has supported long-term studies, improved mutation detection strategies, and facilitated the implementation of modern techniques such as multigene panel sequencing. Overall, these efforts have significantly improved understanding of the genetic basis of colorectal polyposis in Poland and contributed to more effective, personalized approaches to diagnosis, patient management, and surveillance. The research in part was financed by state budget funds granted by the Minister of Science and Higher Education within the “Science for Society II” Program (Poland) framework, no. NdS-II/SP/0139/2024/01. amount of funding 1 980 000 zł total value of the project 1 980 000 zł

C2.0

Beyond NF1: The Diagnostic Value of Reanalysis and Expanded Panels in Neurofibromatosis Type 1 Testing.

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Słowa kluczowe: Neurofibromatosis Type 1, RASopathy-associated genes, deep intronic mutation analysis

Introduction: Neurofibromatosis type 1 (NF1) is a disorder belonging to RASopathies characterized by pigmentary changes. Molecular investigations of patients with this phenotype are conducted at the Department of Medical Genetics, Institute of Mother and Child since 10 years. The diagnostic algorithm includes targeted next-generation sequencing (tNGS), MLPA and RNA sequencing. **Methods:** Our database currently includes 1,036 probands who underwent targeted NF1 testing. This cohort includes patients that fulfil clinical diagnostic criteria for NF1 as well as individuals presenting only with café-au-lait macules. The molecular testing has been done using KAPA HyperCap Workflow (Roche) and sequencing was performed using Illumina technology. **Results:** The diagnosis of NF1 has been

confirmed in 494 patients (47,7%). An expanded panel and data reanalysis was performed for 197 individuals with clinically diagnosed NF1 and negative results of NF1 testing. This analysis revealed pathogenic variants in LZTR1 (7 patients), SPRED1 (5 patients), and NF2 (4 patients), that seem to explain the phenotype. Also analysis of gene fragments that encompass known deep intronic NF1 variants revealed the presence of pathogenic variants in 11 patients, including c.1642-449A>G (4), c.1722-24A>G (2), and c.6642+18A>G (1). Copy number variation (CNV) analysis allowed to identify pathogenic rearrangements in 5 patients, including NF1 single-exon or multi-exon deletions, duplication of exons 2-8 of NF1, deletion of exon 5 of the SPRED1 (NM_152594.3:c.423+1471_537del) and deletion of exons 10-12 of NF2. Overall, reanalysis of sequencing data using updated bioinformatic tools and panel versions enabled identification of the molecular cause in an additional 35 patients. Conclusion: Our findings suggest that the analysis of deep intronic NF1 variants is important in patients with NF1 symptoms. The multigene panel should include not only SPRED1 but also other RASopathy-associated genes, particularly LZTR1. Expanded panel sequencing is now implemented as a standard component of NF1 diagnostic evaluation in our lab.

C3.0

Prevalence of Cancer-Predisposing Germline Variants in Children Undergoing Phenotype-Driven Genetic Testing: Results from the Nationwide ONKOPRED Study

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Słowa kluczowe: cancer predisposition, children, germline variants

Background: Germline predisposition contributes substantially to pediatric cancer risk and may account for up to 20% of malignancies. We aimed to evaluate the utility of a custom panel-based next-generation sequencing (NGS) approach for identifying constitutional cancer-predisposing variants in children with malignancies. **Methods:** Between September 2022 and December 2025, 300 children diagnosed with cancer before 18 years of age were enrolled. Eligibility required at least one additional criterion: positive family history of cancer, specific cancer diagnosis, secondary malignancy, associated comorbidities, or excessive treatment-related toxicity. Germline DNA was analyzed using targeted NGS with an in-house panel of 220 cancer predisposition genes. Libraries prepared with the SureSelect XT HS2 DNA Kit (Agilent) were sequenced on a NovaSeq 6000 platform (Illumina) with a mean coverage of 100x. Variant interpretation followed ACMG guidelines. **Results:** The cohort included patients with leukemias (n=73), brain tumors (n=39), lymphomas (n=30), Wilms tumors (n=26), carcinomas (n=22), neuroblastomas (n=21), soft tissue sarcomas (n=19), germinal tumors (n=17), hepatoblastomas (n=12), rhabdoid tumors (n=7), osteosarcomas (n=7), retinoblastomas (n=7), skin cancers (n=6), and other malignancies (n=14). Median age at first diagnosis was 7 years (IQR 3–13). Forty-six patients developed two malignancies and three developed three malignancies. Cancer-predisposing variants were identified in 97/300 children (32.3%). After excluding variants associated primarily with adult-onset cancer susceptibility (n=16), 81 children carried pathogenic or likely pathogenic variants conferring pediatric cancer risk. The most frequently affected genes were TP53 (n=11), DICER1 (n=10), NF1 (n=10), RB1 (n=8), PMS2 (biallelic, n=3), and TRIM38 (n=3). Genetic findings enabled syndrome-specific surveillance in 59 of 81 affected children. Candidate variants in HLTF, MAD1L1, PIK3CG, and CDKN2B were also identified for future functional studies. **Conclusions:** Phenotype-driven targeted NGS testing effectively identifies pediatric cancer predisposition syndromes and has direct implications for patient management, surveillance, and genetic counselling.

C4.O

Diagnostic efficiency of whole-exome sequencing (WES) in identifying hereditary cancer predisposition: an analysis of 370 patients

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Słowa kluczowe: WES; Hereditary cancer predisposition syndromes; Diagnostic yield; RASopathy

Background: The identification of germline cancer predisposition syndromes is important for personalized clinical management and family genetic counseling. Whole Exome Sequencing (WES) has emerged as a comprehensive tool for diagnosing rare genetic conditions, including those with subtle or atypical oncological implications. **Objective:** This study aims to evaluate the diagnostic yield and the molecular landscape of cancer-predisposing variants within a cohort of 370 patients referred for constitutional genetic testing. **Methods:** We retrospectively analyzed WES data from 370 individuals. Variants classified as Pathogenic (P), Likely Pathogenic (LP), and Variants of Uncertain Significance (VUS) in established cancer-predisposition genes were correlated with clinical phenotypes and initial diagnostic suspicions. **Results:** Clinically significant variants were identified in 44 samples. WES demonstrated high diagnostic efficiency in confirming targeted clinical suspicions: RASopathies/Noonan syndrome were molecularly confirmed in 56% of suspected cases (9/16), and Tuberous Sclerosis Complex (TSC) achieved a 100% confirmation rate (2/2). In cases of suspected neurofibromatosis, the diagnosis was molecularly supported in 3 out of 7 patients. Notably, WES revealed crucial incidental findings in patients without prior oncological suspicion. Three pathogenic PTEN variants (associated with Cowden syndrome) were identified in individuals presenting with macrocephaly and speech delay. The low-penetrance CHEK2 variants were frequently detected across highly diverse non-cancer phenotypes, including hypercholesterolemia, autism, and congenital adrenal hyperplasia. Furthermore, rare pathogenic changes in ATM, BRCA2, and PALB2 were identified in patients presenting with atypical clinical features such as microcephaly, joint laxity, or schwannoma. WES also identified hematological predispositions, including variants in SAMD9 and SAMD9L in patients with aplastic anemia and leukocytosis. **Conclusion:** WES is a highly effective modality for confirming clinical diagnoses of hereditary syndromes like RASopathies and phakomatoses. Moreover, the frequent identification of incidental predispositions in genes such as PTEN and CHEK2 underscores the utility of WES in uncovering at-risk patients who do not meet classical clinical criteria, thereby facilitating early oncological intervention and long-term surveillance.

C5.O

Exploring Novel Cancer Predisposition Genes in Children with Secondary Malignancies Using Whole-Genome Sequencing

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Słowa kluczowe: drugie nowotwory, onkologia dziecięca

Background Approximately 1% of children diagnosed with cancer subsequently develop a secondary malignancy. Constitutional genetic predisposition is a major risk factor for multiple primary tumors. This study aimed to identify novel germline genetic variants associated with cancer susceptibility in pediatric patients diagnosed with more than one neoplasm. **Methods** Targeted next-generation sequencing (NGS) was performed in 46 pediatric patients with multiple tumors. Whole-genome sequencing (WGS) was subsequently conducted in 35 patients in whom routine genetic testing had not identified pathogenic variants associated with cancer predisposition. **Results** The median age at diagnosis of the first, second, and third malignancies was 5, 12, and 16 years, respectively. Hematologic and lymphoid malignancies were the most common primary tumors (37%), followed by central nervous system tumors (18%). Leukemias represented the most frequent secondary malignancies (33%). Targeted NGS identified pathogenic or likely pathogenic cancer-predisposition variants in 11 patients (23.9%). WGS revealed two additional pathogenic germline variants and four candidate susceptibility variants. A pathogenic truncating variant in PTPRG (p.Arg307*) was identified in a patient with primitive neuroectodermal tumor followed by ganglioneuroma. A pathogenic MYCL variant (p.Ser165Trp) was detected in a patient with neuroblastoma and histiocytosis. Another patient, diagnosed with pineoblastoma and a tubulovillous adenoma, carried a pathogenic DGCR8 frameshift variant (p.Ile639Tyrfs*4), affecting a key component of microRNA biogenesis. In addition, a constitutional duplication of chromosome 12p (12p13.2–p11.22) was identified in a patient with a germ cell tumor and myelodysplastic syndrome. **Conclusions** Children with secondary malignancies should undergo extended genetic evaluation. Comprehensive genomic analyses, including assessment of both sequence and structural variants, can identify novel cancer-predisposition genes and provide candidates for future functional validation.

C6.O

Dietary patterns in women attending a genetic counseling clinic according to high-risk breast cancer mutation status

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Słowa kluczowe: dietary patterns; breast cancer susceptibility; BRCA1; BRCA2; PALB2; CHEK2; FFQ-6

Introduction: Diet may influence breast cancer risk, but its relevance in women carriers of inherited pathogenic variants remains unclear. **Aim:** To compare dietary patterns and the frequency of consumption of selected food groups between women with high-risk pathogenic variants and the remaining patients. **Methods:** This cross-sectional study included women attending follow-up visits at the Genetic Counseling Clinic National Research Institute of Oncology, Gliwice Branch, Poland. Dietary habits were assessed with the FFQ-6 questionnaire validated for the Polish population. Of 197 available questionnaires, 25 women with unknown genetic status were excluded; 172 women were analyzed. Group A comprised carriers of BRCA1, BRCA2 or PALB2 pathogenic variants (n=105) whereas group B comprised isolated CHEK2 carriers and women without high-risk pathogenic variants (n=67). FFQ items were aggregated into 26 food groups. Principal component analysis with varimax rotation was used to identify dietary patterns. Between-group differences were assessed using nonparametric tests and adjusted logistic regression. **Results:** The mean age was 41.4±12.2 years in group A and 44.2±11.8 years in group B. Breast cancer was present in 60.8% and 46.2% of women, respectively; other baseline characteristics were broadly similar. PCA identified

three dietary patterns explaining 31.7% of the total variance: Westernized traditional, Prudent, and Dairy, breakfast cereals and treats. No significant between-group differences were found for individual food groups after correction for multiple comparisons. In the adjusted model, women with high-risk mutations had lower adherence to the Dairy, breakfast cereals and treats pattern (OR per 1 SD increase 0.65, 95% CI 0.45-0.96; $p=0.028$). No significant associations were observed for the Westernized traditional or Prudent patterns. Conclusions: Among women under oncogenetic care, high-risk pathogenic variant status was not associated with clear differences in the consumption of individual food groups. Carriers of BRCA1/BRCA2/PALB2 pathogenic variants showed lower adherence to one PCA-derived dietary pattern.

C7.0

Low selenium status and cancer risk in BRCA1 pathogenic variant carriers: a prospective study

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Słowa kluczowe: BRCA1; selenium; cancer risk; hereditary cancer; genetic predisposition; biomarker; cancer prevention

Identifying modifiable determinants of cancer risk in BRCA1 pathogenic variant carriers remains an important unmet clinical need. We evaluated the association between selenium status and incident cancer risk in women with pathogenic BRCA1 variants. We conducted a prospective cohort study of 514 women with pathogenic BRCA1 variants recruited from 16 cancer genetics clinics in Poland between Jan 2017 and Mar 2024. Blood selenium

concentrations were measured repeatedly during follow-up. Participants in the intervention cohort received dietary recommendations aimed at lowering selenium intake to below 89 µg/L based on blood measurements. Primary analyses focused on the intervention cohort (n=335); secondary exploratory analyses included an external comparator group that did not receive dietary recommendations, approximately 40% of whom had declined participation in the intervention after enrollment). Associations between selenium status and incident cancer risk were assessed using Cox proportional hazards models. During a mean follow-up of 55.0 months, 23 incident cancers were diagnosed. Low selenium status (<95.83 µg/L) was associated with a significantly increased cancer risk (hazard ratio [HR] 3.69, 95% CI 1.53–8.89; p=0.003). Kaplan–Meier curves showed clear separation between groups (log-rank p=0.02). Low selenium status was associated with increased cancer risk in BRCA1 pathogenic variant carriers. These findings support further investigation of selenium status as a potentially modifiable biomarker of cancer risk in genetically predisposed individuals.

C8.O

Selenium and arsenic status and cancer incidence in women with familial breast cancer predisposition

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Słowa kluczowe: selenium; arsenic; breast cancer risk; familial cancer; trace elements; risk index; gene–environment interaction; oxidative stress

Women with a family history of breast cancer have an increased cancer risk. Identification of modifiable environmental factors, including trace element status, may improve risk stratification. Objective: To evaluate whether selenium (Se) and arsenic (As) status, individually and jointly, are associated with cancer risk. In this multicenter intervention study, 6134 women aged ≥ 40 years with hereditary breast cancer predisposition (BRCA1-negative) were recruited from 16 genetic clinics in Poland. Participants were stratified by baseline Se status and assigned to dietary modification, selenium supplementation, placebo, or non-randomized comparator cohorts. Blood Se and As concentrations were measured repeatedly during follow-up (6–60 months). Primary analyses evaluated cancer risk across combined Se and As strata within a predefined optimal Se range (98–108 $\mu\text{g/L}$), including genotype-stratified analyses for CAT rs1001179. Exploratory analyses included a composite risk index (Risk Index (RI) = Se + As $\times$ 50). Associations with incident cancer were assessed using multivariable Cox models. Among 6134 participants, 4847 completed follow-up (79.0%). The lowest cancer risk was observed in women with Se levels between 98–108 $\mu\text{g/L}$ and As levels 0.57–0.75 $\mu\text{g/L}$. Compared with higher As (>1.07 $\mu\text{g/L}$), this group had a lower hazard of cancer (HR 3.46; 95% CI 1.28–9.32). Associations were stronger when Se, As, and CAT genotype were analyzed jointly (HR 4.74; 95% CI 1.44–15.53). In an exploratory analyses, the RI was associated with cancer risk (HR 4.51; 95% CI 1.72–11.83), with the strongest association observed in the combined RI and genotype subgroups (HR 8.49; 95% CI 2.03–35.49). Cancer risk varied across combined selenium and arsenic profiles within an optimal selenium

C9.0

Germline testing practices across ERN GENTURIS member countries

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Słowa kluczowe: Hereditary cancer; Germline genetic testing; Genetic tumour risk syndromes; ERN GENTURIS; Multigene panel testing; Next-generation sequencing; Polygenic risk scores; Whole-exome sequencing; Whole-genome sequencing; Genetic counselling; Access to genetic testing; Laboratory accreditation.

Germline genetic testing practices for hereditary cancer vary across the European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS) member countries. We surveyed experts in genetic testing from 20 EU member countries and Norway to assess multi-gene panel usage, availability of genome-wide sequencing, first-tier testing approaches, implementation of polygenic risk scores, and the roles of non-genetic healthcare professionals. National experts and members of the ERN GENTURIS completed a structured questionnaire covering founder germline pathogenic variants (gPV) testing, panel testing for common genetic tumour risk syndromes, use of whole-exome sequencing (WES) and whole-genome sequencing, polygenic risk score implementation, use of formalin-fixed paraffin-embedded tumour samples, laboratory accreditation, and the clinical roles of physicians, genetic counselors, and nurses. Significant inter-country heterogeneity was observed. Most countries rely on next-generation sequencing (NGS) multi-gene panels. Founder gPV testing is first-line in a few high-prevalence populations (e.g., BRCA1/2 founders). All 21 countries offer NGS panel tests for hereditary breast and ovarian cancer, and ≥ 19 countries do so for colorectal and prostate cancers. However, NGS panel size and gene composition exhibit substantial variability. WES is available in 12 countries on a routine basis. Most countries implemented genetic testing on stored tumour tissue from deceased patients. In all countries, clinical geneticists can order germline genetic tests, and in 9 countries, any physician can do so. These findings show differences in accessibility to germline genetic testing of hereditary cancer in Europe. We propose EU-wide guidance via pathways, standards of care, and sharing of best practices to further optimize access to hereditary cancer genetic molecular diagnostics.

C10.O

RUO Under Scrutiny: Validation of Molecular Laboratory Testing Methods in Light of IVDR (EU) 2017/746 and ISO 15189:2023

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Słowa kluczowe: ISO 15189

The implementation of Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) has introduced new regulatory requirements for medical diagnostic laboratories regarding the use of in-house developed methods and Research Use Only (RUO) reagents in clinical diagnostics. Genetic laboratories employing commercial RUO kits or laboratory-developed reagent configurations must comply with the requirements of Article 5(5) of the IVDR, creating significant organizational and documentation challenges. The aim of this study was to develop a practical documentation and validation framework for in-house IVD methods in a medical genetics laboratory, compliant with the IVDR and PN-EN ISO 15189:2023. A comprehensive documentation package was developed, including: a manufacturing procedure for in-house IVD devices incorporating risk management in accordance with PN-EN ISO 14971; an analytical procedure (illustrated using a Real-Time PCR assay for mutation detection); a method validation and verification procedure; and a validation protocol assessing analytical sensitivity, analytical specificity, precision, repeatability, and reproducibility. In addition, a template for the public declaration required under Article 5(5)(f) of the IVDR was prepared. The proposed documentation framework fulfills the General Safety and Performance Requirements (GSPR; Annex I of the IVDR) and complies with the requirements of PN-EN ISO 15189:2023. The presented solutions enable diagnostic laboratories to use RUO reagents for clinical purposes in a legally compliant manner.

C11.O

From DNA to reliable WGS results: optimizing wet-lab parameters and quality control during NGS platform implementation

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Słowa kluczowe: Whole-Genome Sequencing (WGS), Next-Generation Sequencing (NGS) implementation, pre-analytical quality control, wet-lab optimization,

Implementing routine whole-genome sequencing (WGS) into laboratory diagnostics requires a robust technical workflow and strict standardization. The aim of this study is to present the protocol for quality control, automation, and scaling of the WGS workflow within the project "Digital Medicine: An innovative approach to diagnostics and therapy based on science – RCMC DISRUPTOR at UMW" under project no. 2023/ABM/02/00003–00. We analyzed laboratory metrics from 79 pediatric patients with rare diseases. DNA isolation was performed using the Auto-Pure S32 system, library preparation was executed on the Hamilton NGS STARlet pipetting station, and sequencing was carried out on the Illumina NextSeq 2000 platform. Quality control was monitored via the NanoDrop Lite Plus, Quantus Fluorometer, and 4150 TapeStation. While whole blood served as the primary matrix, dry buccal swabs were utilized in specific cases. Key determinants for sequencing success included a minimum DNA concentration of ≥ 3 ng/ μ l and an A260/280 ratio ≥ 1.8 , but primarily the selection of adapter combinations (indexes) and library loading optimization onto the Flow Cell P4. It was demonstrated that an improper signal balance between the i7 and i5 indexes (increased share of nucleotides read in the same fluorescence channel) increases the number of reads classified as demultiplexing mismatches (One Mismatch), despite high Q30 values. Furthermore, over-clustering the Flow Cell P4 due to suboptimal loading concentrations degraded read quality and increased index identification errors. Introducing library pool molarity control, precise pooling, and run monitoring effectively eliminated these issues. During the initial implementation, the first 4 runs yielded Q30 scores of 86–88% and a total yield of 530–590 Gbp. Following simultaneous optimization of index signal balance and loading conditions, subsequent runs achieved stable Q30 scores $>90\%$ (specification: $\geq 90\%$), an increased total yield of 590–650 Gbp (specification: ≥ 540 Gbp), and a low proportion of One Mismatch reads, while consistently maintaining a 30x mean genome coverage.

C12.O

Should every variant of uncertain clinical significance (VUS) be reported in postnatal diagnostics using aCGH?

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Słowa kluczowe: array comparative genomic hybridization (aCGH), copy number variants, postnatal diagnosis, variant of uncertain significance

Copy-number variants of uncertain clinical significance (VUSs) detected by microarray analysis include genomic losses or gains that cannot be straightforwardly classified as disease causing or benign due to limited evidence of gene(s) function, incomplete penetrance, or variable expressivity of the region, and require careful management due to their ambiguous clinical impact. In Europe, reporting of CNVs of uncertain significance is regulated by recommendations from organizations like the European Society of Human Genetics and, in certain countries, adapted by national bodies (e.g., Association for Clinical Genomic Science in the UK). However, not all countries have such guidelines established. According to the 2024 British recommendations, only VUSs with a high probability of being causally-related to the patient's phenotype should be included in aCGH result report. This includes variants for which additional evidence allowing reclassification to likely pathogenic/pathogenic is likely to emerge in the future. We present selected examples of variants of uncertain significance detected and reported in line with Laboratory's internal policy in postnatal microarray settings. These examples include: 3p22.1 duplication encompassing exons 1-2 of the MYRIP gene, Xp11.2p11.23 duplication encompassing exons 2-5 of the ZNF674 gene, 4q13.1 triplication encompassing exons 3-17 of the EPHA5 gene, and Xp22.11 deletion spanning the DDX53 gene. We present these variants in the context of clinical presentation and discuss whether they should be reported at all. Currently, there are no national recommendations on copy-number reporting in Poland, resulting in inconsistency in reporting strategies among different laboratories. This creates broader implications for patients and their families, often adding anxiety and confusion when receiving inconclusive report. Additional key challenges include clinical management dilemmas and can lead to unnecessary prenatal testing. We therefore propose an open discussion on the urgent need to create Polish recommendations on CNV VUSs reporting.

C13.O

The genetic, chromosomal, and epigenomic landscape of male infertility

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Słowa kluczowe: male infertility, chromosome translocations, sperm epigenome

Infertility is estimated to affects approximately 12-15% of couples of reproductive age worldwide, with about 7% of men and 12% of women experiencing difficulties in achieving reproductive success. Male factors contribute to ~20-30% of all infertility cases. There broad etiological spectrum of male side of infertility includes genetic factors, chromosomal aberrations, epigenetic alterations, hormonal disturbances, infections, or functional defects of the male reproductive tract. Genetic causes are responsible for ~10-15% of male infertility. It is estimated that nearly 2,000 genes are involved in spermatogenesis. To date, approximately 950 causative variants characterized in ~400 genes have been more precisely linked to the observed infertile phenotypes. Chromosomal aberrations represent another key component of male infertility. Their frequency in infertile men is several times higher than in the general population, averaging about 3.5% overall and up to 20% among men with azoospermia. Nevertheless, in about 25% of cases, the etiology of male infertility remains idiopathic. In recent years, accumulating evidence has pointed to an important contribution of epigenomic dysregulation, particularly within this idiopathic subgroup. Epigenetic alterations have been documented both during the course of spermatogenesis and in mature spermatozoa, including aberrant DNA methylation patterns, abnormal post-translational histone modifications, and changes in the content and regulation of non-coding RNAs. In this presentation the review of current knowledge on the role of chromosomal and epigenomic aspects of male infertility will be summarized, with the potential suggestions for improving diagnosis and risk stratification in affected men. Funding: National Science Centre in Poland 2020/38/E/NZ2/00134

C14.O

Implementation of a Quick and Coordinated Genetic Diagnostic Program for Newborns and Infants with developmental delay and congenital defects: Results and Conclusions from Three Years of Experience

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Słowa kluczowe: rare diseases, coordinated healthcare, diagnostic yield, pediatric genetics

Introduction: Congenital malformations and developmental delay are a major group of rare diseases, affecting fewer than 1 in 2,000 individuals but collectively impacting up to 8% of the population. Most have a genetic basis and manifest in childhood, often requiring complex diagnostics, long-term treatment, and multidisciplinary care. Early detection and coordinated management are essential to improve outcomes and quality of life. The aim of this study was to evaluate the effectiveness of a coordinated genetic diagnostic program for children with congenital anomalies and suspected rare diseases. **Materials and Methods:** Patients with suspected rare diseases were referred to the University Centre for Rare Diseases (UCRD), where medical records were reviewed by a multidisciplinary Medical Council. Based on case evaluation, patients underwent targeted diagnostics, including specialist consultations and genetic testing (array comparative genomic hybridization [aCGH], next-generation sequencing [NGS] panels, or whole-exome sequencing [WES]). Coordinated care, telemedicine, and psychological support were provided when indicated. **Results:** From March 2023 to June 2026, a total of 1,201 patients with congenital anomalies requiring further diagnostic evaluation

were enrolled in the program. Patient cases were discussed during 143 multidisciplinary meetings, resulting in 228 patients being selected for genetic testing. A molecular diagnosis was established in 65% of tested patients. The lowest diagnostic yield was observed for aCGH testing (40%), whereas WES achieved the highest diagnostic yield, with a diagnosis established in 75% of tested patients. **Conclusions:** A coordinated multidisciplinary approach combined with targeted genetic testing achieved a high diagnostic yield in children with congenital anomalies and suspected rare diseases. Pre-test clinical assessment by a multidisciplinary medical council optimized test selection, improving diagnostic efficiency and reducing unnecessary costs. Early genetic diagnosis facilitated personalized patient management, genetic counselling, and access to appropriate support services for affected families.

C15.O

Nine months of pilot population-based newborn screening for spinal muscular atrophy and severe combined immunodeficiency using a combined assay in Poland.

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Słowa kluczowe: spinal muscular atrophy, severe combined immunodeficiency, newborn screening

Introduction: Spinal muscular atrophy (SMA) and severe combined immunodeficiency (SCID) are rare disorders in which early diagnosis is essential for prompt treatment and better outcomes. In Poland, SMA was included in the Newborn Screening Programme in April 2021. Since September 2025, a pilot screening is performed for SCID. Here, we present nine months of our experience with combined SMA and SCID newborn testing. **Methods:** DNA is extracted from dried blood spots if parent agrees for molecular testing. Since September 2025, a qPCR based test SPOT-it TREC, KREC & SMN1 Screening Kit (ImmunoID) is used for SMA and SCID screening. Presumptive positive results for SMA are confirmed with a PCR-based AmpliEx SMA Plus Kit (Asuragen) and MLPA with P021 kit (MRC-Holland). Infants with confirmed SMA are referred to pediatric neurology centers for neurological assessment, confirmatory testing and treatment qualification based on SMN2 copy number and phenotype. Newborns with abnormal SCID results are referred to Immunology Clinic for clinical and immunological evaluation, and if the results are consistent with inborn errors of immunity, molecular testing is performed. **Results:** By June 2026, 174737 newborns had been tested with the combined assay. In this group, 8 patients were diagnosed – 4 with SCID (3 - RAG1 deficiency, 1 - JAK3 deficiency) and 4 with combined immunodeficiencies (2 - Nijmegen syndrome, 1 - CHARGE syndrome, 1 - RMRP-related syndrome). In addition, 19 new SMA cases were detected by the combined test giving a total number of 165 children diagnosed with SMA by the newborn screening (1266800 patients screened). All underwent clinical assessment and treatment according to current policy.

C16.O

PURA-related neurodevelopmental disorder: clinical delineation and genotype-phenotype study in further seven individuals

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Słowa kluczowe: PURA, neurodevelopmental syndrome, NGS sequencing

Background: PURA-related neurodevelopmental disorder (PURA-NDD; OMIM #616158) is a rare autosomal dominant condition caused by pathogenic variants in the PURA gene, encoding Pura, a multifunctional protein involved in DNA replication, transcriptional regulation, and RNA transport. The clinical presentation is characterized primarily by neonatal hypotonia, global developmental delay, intellectual disability, feeding difficulties, and epilepsy, along with additional features such as respiratory insufficiency, movement disorders, hypersomnolence, and variable dysmorphic traits. Although PURA-NDD presents with a relatively consistent core phenotype, considerable clinical variability between affected individuals frequently complicates diagnosis based solely on clinical presentation. **Patients and methods:** We present a cohort of seven patients with PURA-NDD identified through a genotype-first diagnostic strategy using comprehensive genomic testing (NGS panel). They were initially referred due to nonspecific neurodevelopmental abnormalities, such as neonatal hypotonia, respiratory distress, and epilepsy, without a clearly directed clinical diagnosis. **Results:** Molecular analysis identified seven heterozygous variants in PURA, of which three were novel: c.311T>G p.(Met104Arg), c.515A>C p.(Gln172Pro), and c.885delinsGC p.(His296Profs21). The remaining variants included previously reported missense and frameshift changes associated with PURA-NDD, as well as one ClinVar-reported variant. **Conclusions:** Our results broaden the known mutational spectrum of PURA and emphasize the value of combining molecular findings with clinical data for accurate variant interpretation. This study contributes to the growing field of molecular genetics of rare disorders, illustrating how genomic approaches improve diagnostic effectiveness, refine clinical characterization, and deepen understanding of disease-causing mechanisms.

C17.0

Phenotypic characteristics of Polish patients with AGO1 and AGO2 mutations.

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Słowa kluczowe: AGO1, AGO2, Argonaute syndromes, NEDLBAS, Lessel-Kreienkamp syndrome, neurodevelopmental disorders

Phenotypic characteristics of Polish patients with AGO1 and AGO2 mutations. In eukaryotes, Argonaute family proteins (AGO1–4) bind various classes of small non-coding RNAs (miRNAs, siRNAs, piRNAs) to form RNA-induced silencing complexes (RISC) within RNA interference (RNAi) pathways. This study focuses on AGO1 and AGO2. Despite high structural homology, they differ significantly in function: AGO1 acts primarily as a scaffold protein, whereas the PIWI domain of AGO2 possesses unique catalytic activity enabling direct cleavage of target mRNA transcripts. Since the early 21st century, the involvement of AGO proteins in the pathogenesis of neurodevelopmental, oncological, and immunological disorders has been increasingly documented. The first patient with an AGO1 mutation was reported in 2012, and in 2021, the NEDLBAS syndrome was formally defined. It manifests as intellectual disability, delayed expressive language development, autism spectrum disorder (ASD) behaviors, feeding difficulties, and dysmorphic features (including microcephaly, a broad nasal bridge, and a round face). Similarly, for the AGO2 gene, Lessel-Kreienkamp syndrome (LESKRES) was formally defined in 2020. The clinical presentation of both entities (collectively termed Argonaute syndromes) overlaps significantly and exhibits profound clinical heterogeneity, making distinct differentiation challenging. The objective of this study is to describe and compare the phenotype of Polish patients with mutations in the AGO1 and AGO2 genes. A retrospective analysis and phenotypic comparison of Polish patients with AGO1 and AGO2 mutations were performed. Psychomotor development, neurological profile (including EEG recordings and neuroimaging studies), and metabolic profile were evaluated. The presented clinical characterization of the Polish cohort provides a better understanding of rare phenotypes within the Argonaute syndromes, facilitating their future diagnosis and the personalization of medical care.

C18.O

Clinical evolution and quality of life in Kabuki Syndrome: a 2-year observational study of 45 genetically confirmed Polish patients.

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Słowa kluczowe: Kabuki syndrome, Quality of life, caregiver burden, multidisciplinary care, genotype-phenotype correlation, Polish group

Background: Kabuki syndrome (KS) is a rare multisystem neurodevelopmental disorder caused by pathogenic variants in KMT2D or KDM6A. This study aims to evaluate quality of life (QoL) in a genetically confirmed Polish patient group and examine longitudinal trends alongside associations between multidisciplinary interventions, patient functioning, and caregiver burden. **Methods:** We conducted a prospective observational study of 45 individuals with molecularly confirmed KS (43 children, 2 adults) recruited between 2020 and 2023. Caregivers completed a 100-item QoL questionnaire. Participants underwent multidisciplinary in-person clinical evaluations and a 2-year follow-up. Interventions analyzed included speech, physio-, dog, and music therapies, nutritional care, and recombinant growth hormone treatment. Data were analyzed using descriptive statistics, correlation analyses, and subgroup comparisons. **Results:** Caregivers reported substantial QoL impairment across physical, cognitive, and psychosocial domains. The mean total fatigue score was 2.82/4 (indicating mild-to-moderate fatigue), with cognitive fatigue being particularly prominent. Child fatigue strongly correlated with family impact ($p < 0.001$), though perceived family burden decreased with patient age ($p < 0.001$). Multidisciplinary therapies were widely utilized and associated with reported improvements in motor development and daily functioning. Distinct genotype-related differences in hypoglycemia risk were observed [e.g., occurring predominantly in KMT2D variants]. **Conclusion:** QoL in KS is multidimensional and intrinsically linked to caregiver well-being. Integrated, multidisciplinary care may significantly mitigate disease burden. Early, coordinated, family-centered interventions appear essential to optimizing long-term patient and family outcomes.

C19.0

Exome sequencing for the genetic evaluation of fetuses with craniofacial abnormalities.

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Słowa kluczowe: craniofacial abnormalities, prenatal diagnosis, exome sequencing

Background: The development of craniofacial structures is a highly complex process regulated by both genetic and environmental factors. It involves pluripotent neural crest cells, which migrate to the developing head and neck region and contribute to the formation of the pharyngeal arches, giving rise to bone, cartilage, and muscle tissues. The most common craniofacial abnormalities are orofacial clefts. Other anomalies include microcephaly, craniosynostosis, micrognathia, external ear malformations, and hypertelorism. Despite the identification of numerous genes associated with craniofacial malformations, many patients remain without a confirmed genetic diagnosis. Prenatal exome sequencing has improved the identification of genetic causes of fetal structural anomalies, increasing diagnostic yield and supporting genetic counseling. **Material and methods:** In this study, we aim to recruit a cohort of 40 fetuses with craniofacial abnormalities identified by prenatal ultrasound. Among the fetuses enrolled thus far, all presented with dysmorphic features and craniofacial anomalies. Two fetuses exhibited features consistent with Pfeiffer syndrome, while one fetus exhibited features consistent with Meckel syndrome. To date, exome sequencing analysis has been performed in seventeen fetuses. Genomic DNA was isolated from amniotic fluid cells, chorionic villi, or umbilical cord tissue. **Results:** Exome sequencing revealed pathogenic variants in four patients, likely pathogenic variants in two patients, and a variant of uncertain significance in one patient. We identified variants in the *MKS1*, *FGFR2*, *FLNB*, *OFD1*, *IRF2BPL* and *ZFHX4* genes. All clinically relevant variants detected by exome sequencing were subsequently confirmed by Sanger sequencing. **Conclusion:** Our findings support the use of prenatal exome sequencing in fetuses with craniofacial anomalies. The integration of detailed prenatal ultrasound assessment with molecular testing improves diagnostic yield and facilitates genotype–phenotype correlation. Further analysis of a larger cohort will help define the genetic landscape of these defects and optimize genetic counseling and prenatal care.

C20.O

Application of molecular methods in the differentiation and characterization of bone tumors in children – our own experience

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Słowa kluczowe: NGS, FISH, osteosarcoma, Ewing sarcoma

Malignant bone tumors account for ~7% of childhood cancers, most commonly osteosarcoma, Ewing sarcoma, and chondrosarcoma. Fluorescence in situ hybridization (FISH) remains the gold standard, while next-generation sequencing (NGS), including RNA sequencing (RNA-

seq), enables identification of rare fusion partners and assessment of biomarkers such as homologous recombination deficiency (HRD), microsatellite instability (MSI), and tumor mutational burden (TMB). Here we present the preliminary results of an ongoing multicenter collaboration in cytogenetic and molecular diagnostics of pediatric bone tumors. In this initial cohort, 14 samples from patients with suspected or confirmed bone tumors, collected over 8 months, were analyzed. First, FISH probes were used to identify rearrangements characteristic of Ewing sarcoma and osteosarcoma. Among 5 Ewing sarcoma samples, 1 did not yield a diagnostic FISH result with the EWSR1::FLI1 probe; the remaining were positive and confirmed by RNA-seq. The non-diagnostic sample contained 80% tumor necrosis. In one Ewing sarcoma case, NGS additionally revealed a STAG2 deletion. Among 9 osteosarcoma samples, 3 did not yield a diagnostic FISH result; in one, RNA quality allowed a result by RNA-seq. MDM2/CDK4 amplification was tested in 5 samples, all normal. TP53 and RB1 deletion analyses in 3 patients identified an RB1 deletion in one. In osteosarcoma samples with high nucleic acid integrity, NGS revealed NF1 c.7457+2T>G in one patient; RECQL4 c.2296+1del with ATR c.2320del in a second; TP53 c.842A>T in a third; TP53 c.1014C>A with high HRD in a fourth; and TP53 c.711G>A with intermediate HRD in a fifth. Integrating classical and modern techniques substantially increases diagnostic precision and enables identification of therapeutic targets. The main limitation is the quality and amount of tissue material, highlighting the role of standardized pre-analytical procedures. Although preliminary, our data support multicenter cooperation and advanced molecular diagnostics as essential for personalized medicine in these rare, heterogeneous pediatric tumors.

C21.0

Breast cancer in males report on 48 cases.

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Słowa kluczowe: breast cancer, male, BRCA

Introduction: Male breast cancer accounts for 1% of all breast cancers. Its low frequency leads to a lack of awareness, resulting in significant diagnostic delays. Additionally, this limits the available evidence, which primarily uses diagnostic-therapeutic algorithms based on women. Objectives: To analyze the prevalence, clinical presentation, anatomical and pathological characteristics. Materials and methods: A observational, descriptive, retrospective study was conducted in NIO PIB Gliwice Branch, from 2017 to 2025 including 48 men with a pathological diagnosis of breast cancer. Patients had a genetic consultation. During the patient interview a written, informed consent and a blood sample were obtained from all subjects. All patients were of European ancestry and ethnic Poles. Results: A total of 48 patients were included. The pathogenic variants in BRCA genes were detected in 6 patients (10,6 %), and 1 in CHEK2 gene. The most common clinical presentation was a palpable mass in 19 males (40,4%) . Invasive ductal carcinoma was the most frequent type in 44 (93,6%), and luminal A and luminal B were the predominant subtype (47.76%). Surgery was the most utilized treatment; mastectomy was performed in 37 patients (77,08%). Conclusions MBC is still poorly understood and has a significantly lower survival benefit. MBC diagnosis is challenging, not only because of the lack of screening, limited awareness, and education, but also because of the general inclination among men to delay care. There is need for dedicated research to fully explain the various aspects related to this disease

C22.O

From Validation to Clinical Application: Duplex Sequencing for Detection of Low-Level Mosaicism

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Słowa kluczowe: mosaicism, duplex sequencing, rare disorders

Background: Postzygotic mosaicism is increasingly recognized as one of the contributors in genetic disorders and cancer, yet variants present at low variant allele fractions (VAFs) may escape detection by conventional next-generation sequencing. Duplex sequencing substantially improves error correction and enables highly sensitive variant detection. We optimized and validated duplex sequencing workflow for the detection of low-level mosaic

variants across clinically relevant genes. **Methods:** Targeted duplex sequencing assays were optimized and validated for the PIK3CA, TP53, NF2, SMARCB1, LZTR1, TSC1 and TSC2 genes. Analytical validation included positive and negative controls and serial dilution experiments to establish assay sensitivity and detection thresholds. Variant-calling thresholds were defined individually based on gene-specific background error profiles and sequencing performance metrics. **Results:** As a proof-of-principle application, duplex sequencing of PIK3CA and TP53 identified multiple pathogenic hotspot variants in uninvolved mammary tissue from breast cancer patients, with VAFs as low as 0.01%. Analytical validation of the NF2, SMARCB1, and LZTR1 assay using 35 control samples revealed no pathogenic variants in NF2 and SMARCB1, while a single low-level splice variant (0.11% VAF) was detected in LZTR1. Serial dilution experiments established a detection threshold of 0.06% VAF. Analysis of 102 patients with suspected schwannomatosis identified mosaic NF2 variants in 11 individuals; blood and tumor findings were fully concordant in all eight cases with available tumor material. Validation of the TSC1/TSC2 assay showed no pathogenic variants in 19 control samples, while detection thresholds for TSC1/TSC2 were established at 0.22% and 0.34% VAFs, respectively. Among 20 individuals with suspected tuberous sclerosis complex, pathogenic mosaic TSC2 variants were identified in five. **Conclusions:** The optimized duplex sequencing workflow provides a robust method for detecting low-level mosaic variants. Its implementation in clinical settings may improve the diagnostic yield for mosaic conditions. **Funding:** The study was supported by the National Centre for Research and Development (EJPRD/IV/36/EURONET-NF/2023; LIDER/45/0234/L-12/20/NCBR/2021) and by the Foundation for Polish Science (MAB/2018/6).

C23.O

The Essential Role of DNA/RNA Next-Generation Sequencing, Rather than Immunohistochemistry, in the Molecular Subtyping of Intrahepatic Cholangiocarcinoma.

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Słowa kluczowe: intrahepatic cholangiocarcinoma, molecular subtypes, FGFR2 fusion, next-generation sequencing (NGS)

Background: Intrahepatic cholangiocarcinoma (iCCA) is an aggressive biliary malignancy with poor 5-year survival. Recent studies have identified distinct genomic subtypes, including tumors with IDH1 mutations or FGFR2 fusions, that may benefit from targeted therapy. This study used integrated DNA and RNA next-generation sequencing (NGS) to characterize the molecular landscape of a large iCCA cohort and evaluated FGFR2 immunohistochemistry (IHC) as a screening method for FGFR2 fusions. **Methods:** Tumor samples were analyzed using a 500-gene DNA NGS panel and an ArcherDx targeted RNA fusion sequencing assay, then assigned to molecular subgroups. FGFR2 protein expression was assessed by IHC using four antibodies targeting different epitopes, and results were compared with DNA/RNA NGS findings. **Results:** Partially degraded DNA and RNA were successfully extracted from 140 and 119 tumors, respectively. Seventy-four tumors harbored mutations in major oncogenes (ABL1, BRAF, EGFR, FGFR2, ERBB2, ERBB4, IDH1, IDH2, KRAS, NRAS, PIK3CA) or tumor suppressor genes (CDKN2A, FBXW7, SMAD4, STK11, TP53). Coexisting oncogene and tumor suppressor mutations occurred in 11 tumors. IDH1 mutations were the most frequent (n=23), followed by RAS and BRAF alterations. NRAS amplification without mutation was identified in three cases, while TP53 was the sole alteration in 11 of 16 TP53-mutated tumors. FGFR2 fusion transcripts were detected in 15 cases (12.6%), most commonly FGFR2::BICC1 (n=5), with multiple additional fusion partners. Tumors with FGFR2 fusions lacked other pathogenic mutations. Oncogenic BRAF isoform transcripts were identified in four FGFR2 fusion-negative iCCAs without mutations in IDH1/2, KRAS/NRAS, BRAF, PIK3CA, or TP53. FGFR2 IHC showed inconsistent results, with both false-negative and false-positive findings. **Conclusions:** Molecular testing identifies therapeutically relevant iCCA subtypes, including tumors driven by FGFR2 fusions. FGFR2 IHC lacks sufficient sensitivity and specificity, supporting comprehensive DNA/RNA-based NGS as the preferred method for selecting patients for targeted therapies.

C24.O

Regulation of mTOR pathway components by miR-199b-5p, a candidate tumor suppressor in T-ALL

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. Aberrant activation of the mTOR signaling pathway has been reported in multiple cancers, including T-ALL, making it a potential therapeutic target. To better identify patients who may benefit from mTOR-targeted therapies, the mechanisms underlying mTOR deregulation need to be clarified, including the role of miRNAs, which remains poorly understood. Using mRNA-seq data from 50 T-ALL patients, we analyzed the expression of 154 mTOR-related genes. Unsupervised hierarchical clustering identified two distinct patient subgroups. Differential expression of DEPTOR and PRAS40, endogenous inhibitors of mTOR complexes, strongly distinguished these clusters, suggesting differences in mTOR pathway activity. Analysis of matched miRNA-seq data identified 42 differentially expressed miRNAs between these groups. Among them, miR-199b-5p was significantly downregulated in patients with presumed higher mTOR activity and also in T-ALL compared with normal thymocytes, suggesting a tumor suppressor role. To assess its function, we stably overexpressed miR-199b-5p in MOLT-4 and DND-41 T-ALL cell lines with low endogenous expression. GFP competition assays demonstrated reduced survival rate upon miR-199b-5p overexpression. In silico analysis identified several mTOR pathway elements as predicted miRNA targets, including mTOR, MLST8, TELO2, RAPTOR, and RHEB, which were validated as miRNA targets by luciferase assays. Immunoblotting confirmed decreased MLST8 protein levels in both cell lines upon miR-199b-5p overexpression. To validate the contribution of MLST8 repression to miR-199b-5p effect, we performed shRNA-mediated MLST8 silencing in both cell lines. MLST8 inhibition reduced survival rate, phenocopying the effects of miR-199b-5p overexpression and supporting MLST8 as its functional downstream target. Because mTOR hyperactivation has been linked to steroid resistance, we tested dexamethasone sensitivity and found that miR-199b-5p overexpression significantly lowered dexamethasone IC50 dose. These findings identify miR-199b-5p as a novel tumor suppressor candidate in T-ALL. Its downregulation may contribute to leukemogenesis through insufficient repression of mTOR pathway components and may indirectly promote steroid resistance. Funding: NCBR: STARTEGMED3/30456/5/ NCBR/2017NCN: 2021/41/N/NZ2/02247, 2014/15/B/NZ2/03394

C25.O

ETV6-Related Hereditary Thrombocytopenia with Cancer Predisposition in Pediatric Acute Lymphoblastic Leukemia: Insights from a Nationwide Cohort of 1811 Patients

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Słowa kluczowe: ETV6, children, acute lymphoblastic leukemia, Whole Genome Sequencing, cancer predisposition

Background: While somatic lesions of ETV6 gene are ample in hematological cancer including pediatric acute lymphoblastic leukemia (ALL), germline aberrations affecting ETV6 gene, causative for hereditary thrombocytopenia 5/leukemia predisposition syndrome (OMIM #616216) have so far been documented in only 35 families worldwide. **Aim:** The aim of this study was to assess the frequency and clinical impact of ETV6 constitutional alterations in a homogeneous, extensively characterized cohort of Polish pediatric ALL patients treated according to two consecutive national protocols. **Material:** The study cohort included 727 pediatric patients treated for ALL according to the cALL-POL 2020 clinical trial and 1084 pediatric patients treated according to the AIEOP-BFM ALL 2017 Protocol. Germline genomic DNA derived from remission blood, bone marrow and buccal swabs. With a combination of high-throughput sequencing technologies [RNA seq and WGS (n=725)] we genetically screened 1213 patients and identified 6 unrelated pediatric ALL patients with potentially pathogenic germline ETV6 variants, including 5 B-ALL and 1 T-ALL case, 5 of whom display chronic/familial thrombocytopenia, corresponding with 0.4% incidence. There were two unrelated patients with a recurrent syndromic p.Pro214Leu variant and 4 patients with variants previously not reported (p.Glu335Lys, p.Arg386Ile, c.164-1G>A, p.Tyr346Cys). Germline ETV6 lesions in pediatric ALL were associated with low initial WBC count, frequent therapy disruptions, B-cell immunophenotype, hyperdiploidy and Ras pathway mutations signature. Leukemia treatment history of all 5 patients with B-ALL was impacted by prolonged thrombocytopenia. One patient developed secondary MDS. **Conclusions:** In conclusion, we documented and analyzed ETV6-related inherited thrombocytopenia (IT) associations in a large-scale, modern technology-driven study of a homogeneous pediatric ALL population, and provide refined prevalence estimates together with tentative clinicobiological associations. We emphasize the need to translate the accumulating evidence into recommendations for cancer surveillance and hematopoietic stem cell transplantation.

C26.O

Genomic landscape and clinical correlates of copy number alterations and loss of heterozygosity in pediatric B-cell acute lymphoblastic leukemia with ETV6::RUNX1 rearrangement

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Słowa kluczowe: pediatric B-ALL, ETV6::RUNX1, copy number alterations, loss of heterozygosity, SNP microarray, relapse

Background: B-cell acute lymphoblastic leukemia (B-ALL) is the most common childhood malignancy, and the t(12;21)(p13;q22) translocation, resulting in the ETV6::RUNX1 fusion, defines one of its most frequent genetic subtypes. Despite its generally favorable prognosis, a subset of patients relapses, and reliable markers of this risk remain insufficiently defined. Aim: To perform a genome-wide characterization of secondary copy number alterations (CNAs) and loss of heterozygosity (LOH) accompanying ETV6::RUNX1 rearrangement, and to assess their relationship with clinical course and treatment response. Methods: We retrospectively analyzed 236 children with ETV6::RUNX1-positive B-ALL (mean age 5.1 years; 50.4% male) treated according to the AIEOP-BFM 2017 protocol. Diagnostic bone marrow samples were examined by SNP-array, enabling detection of segmental aberrations (>10 Mb) and gene-level lesions across 1283 leukemia-associated genes (<10 Mb), and integrated with clinical and treatment-response data. Results: In our cohort, we identified a broad spectrum of secondary CNAs and LOH regions accompanying ETV6::RUNX1 rearrangement. Partial deletions predominated, mainly involving 12p with the non-rearranged ETV6 allele (46.6%), followed by deletions of 6q (25.0%), 9p (15.7%), and 11q (15.3%); whole-chromosome loss was limited to monosomy X (5.1%). Gene-level deletions mainly involved ETV6, CDKN1B,

BCL2L14, ATF7IP, CDKN2A/CDKN2B, and PAX5. Whole-chromosome gains were dominated by trisomy 21 (19.1%) and trisomy 10 (9.7%), whereas partial duplications most frequently affected Xq (24.6%) and 21q containing RUNX1 (12.7%). Copy-neutral LOH occurred in 12p including ETV6 (6.4%) and on chromosome 11 (4.2%). The cohort showed a favorable clinical course, although relapses occurred. Conclusions: SNP-array complements conventional cytogenetics by refining the molecular karyotype and revealing submicroscopic CNAs and copy-neutral LOH, including alterations not detectable by standard karyotyping. These lesions indicate biological heterogeneity despite generally favorable prognosis. Integrated genomic and clinical assessment of ETV6::RUNX1-positive B-ALL may improve diagnostics, refine risk stratification, identify patients requiring closer molecular monitoring, and support earlier identification of patients at higher relapse risk during clinical follow-up after completion of therapy.

C27.0

Clinical and molecular characteristics of inherited bone marrow failures in the Polish population.

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Słowa kluczowe: iBMFs, NGS, SNP-array, OGM, HSCT, Somatic, Malignancy, Inborn Errors of Immunity

Introduction: Inherited bone marrow failure syndromes (iBMFs), which include Fanconi Anemia, Shwachman-Diamond Syndrome, or dyskeratosis congenita, are a heterogeneous group of disorders caused by defects in genes related to DNA repair, ribosome biogenesis and normal hematopoiesis. Defects leading to iBFM are additionally associated with a high risk of clonal transformation, predisposing to hematological malignancies. Treatment of iBFM is challenging and depends on an accurate diagnosis, which requires advanced genetic testing. The aim of this study was to present the molecular diversity of bone marrow failure in the Polish population based on high-throughput studies conducted at Medical Laboratory of Pediatric Oncology and Hematology, Central Clinical Hospital of the Medical University of Lodz, between 2016 and 2026. **Patients and methods:** The study group included 1250 patients and the inclusion criteria were leukopenia, anemia, thrombocytopenia, neutropenia, or pancytopenia. Studies were conducted using deep next-generation sequencing (NGS) followed by high-resolution SNP-arrays and Optical Genome Mapping in selected cases. **Results:** Genetic defects were identified in 420 of the 1,250 (34%) patients. The largest group consisted of patients with congenital neutropenia (27%), followed by patients with thrombocytopenia (25%). Anemia accounted for 22% of diagnoses, of which over one-third were cases of DBA and almost one-fifth were cases of Fanconi anemia. The remaining 26% included telomeropathies, GATA2 deficiency, Monosomy 7 and inborn errors of immunity. The methods used also allowed for monitoring clonal leukemic transformation by identifying somatic mutations. **Conclusions:** The presented results demonstrate the spectrum of genetic alterations leading to bone marrow failure syndromes and emphasize the importance of genetic testing in making a final diagnosis. Precise determination of the molecular basis is essential for selecting appropriate therapy, including hematopoietic stem cell transplantation (HSCT). For patients with iBFM, monitoring acquired clonal mutations is also crucial due to the increased risk of malignancies.

C28.O

Neurofibromatosis type I vel Fanconi anemia – diagnostic odyssey of two patients with biallelic variants in FANCI and FANCL genes

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Słowa kluczowe: Fanconi anemia, Neurofibromatosis type I, MMC test, flow cytometry, NGS

Background: Common features of patients with neurofibromatosis type I (NF1) and Fanconi anemia (FA) include abnormalities of skin pigmentation and high risk of cancers. However, various genetic background and others congenital and systems manifestations are observed. We are presented two patients with long-term clinical suspicion of neurofibromatosis type 1 whose were finally diagnosed with Fanconi anemia. Patient 1: 5-year old girl with clinical suspicion of NF1 was referred to Phacomatosis Clinic to confirm the diagnosis. Her birth weight was 2420 g. Apgar score was 10. During the following years short stature, low weight, café-au-lait spots and hearing problems became apparent. At the 14 years manifestations of MDS were observed and genetic diagnosis of Fanconi anemia was implemented. The results of MMC test on lymphocytes not fully supported diagnosis of FA. There was only slightly increased level of chromosome breaks and a few metaphase spreads with radial figures and 'railroads chromosomes'. On the contrary, the results of MMC test on skin fibroblasts were unequivocal. They revealed that ~90% of cells were damaged. Functional testing of fibroblasts by using flow cytometry indicated hypersensitivity to MMC in the form of an increase of the G2 phase fraction, what confirmed cytogenetic results. Eventually, mutation testing was performed, revealing a biallelic mutation in FANCI gene. Patient 2: 29-year woman with clinical symptoms of NF1 and without hematological manifestations in childhood was referred to Genetic Clinic to confirm the diagnosis. Abnormalities were observed only on patient skin (neurofibromas and café-au-lait spots). Multi-stage molecular analysis of gene NF1 not revealed pathogenic variants. At the 47 years, PV in FANCL gene was detected by WES. MMC test confirmed diagnosis of Fanconi anemia. Comment Characteristic symptoms of FA include light-brown café-au-lait spots and hypopigmented skin-fold freckle-like macules. Because of similar symptoms may exist in patients with other genetic syndromes, eg. NF1 or Nijmegen syndrome comprehensive genetic analysis is required.

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Keywords: anticancer therapies, CAR-T, CRISPR-Cas9, CRISPR-Cas12a2

The heterogeneity of tumour cells limits the efficacy of conventional immunotherapy (anti-CTLA-4, anti-PD-1/PD-L1) and promotes tumour escape from immune surveillance. Approaches are therefore needed that act selectively, on the basis of the molecular profile of the individual tumour cell. At the Hirszfeld Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, CAR-T technology has been developed and preliminary results have been obtained. Within the BECAME project, CAR constructs directed against the plasma cell myeloma antigens BCMA and SLAMF7 were designed. Therapeutic targets were selected using transcriptomic, proteomic and bioinformatic analyses. In vitro assays showed that CAR-T lymphocytes efficiently eliminated myeloma cell lines (MM.1S, U266), as confirmed by live-cell microscopy. CAR-T therapy, however, remains largely confined to haematological malignancies. As a subsequent step, we present the prospect of gene therapy based on the recently described nuclease CRISPR-Cas12a2. This type V nuclease is activated upon recognition of a target RNA transcript and triggers non-specific degradation of double-stranded DNA, leading to apoptotic cell death. This mechanism enables the selective destruction of cells based on their transcriptional profiles, with single-nucleotide resolution and without detectable off-target activity. The method permits the elimination of, for example, cells carrying oncogenic point mutations (e.g. KRAS G12C), cells infected with high-risk viruses (HPV, E6/E7 transcripts), or cells harbouring fusion genes. Integrating the CAR-T findings with the potential implementation of Cas12a2 delineates a coherent development trajectory: from CAR-T lymphocytes to precise, RNA-guided gene therapy for tumours that have hitherto been difficult to treat, including HPV-dependent and fusion-gene-positive malignancies. The presentation will summarise these results, along with directions for development that may broaden the national potential for innovative anticancer therapies.

C30.O

A model of collaboration between clinicians and a patient foundation in the development of translational research on the ultra-rare PACS2 syndrome.

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Słowa kluczowe: PACS2, epilepsy, collaboration, translational research

Heterozygous de novo variants in the PACS2 gene, encoding phosphofurin acidic cluster sorting protein 2, are causative of PACS2-related syndrome, previously also called Developmental Epileptic Encephalopathy 66 (DEE66, OMIM 618067). PACS2 syndrome is an ultra-rare neurodevelopmental disorder characterized by significant phenotypic heterogeneity, including, variable degree of developmental delay and intellectual disability (ID), seizures, hypotonia, behavioral abnormalities, and congenital anomalies, including cerebellar dysgenesis and facial dysmorphism. The small number of patients and the limited availability of research models pose a significant barrier to the development of targeted diagnostics and therapies. The aim of this study is to present a model of collaboration between clinicians, researchers, and a patient foundation, starting with fundamentals—that is, research on the natural history of the disease (with the goal of better characterizing phenotypic variability) to the development of a translational research platform that bridges the gap between basic science, laboratory discoveries, and patient care—all tailored to the needs of the 21st century. The project has established a repository of biological materials comprising patient-derived fibroblast lines, induced pluripotent stem cells (iPSCs), isogenic lines, brain organoid models, and animal models. Projects are being developed in parallel on metabolic profiling, phenotyping of preclinical models, unbiased drug screening, ASO therapy, and RNA and DNA base editing technologies. The model presented here demonstrates that an active patient foundation can serve as a catalyst, and that partnerships between clinicians and researchers enable a faster transition from clinical observation to the initiation of projects aimed at developing causal therapies. The model also demonstrates how to build research infrastructure and international cooperation, thereby establishing a new standard for collaboration in the field of rare genetic diseases.

C31.0

How a patient-led initiative accelerates drug development in ultra-rare MAP2K2-related RASopathy

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Słowa kluczowe: cardiofaciocutaneous syndrome, CFC4, RASopathies, translational research, drug discovery, patient-led initiative

Cardiofaciocutaneous syndrome type 4 (CFC4) is a rare genetic disorder caused by pathogenic variants in the MAP2K2 (MEK2) gene within the RAS/MAPK signaling pathway. Commercial drug development for such ultra-rare diseases is practically nonexistent, restricting families and clinicians to symptomatic care without any option for targeted therapy. To bridge this gap, we established an international, science-focused network to translate the genetic diagnosis of a single patient carrying the MAP2K2 c.619G>A (p.Glu207Lys) variant into actionable therapeutic strategies. Guided by a custom-built research roadmap, this patient-led initiative coordinates parallel project tracks across a multidisciplinary network. We have already generated patient-derived iPSC and fibroblast lines, including familial controls, to serve as an open-access resource for downstream testing. To better define the clinical presentation of this condition, we consolidated scattered global data by publishing a comprehensive literature review alongside a detailed case report. From there, the project has rapidly moved into active experimentation. Structural modeling and in silico molecular simulations of the mutated MEK2 protein have already yielded initial docking insights, while high-throughput drug repurposing via cell painting and phospho-ERK screening is currently underway. Concurrently, custom antisense oligonucleotides (ASO) and self-delivering siRNA platforms are being evaluated in discovery-stage tracks. To enable future in vivo validation of these compounds, targeted zebrafish and mouse models are actively being engineered. In parallel with drug discovery, we focus on deep patient characterization to uncover biomarkers that correlate with clinical manifestations. Using patient blood samples, we perform single-cell RNA sequencing, deep leukocyte immunophenotyping, and vascular-immune profiling. Additionally, an active clinical trial at the Medical University of Warsaw is investigating trametinib as a targeted treatment for pediatric hypertrophic cardiomyopathy in RASopathies. This approach demonstrates how dedicated patient advocacy can successfully fuel global research collaborations and drive drug discovery.

C32.0

Adult phenotype of Cornelia de Lange syndrome: clinical characteristics of a Polish patient cohort

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Słowa kluczowe: CdLS adult phenotype

Background: Cornelia de Lange syndrome (CdLS) is a rare multisystem neurodevelopmental disorder, and its adult phenotype remains insufficiently characterized. **Aim:** The aim of this study was to describe the adult phenotype of CdLS in a Polish patient cohort, representing, to our knowledge, the first analysis of this kind. **Methods:** Adult CdLS patients under our care were assessed using clinical data, medical records and phone or in-person interviews. Data were collected in collaboration with the Polish CdLS family association: Stowarzyszenie Dzieci i Rodzin Cornelia de Lange Syndrom (CdLS) w Polsce. Demographic, genetic, developmental, neurological, gastrointestinal, behavioral and other relevant clinical variables were assessed. Descriptive statistics were used to summarize the cohort. The study was approved by the local Bioethics Committee. **Results:** The study included 48 adult patients with CdLS; mean age 27.3 years (18–49 years). Molecular confirmation was available in 89% of patients, most commonly involving NIPBL gene, followed by SMC1A, SMC3, HDAC8 and RAD21. Anthropometric data confirmed marked growth impairment. Mean adult height was 144.0 cm (80–170), weight - 47.5 kg (20–131), with prominent microcephaly. All patients presented intellectual disability or global developmental delay. Educational history showed that the majority attended special, adapted, home-based or therapeutic education, whereas 8 patients had experience of mainstream schooling or mainstream schooling with support. Frequent clinical features included feeding or gastrointestinal problems, behavioral difficulties, epilepsy or other neurological manifestations, hearing impairment, myopia, musculoskeletal abnormalities and premature greying of hair. One patient developed thyroid cancer. Five patients died due to infectious, gastrointestinal or postsurgical complications. **Conclusions:** This adult CdLS cohort highlights the broad and persistent multisystem burden of the syndrome beyond childhood. Standardized long-term follow-up and systematic data collection may support better clinical care, transition planning and natural history studies in this rare condition.

C33.O

Assessment of circulating microRNA profiles in patients with Ehlers-Danlos Syndrome (EDS)

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Słowa kluczowe: Ehlers-Danlos syndrome, microRNA, non-coding RNA

Introduction: Ehlers–Danlos syndrome (EDS) is a group of hereditary connective tissue disorders characterised by joint hypermobility, skin hyperextensibility, and tissue fragility. Classical EDS is associated with pathogenic variants in the COL5A1 gene, encoding type V collagen. MicroRNAs (miRNAs) are small non-coding RNAs involved in post-transcriptional gene regulation. Members of the miR-29 family are important regulators of collagen synthesis and ECM remodelling. Dysregulation of miR-29 expression may therefore contribute to connective tissue abnormalities observed in EDS. The aim of this study was to evaluate the expression profile of miR-29 (a, b and c) in patients with EDS carrying COL5A1 mutations. Material and Method: The study material consisted of plasma samples from 13 patients diagnosed with EDS (10 females and 3 males) with COL5A1 gene variants identified by NGS. The control group included 13 healthy age- and sex-matched individuals. Circulating miRNA was isolated from plasma samples and analysed using qPCR with SYBR Green and miRNA assay reagents (Qiagen). Relative expression levels were assessed using endogenous and spike-in controls, followed by comparative Ct analysis. Results and Conclusion: Decreased expression of the miR-29 family was observed in EDS patients compared with healthy controls, with the most consistent reduction detected for miR-29a. No significant differences were observed for sex or mutation type. The observed downregulation may represent a secondary molecular response to abnormal collagen organisation and ECM damage caused by COL5A1 variants. Reduced miR-29 expression may result in disturbed collagen turnover, impaired ECM remodelling, and altered fibroblast activity. Moreover, persistent suppression of miR-29 may contribute to chronic low-grade inflammatory signalling, which is associated with ongoing ECM dysregulation. The observed dysregulation may therefore reflect not only an underlying collagen defect but also an additional mechanism contributing to disease progression in hereditary connective tissue disorders. The study was funded by The Ehlers-Danlos Society grant: “miRNA in Ehlers-Danlos syndrome diagnostics (MIREd)”

The Genetic Landscape of Lennox–Gastaut Syndrome in adults

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Słowa kluczowe: developmental and epileptic encephalopathies, Lennox-Gastaut syndrome, genetic syndromes, pediatric patients, adult patients

Introduction: Developmental and Epileptic Encephalopathies (DEEs) are rare, severe neurological disorders characterized by epilepsy together with cognitive, behavioral, and motor impairments. Their etiology may be genetic, acquired, or both, and they can occur in the context of normal or abnormal brain development. The introduction of next-generation sequencing (NGS) has significantly improved diagnostic yield in pediatric cohorts. However, many adults remain undiagnosed, as their clinical evaluation preceded the identification of numerous DEE-associated genes and the routine use of genomic testing. Consequently, data on genotype–phenotype correlations and disease course in adults are still limited. **Rationale:** Lennox–Gastaut syndrome (LGS) is a severe DEE with onset typically between 1 and 8 years of age (peak 3–5 years), characterized by multiple seizure types, cognitive impairment, and specific electroencephalographic abnormalities. The diagnosis is based on clinical features regardless of etiology. Although LGS begins in childhood, most patients survive into adulthood, where symptoms persist, leading to a growing but poorly characterized adult population. Genetic causes are increasingly recognized, with reported frequencies ranging from single digits up to ~30% across cohorts. In this study, we present molecular findings from a cohort of adult patients with LGS who underwent whole-exome sequencing (WES) to identify the underlying genetic causes of their disorder. **Results:** WES analysis was performed in 10 patients according to ILAE criteria. Gene selection was guided by Human Phenotype Ontology (HPO) terms corresponding to key clinical features. No potentially relevant variants were identified only in two cases. In two patients, diagnoses of PURA-related neurodevelopmental disorder and GLUT1 deficiency syndrome were established. In the remaining patients, multiple variants of uncertain significance (VUS) were detected, requiring further clinical correlation and evaluation of inheritance (de novo vs inherited).

C35.0

Personalized therapy in lower extremity artery disease: biomarkers and pharmacogenetics in the COLSTENT study

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Słowa kluczowe: PCSK9; lower extremity artery disease; restenosis; colchicine; pharmacogenetics; atherosclerosis

Introduction: Despite advances in endovascular treatment of lower extremity artery disease (LEAD), restenosis remains a major limitation of therapeutic efficacy. The COLSTENT study evaluates oral colchicine as a strategy to reduce inflammation and neointimal proliferation following stent implantation. An important component of the project is the identification of genetic and biochemical markers enabling personalized therapy. **Aim:** To present preliminary findings indicating the potential role of the PCSK9 gene as a biomarker of atherosclerotic risk and to outline the pharmacogenetic component of the COLSTENT study. **Materials and Methods:** The project includes sequencing of genes involved in lipid metabolism, atherosclerosis, and inflammatory response, including LDLR, APOB, LDLRAP1, SORT1, PCSK9, and APOE, as well as genes encoding interleukins, adiponectin, osteoprotegerin, matrix metalloproteinases, and their tissue inhibitors. Circulating levels of PCSK9, adiponectin (ADIPOQ), and osteoprotegerin (OPG) are assessed together with IL-6, CRP, and lipid profile parameters during 24 months of follow-up. Pilot data were derived from a previous study of 96 individuals evaluated for LEAD, in whom the entire PCSK9 gene was sequenced and circulating PCSK9 levels were measured. The multicenter COLSTENT study involved 284 patients randomized to two treatment arms. **Results:** Five missense variants of the PCSK9 gene were identified. Neither PCSK9 variants nor circulating PCSK9 levels were associated with LEAD diagnosis. However, significant associations were observed with markers of subclinical atherosclerosis and inflammation. The rs11591147 variant correlated positively with carotid intima-media thickness (IMT) and CRP levels, whereas rs505151 was positively associated with HDL-C concentration. One restenosis event was observed. **Conclusions:** Preliminary findings suggest that PCSK9 genetic variability may influence the atherosclerotic phenotype independently of clinically manifest LEAD. The COLSTENT study will enable comprehensive evaluation of genetic and biomarker determinants of response to colchicine therapy and may contribute to the development of personalized anti-restenotic treatment strategies. **Funding:** National Science Centre, Poland (MINIATURA 4, 2020/04/X/NZ5/01543) and the Medical Research Agency, Poland (2022/ABM/03/00046 – COLSTENT).

C36.O

The Genetics of Epigenetics - dissecting the crosstalks between DNA, RNA, and chromatin

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Słowa kluczowe: Genetic Disease, Mono-allelic expression, Epimutation, Promoter Silencing, Epigenetics

Recessive genetic diseases are characterized by bi-allelic loss of function mutations. Vitamin B12 deficiency due to mutations in the MMACHC gene is one of such disorders. We have previously identified a subset of patients – designated as epi-cblC - where apparently only one allele was genetically inactivated, while the other “normal” allele was subject to epigenetic silencing by promoter DNA methylation. More careful analysis by whole genome sequencing determined that this transcriptional silencing was not purely epigenetic but was caused by splice-site mutations in the neighboring gene, PRDX1. Those genetic changes caused RNA-processing defects and transcriptional interference with the disease-causing MMACHC gene. Here in the continuation of this work, we use this model to understand in depth the interplay between transcription and chromatin modifications resulting from defective transcriptional termination and readthrough. We recapitulate the human molecular phenotype in a mouse cell-culture system using a targeted deletion of the terminal splice acceptor site of Prdx1. We then systematically introduce the Prdx1 mutation into distinct epigenetically deficient backgrounds which lack H3K36me2, H3K36me3, and/or de novo DNA methylation, to identify the epigenetic factors that are involved in transcriptionally mediated promoter silencing. We also use ASOs and genetic silencing of Prdx1 to determine the role of antisense RNA and study the reversibility of this secondary epimutation. Together, our findings define a hierarchical silencing cascade where transcription of one gene across the promoter of a neighbouring gene is converted into stable epigenetic memory and establish transcription as an instructive regulatory process that can reshape local chromatin environments and gene expression. This model system provides new insights into the feedback mechanisms governing the interplay of transcriptional and chromatin states in mammalian genomes.

Autophagy Modulation in Huntington's Disease: Insights into Pathogenesis and Therapeutic Potential

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Słowa kluczowe: Huntington's disease; CAG extension; autophagy; cellular models; Genistein

Neurodegenerative diseases constitute a heterogeneous group of disorders characterized by progressive loss of neurons in the central and peripheral nervous systems, often associated with pathological protein aggregation. Huntington's disease (HD) is a monogenic polyglutamine disorder caused by CAG repeat expansion in the HTT gene. Repeat lengths above 36 residues lead to disease manifestation, with severity varying according to repeat length. The mutant huntingtin protein (mHTT) forms toxic aggregates that are difficult to degrade. Autophagy is a key cellular mechanism responsible for the removal of protein aggregates, and its modulation may lead to a reduction of mHTT levels. In this study, HEK293T cells containing different numbers of CAG repeats (HEK, HEK41, HEK53, HEK84) were used as a model for HD. The cells were treated with genistein, a flavonoid that induces autophagy and is capable of crossing the blood–brain barrier, which is critical in neurodegenerative diseases. Transcriptomic analysis revealed an increase in the number of differentially expressed autophagy-related genes with increasing CAG repeat length. Genistein significantly modulated the expression of these genes, partially normalizing their levels relative to control cells. Protein analysis showed increased levels of markers associated with autophagy initiation and autophagosome formation in genistein-treated cells. These results indicate that mHTT affects gene expression in a CAG repeat-dependent manner, and that genistein, by stimulating autophagy, enhances the clearance of toxic aggregates. This study provides new insights into the pathogenesis of HD and highlights the therapeutic potential of autophagy modulation.

C38.O

The SLC19A1 gene: a key player in one-carbon metabolism

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Słowa kluczowe: One-carbon metabolism, Reduced Folate Carrier, SLC19A1, folate, SNV

The main transporter of 5-methyl-tetrahydrofolate into cells is the reduced folate carrier (RFC1) encoded by highly polymorphic SLC19A1 gene. The most widely reported polymorphic variant of the SLC19A1 gene is the c.80G>A substitution (rs1051266). Since 5-methyltetrahydrofolate serves as a cellular methyl group donor for homocysteine remethylation—resulting in methionine production and subsequent methylation processes—the SLC19A1 gene acts as a crucial epigenetic player. To determine whether one-carbon metabolism genes modify the carcinogenic effects of smoking, the relationship between several functional polymorphisms in the MTHFR, CBS, SHMT1, MTHFD1, MTRR, MTR, TYMS DHFR, TCN2, SLC19A1 genes and lung cancer risk was assessed in a study of 132 lung cancer patients and 396 controls from northern Poland. Interestingly, the SLC19A1 c.80AA genotype was associated with an increased risk of lung cancer incidence in women, and a decreased risk in men. This observation led to the haplotype analysis of the SLC19A1 gene. The coding region substitution c.80A and the promoter variant c.-43C (rs1131596) constitute a distinct haplotype. Thus, the SLC19A1 c.-43C–c.80A haplotype is associated with a lung cancer risk in women (c.-43CC versus TT OR, 3.14; 95% CI, 1.32–7.46; $P = 0.010$). Pilot functional analyses in a cellular model revealed higher activity of the c.-133AGG[2]–c.-43C promoter haplotype as compared to c.-133AGG[3]–c.-43T. These results contribute to the discussion regarding introduction of food fortification with folic acid. They add to better understanding of the complex gene-environment interactions within the folate/homocysteine pathway and to understanding of gender-specific role of the SLC19A1 gene in smoking-related lung cancer development.

C39.O

Genetically determined causes of nystagmus in children with pervasive developmental disorders using Whole Exome Sequencing (WES) and RNA sequencing (IMiD research project No. 510-18-50 for the years 2026-2027)

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Słowa kluczowe: Nystagmus, optic nerve hypoplasia, WES, RNA sequencing

Introduction: Nystagmus is defined as involuntary, rhythmic, and repetitive oscillations of the eyes, often followed by a compensatory head posture to reach the so-called nystagmus null zone. It can be classified into congenital nystagmus, most commonly associated with ophthalmic disorders such as bilateral congenital cataracts, optic nerve hypoplasia, and macular hypoplasia, as well as genetic diseases, including retinal dystrophies such as Leber congenital amaurosis (LCA). **Aim:** At the Department of Medical Genetics of the Institute of Mother and Child, a group of 35 children aged 9 months to 14 years presenting with nystagmus and concurrent pervasive developmental disorders was evaluated to find a genetic cause of nystagmus using WES- Whole Exome Sequencing and RNA sequencing. **Material and methods:** The patients underwent comprehensive ophthalmological evaluation (including visual acuity testing, fundoscopy, flash visual evoked potentials [flash VEP/VER], and electroretinography [ERG]), neurological assessment (neurological examination, brain MRI, and EEG), and genetic testing. The genetic examination included Whole Exome Sequencing (WES) and the exclusion of submicroscopic genomic imbalances using chromosomal microarray analysis via array comparative genomic hybridization (array CGH). The some cohort of patients will be qualified for RNA sequencing. **Results:** The 9 patients were diagnosed after Whole Exome Sequencing (WES). It were confirmed pathogenic or likely pathogenic variants in genes: PCARE, PCYT1A, MECP2, CNGA3, COL4A1, and OTX2. For the 4 patients, no diagnostic variants or submicroscopic chromosomal imbalances were detected by microarray analysis. In the remaining patients, variants of uncertain significance (VUS) were identified, which require parental segregation analysis. This latter cohort of patients has been qualified for RNA sequencing. **Conclusion:** Recent studies demonstrate that 31% of splice-site variants of uncertain significance (VUS) can be reclassified as likely pathogenic or likely benign following RNA sequencing, thereby increasing the diagnostic yield for patients by 8% to 36%. RNA sequencing could be helpful for not diagnosed patient also in pediatric group.

C41.O

Development and pilot validation of a targeted NGS panel for analysis of clinically relevant deep intronic and regulatory variants in rare genetic diseases

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Słowa kluczowe: NGS, intronic variants, unresolved cases

Background:Whole-exome sequencing (WES) has become a standard diagnostic tool for rare genetic diseases; however, its diagnostic yield remains limited, typically ranging from 25% to 40%. A substantial proportion of unresolved cases may be explained by pathogenic variants located outside coding regions, including deep intronic, untranslated (UTR), and regulatory sequences. Whole-genome sequencing (WGS) represents the natural next step following negative WES results, as it enables interrogation of both coding and non-coding regions. However, due to high costs, complex bioinformatic analyses, and challenges in variant interpretation, WGS remains difficult to implement in routine diagnostic practice. We therefore developed a targeted NGS panel designed to analyze known disease-associated non-coding variants implicated in inherited disorders.**Methods:**The panel was designed using pathogenic and likely pathogenic non-coding variants reported in ClinVar and HGMD. Target regions included splice-altering variants, regulatory elements, untranslated regions (UTRs), and 38 disease-associated non-coding RNA genes. According to the Roche design report, the panel achieved probe coverage of 83.03% of target regions, with a predicted sequencing coverage of 91.41%.**Results:**The panel was applied to 48 individuals with suspected inherited disorders who remained unsolved after routine molecular testing, including WES. Six rare non-coding variants were identified in six unrelated patients, including four variants previously reported as pathogenic or likely pathogenic in the ClinVar database, one previously described variant, and one novel variant predicted to affect splicing. Three variants were located in genomic regions not covered by routine WES analysis. Further studies are required to determine whether any of the identified variants contribute to disease pathogenesis in the investigated individuals.**Conclusions:**Targeted analysis of disease-associated non-coding regions represents a practical and cost-effective intermediate strategy between WES and WGS. The developed panel expands the spectrum of variants accessible to molecular diagnostics and may facilitate the identification and prioritization of candidate disease-causing variants for further functional studies.

C42.O

Prevalence of genetic variants with therapeutic potential in a Polish Amyotrophic Lateral Sclerosis Cohort: a Single-Center Experience

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Słowa kluczowe: ALS, SOD1, tofersen

Amyotrophic lateral sclerosis (ALS) is a fatal refractory neurodegenerative disease characterised by the degeneration/loss of motor neurons with no effective treatment so far. The discovery of germline pathogenic variants that contribute to ALS development has created promising targets for therapies. The recent approval of tofersen (an antisense oligonucleotide targeting SOD1 mRNA) could alter the natural progression of the disease for individuals carrying a pathogenic SOD1 variant. Advanced clinical trials are carried out for the FUS mRNA and C9orf72 repeat expansion targeting molecules. A cohort of 387 patients referred with a clinical suspicion/diagnosis of ALS was analysed at a single genetic diagnostics centre - patients were referred for testing of at least the SOD1 gene. With next-generation sequencing (NGS) and custom-designed panels (Twist Bioscience), two testing strategies were applied: a basic SOD1/FUS/TARDBP gene panel (264 cases; 68%) and a broader neurodegenerative panel (123 cases; 32%). The entire coding sequence, known deep intronic ClinVar variants and copy number variants (CNVs) were assessed. Furthermore, 191 patients (49% of the cohort) underwent parallel/additional testing for the C9orf72 hexanucleotide repeat expansion using RP-PCR. A causative variant in NGS panel was identified in 17 patients (4.39%) within the cohort, predominantly missense SOD1 variant (11 individuals, 2.84%) followed by TARDBP (2 patients) and single FUS, FIG4, GRN and CSF1R positive cases. Additionally, a C9orf72 hexanucleotide repeat expansion was detected in nine of 191 tested patients (4.71%). 6 patients harbor variant of unknown significance (by ACGS 2024 criteria), predominantly located within basic ALS gene list. These results suggest that prevalence SOD1 variants in Polish unselected patients with ALS suspicion/diagnosis is consistent with current literature, implying that around 3% of these patients might benefit from SOD1-orientated therapy. A further 5% of patients harbor a potential therapeutic target currently evaluated in clinical trials.

C43.O

Genetic heterogeneity of neurotransmitter-related disorders in undiagnosed Polish children with suspected monoamine defects

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Słowa kluczowe: monoamine neurotransmitter disorders, whole-exome sequencing, inborn errors of metabolism, pediatric genetics

Background: Monoamine neurotransmitter disorders are rare inherited conditions that are frequently under-recognized and misdiagnosed. They include primary defects of monoamine biosynthesis, degradation, and transport, as well as secondary disorders associated with dysregulation of monoaminergic pathways. Common symptoms comprise movement disorders, autonomic dysfunction and oculomotor abnormalities. Their presentation often overlaps with other neurological conditions including cerebral palsy and epileptic encephalopathies. **Aims and methods:** The aim of this study was to investigate the genetic heterogeneity in a cohort of 40 undiagnosed pediatric patients with suspected monoamine neurotransmitter disorders using trio whole-exome sequencing. Inclusion criteria comprised abnormal cerebrospinal fluid (CSF) serotonin/dopamine metabolite levels and/or clinical features suggestive of neurotransmitter disorders. Genetic variants were classified according to ACMG criteria. **Results:** A molecular diagnosis was established in 43% (17/40) of patients. It included 8 patients with primary monoamine neurotransmitter disorders caused by pathogenic variants in TH, DDC, MAOA and GCH1 genes. In two patients with GCH1 deficiency, additional pathogenic variants in NEXMIF and HPRT1 were found, resulting in complex phenotypes not previously reported. Secondary defects were identified in 9 patients and included variants in EBF3, EEF1A2, STXBP1, DEAF1, MECP2, WASF1, NKX2-1, GNAO1, and YY1 genes, reflecting diverse mechanisms leading to dysregulation of the monoaminergic pathways. Among 22 identified pathogenic/likely pathogenic variants, 9 arose de novo and 3 were novel

(NEXMIF:p.Asp155GlnfsTer2, EBF3:p.Gly459Ser and MAOA:p.Ala272Val). Conclusion: We have identified both known and novel disease-causing variants in genes responsible for primary and secondary monoamine neurotransmitter defects. Our findings underline the importance of the integration of genomic, biochemical, and clinical data to enable proper diagnosis and refine genotype-phenotype correlations in variable group of disorders. This is the first study characterizing the genetic heterogeneity in a cohort of Polish pediatric patients with suspected monoamine neurotransmitter disorders.

C44.O

DYRK1A Syndrome in a Polish Cohort: Phenotypic Characteristics and Parental Quality of Life

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Słowa kluczowe: DYRK1A syndrome, neurodevelopmental disorder, intellectual disability, rare genetic disease, parental quality of life

Background: DYRK1A syndrome is a rare autosomal dominant neurodevelopmental disorder caused by haploinsufficiency of the DYRK1A gene. Despite growing international interest, no systematic clinical description of Polish patients has been published to date. **Objectives:** To expand the phenotypic description of DYRK1A syndrome based on a Polish cohort of patients with molecularly confirmed diagnosis, and to evaluate the impact of the condition on parental quality of life. **Methods:** Ten patients with molecularly confirmed DYRK1A syndrome were enrolled. Clinical data were collected using a dedicated 266-item questionnaire covering perinatal history, developmental milestones, comorbidities, and diagnostic pathway. Parental quality of life was assessed using the PedsQL Family Impact Module in six participating families. **Results:** All patients presented with global developmental delay, severe speech impairment, and a distinctive dysmorphic facial gestalt including microcephaly and deep-set eyes. Ophthalmological abnormalities were present in nine patients (90%), and sensory processing disorders in eight (80%). Epilepsy, autism spectrum disorder, and congenital cardiac defects were each diagnosed in five patients (50%). Abnormal muscle tone was universal (hypertonia 70%, hypotonia 30%), and abnormal gait was observed in six patients (60%). Significant diagnostic delay was noted, with age at diagnosis ranging from 2 years 9 months to 13 years. Quality of life assessment revealed burden across all domains; worry about the child's future (2.33/4), daily activity impairment (2.17/4), and caregiver emotional functioning (2.13/4) were most severely affected. In all families, mothers served as sole primary caregivers and one parent had resigned from employment. Nine of ten families reported no opportunity for rest or leisure, and 70% rated available medical care as average or poor. **Conclusions:** This first Polish cohort confirms the multisystem nature of DYRK1A syndrome and highlights the substantial burden experienced by affected families. Significant diagnostic delays underscore the need for earlier implementation of genomic testing in children presenting with unexplained neurodevelopmental disorders.

C45.O

Molecular background of Emery-Dreifuss muscular dystrophy – insights into progenitor muscle cells disfunction.

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Słowa kluczowe: nuclear envelope, emerin, rare diseases, iPSCs, muscles, transcriptomics, EMD mutations

Mutations in genes encoding nuclear envelope proteins, including emerin, lamins A/C, and nesprins, lead to Emery-Dreifuss muscular dystrophy (EDMD), a disorder with a poorly understood molecular basis and pathogenesis. We therefore focused on the early stages of myogenesis to evaluate their role in disease development. As patient-derived muscle precursor cells are severely limited and animal models poorly recapitulate the disease phenotype, there was a need for a more physiologically relevant model. Cells from EDMD patients with EMD mutations c.153del (resulting in the absence of emerin) and c.451dup (generating truncated emerin) were reprogrammed into induced pluripotent stem cells and then differentiated in vitro into muscle cells at different stages of myogenesis: satellite cells (muscle precursor cells), myoblasts, and myotubes. We performed comprehensive functional assays alongside transcriptomic profiling. We observed that emerin deficiency was associated with delayed muscle differentiation, whereas expression of truncated emerin surprisingly accelerated this process. Muscle progenitor cells emerged as the earliest affected stage, exhibiting altered apoptosis and migration rates, as well as widespread dysregulation of gene expression patterns, for both protein-coding and microRNA-coding genes. We propose that dysfunction of satellite cells may represent a primary pathogenic event driving the development of the EDMD phenotype.

C46.O

ReNU Syndrome in a Polish Cohort: Phenotypic Characteristics, Diagnostic Challenges, and Clinical Implications

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Słowa kluczowe: ReNU syndrome, neurodevelopmental disorder, RNU4-2, intellectual disability

Introduction: ReNU syndrome is a recently described neurodevelopmental disorder caused by pathogenic variants in the RNU4-2 gene. Owing to its recent discovery, the full clinical spectrum of the condition remains incompletely characterized. The aim of this study was to describe the phenotype of Polish patients with ReNU syndrome and to evaluate the diagnostic and clinical challenges associated with this disorder. **Materials and Methods:** Eleven individuals with molecularly confirmed ReNU syndrome from Poland, were enrolled in the study. Clinical data were collected using a dedicated questionnaire and correlated with genetic findings and previously published reports. **Results:** The most frequently identified pathogenic variant was n.64_65insT, detected in 82% of patients. Developmental motor delay and hypotonia were observed in nearly all affected individuals, while severe speech delay was present in 82% of patients carrying the common variant. Childhood-onset osteoporosis was diagnosed in two patients, expanding the spectrum of skeletal manifestations associated with ReNU syndrome. In most cases, the final diagnosis was preceded by a prolonged diagnostic odyssey involving multiple genetic investigations, including chromosomal microarray analysis, MLPA, whole-exome sequencing, and repeated data reanalysis before identification of the causative RNU4-2 variant. **Conclusions:** The Polish cohort confirms that ReNU syndrome is a multisystem disorder requiring coordinated multidisciplinary care. Early consideration of RNU4-2 testing in children presenting with developmental delay may significantly shorten the diagnostic pathway and reduce the burden associated with prolonged diagnostic odysseys.

C47.O

Genetic landscape of Usher syndrome in a Polish cohort: high diagnostic yield and functional validation of splice-altering variants

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Słowa kluczowe: Usher syndrome, NGS, hearing loss, retinitis pigmentosa

Background/Objectives: Usher syndrome is one of the most common rare diseases characterized by the coexistence of hearing loss (HL) and retinitis pigmentosa (RP). Currently, four types of Usher syndrome have been identified, which are genetically and clinically heterogeneous. The aim of this study was to characterize the genetic background of Usher syndrome in a group of Polish patients. **Methods:** A total of 90 patients with hearing loss (HL) and retinitis pigmentosa were recruited to the study. The DNA was isolated from blood and genetic testing was performed using: qPCR genotyping, targeted sequencing with the multigene panel. Segregation analysis were performed on available samples, using Sanger sequencing and qPCR. The minigene assay was performed for five variants in USH2A and two variants in ADGRV1 that were predicted to affect transcript splicing. **Results:** The cause of Usher syndrome was identified in 97% (87/90) patients. In 73% (66/90) of the individuals, causative variants were located in the USH2A gene. Variants in MYO7A accounted for 8% (7/90) of diagnoses, followed by PCDH15 (7%) and ADGRV1 (6%). Variants in CLRN1, PDZD7, and USH1C were each identified in 1% of patients. The minigene assay confirmed splicing alterations for all investigated variants. **Conclusion:** Genetic testing of patients should be based on high-throughput tests that enable simultaneous identification of SNVs and CNVs. USH2A variants were the major cause of Usher syndrome in the studied Polish cohort. The gathered data can serve as a starting point for further genotype-phenotype association analyses. **Grant:** 2020/37/N/NZ5/02800 National Science Centre, Poland 2024/ABM/03/KPO/KPOD.07.07-IW.07-0141/24-00 Medical Research Agency, Poland

C48.O

Contribution of functional studies to variant interpretation in isolated and syndromic hearing loss

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Słowa kluczowe: Hereditary hearing loss, Variant interpretation, functional validation, RNA splicing, disease mechanisms, precision medicine

Introduction: Interpretation of genetic variants remains one of the major challenges in hereditary hearing loss diagnostics. Although genomic technologies have substantially improved diagnostic yield, assessment of variant pathogenicity often requires experimental validation. Owing to the diversity of molecular mechanisms underlying hearing loss, different classes of variants require distinct functional approaches. **Methods:** Variants identified in patients with isolated and syndromic hearing loss were investigated using complementary functional models. Predicted splicing variants in genes including EYA1, CHD7, SLC26A4, EYA4, MYO6, MYO7A and MYO15A and USH2A were evaluated using minigene assays. In addition, variants in GRHL2, NLRP3 and TBC1D24 were investigated using structural and functional analyses, cellular assays and zebrafish models, respectively. **Results:** Functional studies revealed diverse molecular consequences of the analyzed variants. Minigene assays demonstrated exon skipping, exon shortening, intron retention and activation of cryptic splice sites as mechanisms underlying aberrant RNA processing. In contrast, no effect on splicing was observed for a MYO15A variant, providing evidence against its predicted molecular consequence. Structural and functional analyses of a GRHL2 variant revealed impaired transcriptional activity, destabilization of the dimer interface and altered DNA-binding dynamics. Functional studies of an NLRP3 variant demonstrated increased inflammasome activation and enhanced IL-1 β secretion, whereas zebrafish experiments confirmed the deleterious effect of a TBC1D24 variant on inner ear development and function. **Conclusions:** Different classes of genetic variants require distinct functional approaches for accurate interpretation. Integration of complementary studies at the RNA, protein, cellular and organismal levels supports variant classification and enables translation of genomic findings into biological insight and disease mechanisms, and facilitates the implementation of precision medicine approaches in hereditary hearing loss. This work was funded by National Science Centre, Poland, grants number 2021/41/B/NZ5/04390 and 2020/37/N/NZ5/02800.

C49.O

From Targeted Panels to Whole-Genome Sequencing: Genomic Diagnostics of Hereditary Hearing Loss in a Cohort of 1022 Patients

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Słowa kluczowe: hearing loss, next-generation sequencing, rare diseases

Background:The implementation of next-generation sequencing (NGS) has transformed the molecular diagnosis of hereditary hearing loss (HL), enabling a transition from targeted multigene panels to broader genomic approaches, including whole-exome sequencing (WES) and whole-genome sequencing (WGS). This study evaluated the diagnostic performance of different NGS strategies and characterized the genetic spectrum of HL in a large clinical cohort.**Methods:**A total of 1022 DFNB1-negative HL patients underwent genetic testing using custom NGS panel (n=780), TruSight One panel (TSO; n=38), WES (n=195), and WGS (n=9). Cases were considered solved when pathogenic or likely pathogenic variants explaining the phenotype were identified. **Results:**A molecular diagnosis was established in 481 patients (47%). Diagnostic yield was 45% for targeted panels (351/780), 53.8% for WES (105/195), 58% for TSO (22/38), and 33% for WGS (3/9). The most frequently implicated genes were USH2A (n=68), SLC26A4 (n=32), MYO15A (n=27), TMC1 (n=17), MYO6 (n=17), EYA1 (n=15), STRC (n=15), CHD7 (n=14), WFS1 (n=13), and MYO7A (n=13). Additional recurrent genes included MITF, TBC1D24, TMPRSS3, PCDH15,TECTA, CDH23, GSDME, OTOG, POU4F3, and PAX3. Beyond common causes of nonsyndromic hearing loss, genomic testing enabled the diagnosis of are syndromic disorders, including Usher, CHARGE, Branchio-Oto-Renal, Waardenburg, Wolfram, Treacher-Collins, Alport, Perrault, Pendred, and Barakat syndromes. WGS identified non-coding variants in ATP11A, EYA4 and POU3F4 genes.**Conclusions:**NGS-based diagnostics provides a high diagnostic yield in hereditary HL. Broader approaches, particularly WES and WGS, outperformed targeted panels. The cohort showed marked genetic heterogeneity with USH2A as the most frequently involved gene. Comprehensive genomic testing improves the detection of rare genetic etiologies and supports precision diagnostics.**Funding:**This work was funded by National Science Centre, Poland, grants numbers 2016/22/E/NZ5/00470, 2017/27/N/NZ5/02369, 2020/37/N/NZ5/02800, 2021/41/B/NZ5/04390 and by the Medical Research Agency (ABM) under the National Recovery and Resilience Plan (KPO), grant number 2024/ABM/03/KPO/KPOD.07.07-IW.07-0141/24-00.

C50.0

First transcriptomic analysis of retinal samples from living patients with high myopia

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Słowa kluczowe: high myopia, human retina, transcriptomic analyses, RNA sequencing

Background: High myopia (HM), an eye disorder with a refractive error of ≤ -6.0 diopters (D) and axial length of the eyeball greater than 26.0 mm, has a multifactorial etiology, with genetic and environmental factors involved. Axial elongation of the eyeball leads to structural changes and thinning of the retina, choroid, and sclera in the posterior segment of the eye. In this project, we aimed to focus on human retina and assess its transcriptome in samples from Polish patients diagnosed with HM. **Materials and methods:** Total RNA was extracted from retinal samples obtained intraoperatively during pars plana vitrectomy performed for rhegmatogenous retinal detachment in Polish patients with HM and patients without HM diagnosis (non-HM controls). After assessment of RNA quality and quantity, samples were subjected to bulk RNA sequencing (RNA-seq). RNA-seq raw data were processed and differential expression analysis and following enrichment analyses were conducted. Deconvolution of bulk RNA-seq data was performed to identify retinal cell populations within the studied samples based on the expression of known cell-type marker genes. Subsequently, differences in cell composition and cell type-specific gene expression were assessed between HM and non-HM samples. **Results:** Differentially expressed genes in retinal samples from HM patients (FDR p -value < 0.01 ; log fold change > 2.0) were identified. Statistically significant up- and downregulated genes were enriched in biological processes and signaling pathways related to e.g. phototransduction, visual perception, as well as various eye disorders. The deconvolution of RNA-seq data showed differences in cell composition between the studied samples. **Conclusions:** This is the first RNA-seq study on retinal samples obtained from living HM patients. While previous studies have mainly focused on animal models of induced myopia, our findings provide valuable insight into the molecular mechanisms underlying HM in human tissue and highlight the importance of studying this condition in clinically relevant samples. **Funding:** National Science Centre in Poland (2019/35/N/NZ5/03150 to JSJ).

C51.O

Molecular Characterization of Oculocutaneous Albinism in a Single-Center Pediatric Cohort Using Next-Generation Sequencing

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Słowa kluczowe: oculocutaneous albinism (OCA), ocular albinism (OA), TYR, next-generation sequencing

Background: Oculocutaneous albinism (OCA) is a clinically and genetically heterogeneous disorder characterized by reduced or absent pigmentation of the skin, hair, and eyes. Pathogenic variants in seven genes (DCT, LRMDA, OCA2, SLC24A5, SLC45A2, TYR, and TYRP1) are associated with autosomal recessive OCA, whereas pathogenic variants in GPR143 cause X-linked ocular albinism (OA). **Materials and Methods:** We analyzed 32 Polish pediatric patients from unrelated families with clinically suspected OCA/OA. Molecular analysis was performed using a targeted next-generation sequencing (NGS) multigene panel covering known OCA/OA-associated genes. **Results:** A molecular diagnosis was established in approximately 50% of patients with clinically suspected OCA/OA. The most frequently affected gene was TYR, with pathogenic variants identified in 10 patients, consistent with with OCA1. In this group, the common functional TYR variants c.575C>A and c.1205G>A formed a pathogenic haplotype a pathogenic haplotype that consistently segregated with autosomal recessive inheritance in combination with other pathogenic TYR variants, including one novel variant, c.915_916del, and six previously reported variants: c.1037-7T>A, c.650G>A, c.1217C>T, c.1101C>A, c.1A>G, and c.1118C>A. Pathogenic variants were also identified in OCA2, GPR143, SLC24A5, SLC45A2, and TYRP1. Novel variants included: TYRP1 c.421_425del; OCA2 c.1166C>A and c.1298C>T; SLC24A5 c.342G>T; SLC45A2 c.276_277del. Six patients carried only a single pathogenic variant in OCA2, SLC45A2, or TYRP1, leaving the molecular diagnosis unresolved. **Conclusions:** The TYR haplotype comprising the common functional variants c.575C>A and c.1205G>A is strongly associated with a confirmed diagnosis of albinism. Routine reporting and interpretation of these variants, particularly in patients harboring a single pathogenic TYR allele, may improve the molecular diagnostic yield. Integrating the analysis of both rare pathogenic variants and common functional alleles may enhance diagnostic accuracy and facilitate genetic counseling for families affected by albinism.

C1.1.P

Genotype-Phenotype Correlation in VHL-Associated Hemangioblastoma Patients

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Słowa kluczowe: Hemangioblastoma, von Hippel Lindau Syndrome, Central Nervous System, Genotype-phenotype correlation

Introduction Von Hippel-Lindau (VHL) disease is an autosomal dominant disorder classified as a phakomatosis, caused by a germline pathogenic variant in the VHL tumour suppressor gene. Since hemangioblastomas are a primary manifestation, patients diagnosed with these tumours require a genetic diagnosis. Current knowledge indicates a possible correlation between genotype and phenotype, which could have significant clinical implications.

Material and methods The study included 27 patients (10 women and 17 men) diagnosed with hemangioblastoma and confirmed VHL syndrome, treated at the National Institute of Oncology in Gliwice between 2000 and 2026. The median age of the group was 42 (IQR: 19; range: 9–79). Gene variants were identified using NGS or MLPA, and clinical manifestations were confirmed via radiological imaging or histopathological examination.

Results Central nervous system (CNS) hemangioblastomas were the most common manifestation, diagnosed in 20 patients; only one patient presented with an isolated eye tumour, and six had both CNS and eye involvement. Seven patients manifested a single tumour, while 20 developed numerous lesions. Eleven patients presented only hemangioblastomas, while 16 presented other malignancies within the VHL spectrum. Five patients from two unrelated families with a whole VHL gene deletion presented exclusively with hemangioblastomas. Other variants correlated only with hemangioblastomas were p.(Arg69Alafs*63), p.(His115Tyr), p.(Arg167Gln), p.(Leu178Pro), p.(Phe136Ser), and p.(Ser65Ter) - each identified in a single patient.

Conclusions The analysis revealed distinct genotype-phenotype correlations, indicating that specific genetic variants may exhibit an association with particular milder or more severe phenotypes. However, because VHL syndrome is a rare disease, analyses are performed on a limited sample size, and final conclusions should be drawn with caution.

C1.2.P

Hepatoblastoma in course of Familial adenomatous polyposis

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Słowa kluczowe: Hepatoblastoma, familial adenomatous polyposis, APC gene, pediatric cancer, early diagnosis, genetic testing, surveillance

Hepatoblastoma is the most common primary malignant liver tumor in early childhood, typically diagnosed before the age of four years, with a median age of approximately 18 months. Although most cases are sporadic, an increased incidence has been observed in association with hereditary cancer predisposition syndromes, particularly familial adenomatous polyposis (FAP). Children with FAP have an estimated 200-fold higher risk of developing hepatoblastoma compared to the general population, making it one of the earliest extracolonic manifestations of the disease. In this study, clinical and molecular data from five pediatric patients diagnosed with hepatoblastoma in the course of FAP were analyzed. Pathogenic variants in the APC gene were identified in all cases. Importantly, hepatoblastoma was diagnosed in infancy in each patient, between 3 and 18 months of age. The detected variants included two recurrent mutations, c.3164_3165delTA and c.2626C>T but c.3164_3165delTA was observed in two unrelated patients. Family histories revealed features consistent with FAP, including early-onset colorectal cancer as well as extracolonic manifestations such as thyroid cancer. Molecular diagnostics were performed using high-resolution melting (HRM) analysis followed by Sanger sequencing. The identification of pathogenic variants in all analyzed cases confirmed the occurrence of hepatoblastoma in the context of FAP. As hepatoblastoma develops very early, often before other clinical features of FAP become apparent, early genetic testing is crucial, as it enables timely surveillance and helps prevent delayed diagnosis of hepatoblastoma. This allows for appropriate monitoring and early intervention, as well as screening of at-risk family members. Integrating genetic diagnostics into clinical management may therefore improve outcomes and reduce the risk of advanced disease in affected families. The research in part was financed by state budget funds granted by the Minister of Science and Higher Education within the "Science for Society II" Program (Poland) framework, no. NdS-II/SP/0139/2024/01. amount of funding 1 980 000 zł total value of the project 1 980 000 zł

Comparison of Pathogenic/Likely Pathogenic Variants and Variants of Uncertain Significance in Hereditary Cancer Predisposition Genes among Patients with Colorectal and Endometrial Cancer

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Słowa kluczowe: colorectal cancer, endometrial cancer, hereditary cancer, NGS, PV/LPV, VUS

Although approximately 80% of colorectal cancers are sporadic, family history remains an equally important risk factor. Hereditary cancer predisposition syndromes, such as Lynch Syndrome (HNPCC), Familial Adenomatous Polyposis (FAP), and MUTYH-Associated Polyposis (MAP), significantly increase the risk of colorectal cancer. The diagnosis of one of these syndromes or a family history of colorectal cancer diagnosed before the age of 50 constitutes an indication for genetic counseling and consideration of germline testing. Next-generation sequencing (NGS) enables the detection of genetic variants that may contribute to disease development, including pathogenic variants (PV) as well as variants of uncertain significance

(VUS), the interpretation of which remains challenging. The study group consisted of 522 patients, including 422 women and 100 men. Among them, 406 individuals were diagnosed with colorectal cancer, endometrial cancer, or other malignancies, whereas 116 were referred for testing due to a positive family history of cancer. All patients underwent germline NGS analysis of the following genes: MLH1, MSH2, EPCAM, MSH3, MSH6, PMS2, STK11, SMAD4, BMPR1A, MUTYH, APC, and PTEN, using a custom KAPA HyperCap Target Enrichment Probes panel (Roche) designed for sequencing on the MiSeqDx (Illumina). Variant analysis was performed using SeqPilot (JSI Medical Systems), and variants were classified according to ACMG guidelines. A total of 32 PV/LPV variants and 76 VUS were identified. Among PV/LPV variants, alterations leading to loss of protein function predominated. In contrast, VUS were almost exclusively missense variants. The highest number of variants was detected in mismatch repair (MMR) genes, both in the PV/LPV and VUS groups. Among VUS, some variants demonstrated a high predicted risk of affecting protein function; however, insufficient evidence was available to classify them as pathogenic. Comparative analysis of these two groups may support the interpretation of newly detected variants in patients undergoing genetic testing for suspected hereditary predisposition to colorectal cancer.

C1.4.P

Clinical significance of ATM and PARP1 germline variants and their association with prostate cancer risk in Polish men – preliminary results.

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Słowa kluczowe: ATM, PARP1, prostate cancer, NGS, clinical significance

Background/Objectives: Prostate cancer (PC) is a complex malignancy affecting millions of men worldwide. We investigated the association between ATM and PARP1 germline variants and PC risk, as well as their impact on clinical characteristics and overall survival in Polish men with localized PC who are eligible for radical treatment. **Material:** DNA from 97 men with PC recruited throughout the whole Poland and from 100 cancer-free male volunteers, healthy at the time of enrollment. **Methods:** NGS, Sanger sequencing. **Results:** Nine rare germline variants in the ATM and PARP1 genes were identified, each in a single PC patient. These included two pathogenic variants (ATM c.7630-2A>C and c.8947dup), five variants of uncertain significance (ATM c.8762C>T, c.7322C>T, c.4631A>G; PARP1 c.2214C>A and c.2278-4C>T), one likely benign (PARP1 c.1129C>T), and one benign (PARP1 c.942C>T). The ATM c.8947dup and PARP1 c.2214C>A are newly identified variants. Bioinformatic analysis was performed using the Franklin clinical database. Among 7 carriers of variants of uncertain significance or pathogenic (VUS/P), 3 originated from 21 families fulfilling hereditary prostate cancer (HPC) criteria, whereas 4 originated from 76 families not meeting HPC criteria ($p=0.17$, trend). Among VUS/P carriers, PC was diagnosed in 5 of 58 patients aged ≤ 60 years and in 2 of 39 patients aged >60 years ($p=0.5$, NS). A family history of at least one malignancy was reported in 4 of 7 VUS/P carriers and in 57 of 90 non-carriers of tested gene variants ($p=0.7$, NS). **Conclusions:** The preliminary results presented here require further investigation of ATM and PARP1 germline variants to clarify their role in prostate cancer pathogenesis, risk stratification, and prognostic assessment, including recurrence after radical prostatectomy. In the analyzed cohort of PC patients, no association was observed between the presence of rare germline (VUS/P) variants of ATM or PARP1 variants and the evaluated clinical characteristics of PC.

C1.5.P

Detection of large genomic rearrangements (LGRs) in the BRCA1 gene using MLPA in patients with breast and ovarian cancer

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Słowa kluczowe: BRCA1, MLPA, LGRs, CNVs, breast cancer, ovarian cancer, family screening

Background: Germline mutations in the BRCA1 gene significantly increase the risk of developing breast and ovarian cancer (BC and OC). Although most pathogenic variants are point mutations, large genomic rearrangements (LGRs) constitute an important part of the mutational spectrum. Standard sequencing techniques may fail to detect copy number variations (CNVs); therefore, dedicated methods are required for their identification. **Aim:** To determine the frequency and characterize large deletions and duplications in the BRCA1 using multiplex ligation-dependent probe amplification (MLPA) in a phenotypically diverse group of high-risk patients, and to assess their familial transmission. **Materials and Methods:** The study included 122 high-risk patients whose previous routine genetic testing revealed no point mutations in the BRCA1. The cohort was divided into two subgroups: patients diagnosed with breast cancer (n = 69) and ovarian cancer (n = 53). Analysis of large genomic rearrangements was performed using MLPA with commercial diagnostic kits from MRC Holland. **Results:** LGRs in the BRCA1 were identified in 7 of 122 patients (5.74%). In the BC group, LGRs were detected in 5 of 69 patients (7.25%), including four deletions and one duplication. In the OC group, LGRs were identified in 2 of 53 patients (3.77%), including one deletion and one duplication. For the patient carrying a deletion of exons 1–2, cascade testing revealed the same alteration in both her daughter and nephew. In the family of a patient with an exon 22 deletion, the same alteration was also confirmed in her daughter. **Conclusions:** LGRs represent a substantial proportion of pathogenic BRCA1 alterations (5.74%) among hereditary high-risk patients with negative point mutation testing results. The frequency of LGRs was higher in the breast cancer subgroup (7.25%) than in the ovarian cancer subgroup (3.77%). The identification of LGRs in relatives of affected patients highlights the importance of MLPA analysis in family screening and in optimizing preventive strategies for healthy mutation carriers.

C1.6.P

Evidence for Genotype-Specific Optimal Blood Lead Levels for Cancer Risk: MKI67 rs11016073 and APOB rs1367117 in a Female Prospective Cohort

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Słowa kluczowe: APOB rs11016073; MKI67 rs1367117; Pb; cancer risk; carcinogen; non-occupational exposure; prospective study

This study's aim was to clarify the regulatory roles of the MKI67 rs11016073 and APOB rs1367117 polymorphisms in the relationship between blood Pb levels and cancer risk. Blood Pb concentrations were measured using inductively coupled plasma mass spectrometry, and genotyping was performed by real-time PCR with TaqMan probes. Cancer incidence was assessed during a mean follow-up of six years and two months. During follow-up, 210 incident cancers were diagnosed among 2782 women. Pb exposure was categorized into quartiles (Q1: <9.44 µg/L; Q2: 9.44-12.58 µg/L; Q3: 12.59-17.16 µg/L; Q4: >17.16 µg µg/L). The association between Pb levels and cancer risk was strongly genotype dependent. Women carrying APOB non-GG and MKI67 non-AA genotypes exhibited the lowest breast cancer risk at the highest Pb levels (Q4), whereas carriers of APOB GG and MKI67 AA showed the lowest risk at the lowest Pb levels (Q1). Age-stratified analyses further demonstrated genotype-specific differences in optimal Pb exposure ranges, particularly for breast cancer. Cancer risk associated with Pb exposure is not uniform but depends on genetic background. These findings identify genotype-specific optimal blood Pb levels and suggest that incorporation of MKI67 and APOB genotyping may improve risk stratification and interpretation of non-linear Pb-cancer associations.

C1.7.P

All-Cause Mortality and Cancer Risk Dependent on Blood Se Level and HRG rs10770 Genotypes on a Prospective Cohort of Women with Familial Breast Cancers

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Słowa kluczowe: HRG rs10770; Se levels; all-cause mortality; cancer risk; familial breast cancers; prospective study

The aim of this study was to investigate whether the effect of blood selenium (Se) levels on all-cause mortality and cancer risk is modified by the HRG rs10770 genotype. The study included 2,782 initially cancer-free women from families with familial breast cancer registered at the Hereditary Cancer Center in Szczecin, Poland. Participants were aged ≥ 40 years and were recruited between September 2010 and March 2024. Women carrying BRCA1 pathogenic variants and those with a previous cancer diagnosis were excluded. Blood Se concentrations were measured using inductively coupled plasma mass spectrometry, and HRG genotyping was performed using real-time PCR with TaqMan probes. After a mean follow-up of 6.2 years, 89 deaths and 210 incident cancers were recorded. Significant differences were observed in the association between Se levels and health outcomes according to HRG genotype. Among women aged >50 years, those with Se levels in the lowest quartile (Q1) had a significantly higher all-cause mortality risk than women in the highest quartile (Q4) (HR = 3.07; 95% CI: 1.53–6.16; $p = 0.001$). Among carriers of the HRG non-TT genotype, the association was particularly strong: women with Se levels in Q1 had a markedly increased mortality risk compared with those in Q2–Q4 (HR = 7.68; 95% CI: 2.31–25.47; $p = 0.0008$). In contrast, among TT carriers, increased mortality risk was observed only for Q1 versus Q4 (HR = 2.40; 95% CI: 1.09–5.31; $p = 0.029$). For cancer risk, the strongest associations were observed among women aged <50 years. TT genotype carriers with Se levels in Q1 had a significantly higher cancer risk than those in Q2 (HR = 4.15; 95% CI: 1.55–11.06; $p = 0.004$). No significant associations were found among non-TT carriers. These findings suggest that both all-cause mortality and cancer risk are influenced by an interaction between blood Se levels and HRG genotype.

C1.8.P

Toward More Efficient Genetic Screening in Hereditary Cancer: Evidence from Founder Mutation Screening and Extended Multigene Panel Analysis

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Słowa kluczowe: Hereditary cancer syndromes, Cancer predisposition genes

Introduction: Genetic testing is essential in hereditary cancer risk assessment. While targeted screening for recurrent pathogenic variants is widely used, extended next-generation sequencing (NGS) panels may improve the detection of clinically significant variants. **Aim:** To evaluate the diagnostic yield of a two-step strategy comprising founder mutation screening followed by extended multigene NGS analysis. **Materials and Methods:** Between 2023 and 2026, 2270 patients underwent genetic testing for hereditary cancer predisposition. The screening panel included recurrent variants in BRCA1, BRCA2, CHEK2, PALB2, and NOD2. Patients with negative screening results who met clinical criteria underwent extended NGS testing. Indications included breast cancer (75.11%), familial cancer predisposition (20.08%), other malignancies (2.95%), ovarian cancer (1.49%), and prostate cancer (1.05%). **Results:** Pathogenic or likely pathogenic (P/LP) variants were identified in 478/2270 patients (21.1%) by founder mutation screening. The most frequent findings were NOD2 c.3019dup (9.2%), CHEK2 variants (9.6%), BRCA1 variants (1.85%), and PALB2 variants (0.35%). Among 559 patients subsequently tested by NGS, P/LP variants were detected in 97 (17.4%). The most frequently affected genes were BRCA2 (3.57%), BRCA1 (1.61%), NBN (1.07%), ATM (0.89%), and RAD51C (0.89%), while all remaining genes accounted for 12.16% of detected variants. Overall, 575/2270 patients carried a P/LP variant, corresponding to a detection rate of 25.3%. **Conclusions:** A substantial proportion of clinically actionable BRCA1 and BRCA2 variants were identified only through NGS after negative founder mutation screening. BRCA2 was the most frequently affected highpenetrance gene detected by NGS, with 20 P/LP variants identified among screening-negative individuals. These findings highlight the limitations of founder mutation testing and support comprehensive BRCA1/BRCA2 sequencing as a first-line approach, particularly in breast and ovarian cancer patients. Broader multigene panel testing may further improve diagnostic yield.

C1.9.P

Copy number variants in PALB2 gene detected in oncology panel diagnostic tests.

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Słowa kluczowe: CNV, PALB2 gene, NGS, MLPA

IntroductionThe PALB2 gene interacts with both BRCA1 and BRCA2, playing a role in maintaining genomic stability via homologous recombination repair of DNA double-strand breaks. Pathogenic variants in PALB2 confer an increased risk of breast and ovarian cancers. Consequently, cancer patients harboring germline pathogenic PALB2 variants may benefit from targeted therapy with PARP inhibitors.
Methods and materialThe multigene cancer panel testing was performed using next-generation sequencing (NGS) to detect germline SNVs, indels and copy number variants (CNVs) in the BRCA1, BRCA2 and PALB2 genes. A total of 5262 patients underwent cancer genetic testing using NGS between 2020-2026 years. We employed the Agilent SureSelect XT HS2 Custom Panel (Illumina), and the Accel-Amplicon Custom NGS Panel (Swift Biosciences). CNVs detected by NGS were subsequently confirmed using multiplex ligation-dependent probe amplification (MLPA). The MLPA technique was also used to verify the PALB2 deletions in family members.
Results and ConclusionIn our cohort, CNVs in the PALB2 gene were detected in 5 patients. Three deletions were detected as secondary findings during BRCA1/2 testing, one deletion was detected in routine sequencing of cancer panel including BRCA1, BRCA2, PALB2 and CHEK2 genes, another deletion was detected in patient's sibling. The most frequently detected exon rearrangements were exon 11-12 deletions. All CNV variants were classified as pathogenic and interpreted as crucial to cancer risk assessment and the introduction of targeted therapy. Routine assessment of CNV variants in PALB2 should be conducted using standard gene analysis methods.

C2.1.P

Improving the interpretation of intronic variants in genetic diagnostics through targeted mRNA sequencing

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Słowa kluczowe: mRNA sequencing, intronic variants, VUS, genetic diagnostics,

High-throughput genetic diagnostics using NGS methods in patients with suspected rare diseases frequently reveal a large number of intronic variants. These are often private variants of uncertain clinical significance (VUS), posing substantial challenges for interpretation in routine genetic diagnostics. Targeted RNA sequencing using the Sanger method enables assessment of the functional impact of such variants at the transcriptional level during gene expression. Analysis performed for three patients demonstrates the utility of this approach. In the first patient, the entire transcript of the IKBKG (MIM: 300248; variant: NM_001099857.5(IKBKG):c.1056-6C>T) was analyzed. No pathogenic variants were identified within the coding sequence of the examined transcript based on the analysis of patient samples (mRNA isolated from blood). In the second patient, a fragment of the MORC2 transcript (exons 16–21; MIM: 616661; variant: NM_001303256.3(MORC2):c.1813-17_1813-15delinsCAG) was analyzed. mRNA sequence analysis revealed a deletion at the beginning of exon 19, encompassing 95 nucleotides (r.1813_1907del), resulting in a shift of the open reading frame (ORF) and introduction of a premature stop codon (p.(Glu605ThrfsTer30)). In the third patient, the effect of the variant NM_000256.3(MYBPC3):c.821+5G>A in the MYBPC3 gene (MIM: 600958) on mRNA splicing was evaluated by analyzing exons 4–12. The study revealed skipping of exon 7 (r.773_821del), consistent with previous reports indicating that this variant may lead to exon skipping during pre-mRNA splicing. This results in a shift of the open reading frame (ORF) and premature termination of translation (p.(Glu258_Thr274delinsGlyfsTer26)). The application of mRNA sequencing enables expansion of diagnostic procedures for patients lacking molecular confirmation of a clinical diagnosis by allowing functional evaluation of intronic VUS variants and their impact on gene expression.

C2.2.P

Diagnostic pitfalls of NGS - deletions and hypomorphic variants in the diagnosis of genetic diseases - case report

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Słowa kluczowe: Next-generation sequencing, CNV, hypomorphic variants

Introduction:Next-generation sequencing (NGS) is a standard tool in molecular diagnostics, enabling multigene analysis in patients with complex phenotypes. However, detection and interpretation of copy number variants (CNVs) in highly homologous regions remain challenging. In addition, hypomorphic variants may be overlooked because of their relatively high population frequency. We present a case illustrating these diagnostic pitfalls in the ATAD3 gene cluster leading to Harel-Yoon syndrome (HAYOS, OMIM#617183).
Materials and Methods:Patient: A two-year-old female with delayed speech development, congenital cataracts, and alternating hemiplegia.NGS sequencing: Exome libraries were prepared using KAPA EvoPlus V2 and KAPA HyperExome (Roche) and sequenced on NovaSeq6000 (Illumina, 2×100 bp). Variant analysis was performed using the DMG IMiD pipeline (GRCh38/hg38).CNV analysis: CNVs were detected using CODEX, CoNIFER, cn.MOPS, ExomeDepth, and XHMM.RT-PCR: RNA was extracted from peripheral blood leukocytes (RNeasy, Qiagen) and reverse-transcribed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche).
Results:We identified a heterozygous ~67.3 kb deletion at 1p36.33 (chr1:1457128–1524397), encompassing ATAD3A (exons 1–11), the entire ATAD3B gene, and part of ATAD3C (exons 8–12), together with an ATAD3A missense variant, c.229C>G (p.Leu77Val), on the second allele. Due to high sequence homology within the ATAD3 cluster, the deletion risked misclassification as a technical artefact. The hypomorphic p.Leu77Val variant may also be overlooked because it is often annotated as benign. RT-PCR confirmed the deletion.
Conclusions:This case underscores the importance of integrating clinical and molecular findings when interpreting variants in complex genomic regions. Careful assessment of CNVs within the homologous ATAD3 locus, combined with evaluation of a hypomorphic ATAD3A allele, enabled the diagnosis of Harel-Yoon syndrome. These findings emphasize the value of phenotype-guided analysis, comprehensive bioinformatic evaluation, and orthogonal validation in rare disease diagnostics.

C2.3.P

Detection of large chromosomal abnormalities from routine whole-exome sequencing data

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Słowa kluczowe: WES, CNV, large chromosomal rearrangements, mosaic

Introduction Whole-exome sequencing (WES) is routinely used for variant detection in clinical genetics. We investigated whether systematic chromosome-level analysis of WES data can increase the diagnostic yield of clinical genetic testing by identifying large chromosomal abnormalities overlooked by standard cytogenetic approaches. Methods and Results Large CNVs were identified by comparing the number of mapped sequencing reads assigned to individual chromosomes between patients. Read-count data generated using samtools idxstats (v1.23) were aggregated and visualized using the MultiQC (v1.33). Deviations in read distribution corresponded to gains or losses involving large genomic regions or entire chromosomes. The application of this algorithm enabled the detection of additional chromosomal aberrations that had not been previously documented in patients' medical records, with a particular enrichment of findings involving sex chromosomes. Furthermore, the algorithm enabled detection of large chromosomal rearrangements, including duplication of 9p, and combined duplication of 18q with terminal deletion of 7q36.3. Importantly, WES-based analysis confirmed mosaic trisomy 17 previously detected by karyotyping and additionally identified mosaic trisomy 9 in a patient in whom both karyotyping and aCGH had failed to detect the abnormality. Overall, large chromosomal CNVs were identified in approximately 0.5% of the 6,385 analyzed samples. This relatively low frequency likely reflects appropriate clinical and phenotypic stratification of the studied cohort. Conclusions Our results demonstrate that appropriately designed bioinformatic analysis of WES data may provide valuable information not only on sequence-level variants but also on large chromosomal abnormalities and mosaic CNVs.

C3.1.P

Clinical Experience with Preimplantation Genetic Testing for Monogenic Disorders (PGT-M): The Importance of Genetic Diagnosis in Reproductive Medicine

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Słowa kluczowe: PGT-M, badania preimplantacyjne, niepłodność

BackgroundPreimplantation genetic testing for monogenic disorders (PGT-M) provides couples at risk of transmitting inherited genetic conditions with an opportunity to achieve a healthy pregnancy while avoiding the transmission of disease-causing variants. Advances in molecular diagnostics, have expanded the range of conditions that can be identified prior to assisted reproductive treatment and have strengthened the role of genetic evaluation in infertility workups.**Methods**We reviewed our clinical experience with couples undergoing PGT-M for various monogenic disorders identified through genetic diagnosis, preconception or infertility-related genetic testing. Individualized PGT-M strategies were developed based on the familial pathogenic variants and validated molecular workflows. Embryos obtained through in vitro fertilization underwent trophoctoderm biopsy followed by genetic analysis to determine their suitability for transfer.**Results**PGT-M enabled accurate identification of embryos unaffected by the familial pathogenic variants, facilitating informed embryo selection and reproductive decision-making. Our experience demonstrates the clinical value of integrating comprehensive genetic testing into fertility care, particularly in couples with unexplained infertility, recurrent pregnancy loss, or a known family history of genetic disease. One representative case involved a couple with a history of five recurrent miscarriages. Infertility-focused NGS testing revealed that both partners were carriers of pathogenic variants in the PMM2 gene, associated with Congenital Disorder of Glycosylation. Subsequent PGT-M performed on embryos from two IVF cycles identified embryos free of the disease-causing variants, ultimately resulting in the birth of a healthy child. Similar approaches were successfully applied across other monogenic indications encountered in our practice.**Conclusions**Our experience underscores the importance of genetic testing in identifying the molecular causes of reproductive failure and enabling personalized reproductive management. PGT-M represents an effective strategy for reducing the risk of transmitting monogenic disorders and improving reproductive outcomes. Early genetic diagnosis, combined with multidisciplinary collaboration and advanced reproductive technologies, offers affected couples a realistic pathway toward successful pregnancy and the birth of healthy children.

C3.2.P

Chromosome positioning in human spermatozoa from normozoospermic males with decreasing protamination level and retained histone H3

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Słowa kluczowe: male infertility, sperm chromatin, positioning of chromosomes, sperm nuclear order, sperm histones and protamines

The nucleus organization in spermatozoa is unique and different than in somatic cells. The positions of chromosomes in the sperm cell nucleus is not random and plays pivotal role in genomic regulation at the earliest embryogenesis stages. During spermatogenesis histones are replaced with protamines, which allow 6-fold more condensed chromatin packaging and transcriptional inactivation of sperm genome. Among spermatogenetic histones' variants, histone-H3 should be removed before final transformation from spermatid into spermatozoon after series of epigenetic modification rounds. We aimed to assess whether chromosome positioning is altered in human spermatozoa of 5 normozoospermic control males with differing sperm chromatin protamination level, in the context of an elevated histone-H3 retention (H3+) or its lack (H3-). Fixed spermatozoa (3:1 methanol:acetic acid) were evaluated according to protamination status by acidic aniline blue staining (AB), and revealed consecutively decreasing level from 70% to 10%. Fluorescence in situ hybridization (FISH) was applied to analyze the positioning of chromosomes 13/21/18.X/Y, usually checked in prenatal testing (nonlethal ones). Next, on the same spermatozoa, immunofluorescence (IF) staining for histone-H3 was completed. Chromosome positioning was analyzed with motorized fluorescent microscope Leica DM5500 and LAS-X software (Leica), and categorized into two subgroups: H3+ and H3-. Chromosome positions were altered utmost in male with the less protaminated chromatin and H3+ spermatozoa. In contrast, in case with the highest protamination level, sex chromosomes repositioned according to both mean H+ and mean H- toward the nuclear centre, corroborating protective role of this region for these special chromosomes. Interestingly, in H3- subgroup the centre-periphery positioning of chromosome X revealed heterogeneity (no linkage to protamination level). Particular chromosomal shifts in both subgroups (H3+, H3-) revealed a tendency rather in the centre-periphery than in the tail-acrosome axis. Results suggest connection between immaturity level of sperm chromatin and the nuclear architecture, underlining role of the histone-H3 presence and proper protamination. Financing: National Science Centre 2020/38/E/NZ2/00134 (MO)

C3.3.P

Whole genome and methylome sequencing combo reveals candidate genomic variants for defective morphology of the sperm head and tail in brothers with reproductive failure

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Słowa kluczowe: male infertility, genomic variants, sperm methylation, sperm quality

Approximately 30% of the infertility cases originate from the male side only. The aetiology of infertility comprises hormonal disturbances, environmental risk factors, and genetic causes. Genetic background accounts for ~10-15% of reproductive failure; however, ~20-25% of male infertility cases still remain idiopathic. Recent data indicates a rising contribution of epigenetic mechanisms to correct gametogenesis or fertilization processes, including the regulation of imprinting or developmental genes. Consequently, it seems to be justified to investigate infertility within genomic and epigenomic context simultaneously. The aim of our study was to identify genetic variants and differentially methylated regions causative for reduced sperm quality in two families with brothers characterized by decreased sperm concentration and similar abnormalities of sperm head and acrosome morphology. Blood DNA samples (Family-I: 4 brothers; Family-II: 3 brothers, father) were sequenced by whole genome sequencing (WGS, Illumina NovaSeq6000). Sperm DNA samples were evaluated by whole genome methylome sequencing (WGMS, Illumina Infinium Methylation EPICBeadChip 850k array). Details of semen quality, incl. semen parameters and chromatin integrity status were applied (TUNEL, aniline blue, acridine orange stainings). In our study, several genomic and epigenomic variants were found in both families in genes already known as involved in gametogenesis, cilia functioning or linked to processes related with reproduction; thus, it can suggest the background of observed abnormal sperm phenotypes. This study underlines the need for genomic and epigenomic analyses in patients with severely impaired sperm quality. Seemingly identical sperm parameters (microscopic investigation) can arise from distinct genomic or epigenomic background. Hence, integration of WGS and WGMS should be legitimate and encouraged to achieve more detailed diagnostic characterization of the observed abnormalities. Funding: National Science Centre in Poland: 2020/38/E/NZ2/00134 (MO), 2015/17/D/NZ5/03442 (MO), 2015/17/B/NZ2/01157 (MK)

C3.4.P

Complex Chromosomal Rearrangements Detected by Optical Genomic Mapping and Whole Genome Sequencing in an Infertile Male with Normozoospermia

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Słowa kluczowe: male infertility, structural genomic variants, sperm quality, chromosomal aberration

Complex chromosomal rearrangements (CCRs) are structural aberrations involving more than two breakpoints on at least two chromosomes, and have been described in more than 300 publications, so far. Approximately half of cases referred to male CCR carriers, the vast majority of whom are infertile or experience reproductive failures, including only 9 normozoospermic cases. CCR carriership is related to reproductive failures due to disrupted chromosomal pairing and nondisjunction in the pachytene stage of meiosis. Four main CCR categories are systematized acc. to number of chromosomes and breakpoints involved. The purpose of this study was to identify a genomic background of a lack of reproductive success in a unique male CCR carrier with normozoospermia and good quality of sperm chromatin, classified as type III CCR (the presence of at least two independent chromosome aberrations, observed in only 12 cases to date). For the case characterization we applied: sperm quality tests, preimplantation genetic testing for structural rearrangements (PGT-SR; Ion ReproSeq), classic GTG banding, fluorescent in situ hybridization (FISH), optical genomic mapping (OGM), and whole genome sequencing (WGS). Examination of spermatozoa revealed: ~35% of genetically normal/balanced cells for each RCT (meiotic segregation pattern by FISH), 71% of properly protaminated cells (aniline blue staining), and 77% of spermatozoa with high reproductive potential (hyaluronan binding assay, HBA). PGT-SR analysis of five blastocysts showed a variety of trisomy and monosomy events related to translocated fragments. GTG and FISH characterized double reciprocal chromosome translocation, while OGM confirmed the findings and additionally exposed intrachromosomal fusion *ogm[GRCh38] t(2;9)(q32.2;q21.13)(189561373;73326540)*, *ogm[GRCh38] t(12;8)(p12.1;q24.22)(24660805;13094226)*, *ogm[GRCh38] fus(13;13)(q32.3;q21.3)(100997502;70268176)*. WGS did not reveal any SNV variants causative for infertility per se; however, we generated a list of genetic variants related to observed morphological abnormalities of the head of spermatozoa. Funding: National Science Centre in Poland 2020/38/E/NZ2/00134 (to MO) # equal contribution EK and MO are co-presenting authors

C3.5.P

Reproductive significance of pericentric chromosome 9 inversion: high-resolution embryo chromosomal analysis in IVF

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Słowa kluczowe: inversion of chromosome 9, PGT-A, interchromosomal effect, aneuploidy

Research question: Does parental pericentric inversion of chromosome 9 affect embryo chromosomal integrity in IVF when assessed using standard PGT-A combined with high-resolution structural analysis? Key findings: Embryos from inv(9)(p12q13) carriers showed a relatively high aneuploidy rate, but no abnormalities involved chromosome 9. Findings do not support a consistent interchromosomal effect, though individual variability may exist. Background: Pericentric inversion of chromosome 9 occurs in 1–3% of the population and is generally considered a benign variant. Large PGT-A studies report no increased embryo aneuploidy or unbalanced chromosome 9 abnormalities in carriers, arguing against an interchromosomal effect. However, inv(9) appears more frequently in infertile populations, and some carriers show elevated aneuploidy or subtle gamete defects, suggesting possible breakpoint-specific variability. Current guidelines recommend individualized counseling rather than automatic classification as benign. Study design: This retrospective cohort study included 63 embryos from 19 IVF couples undergoing PGT-A between 2024 and 2025, with one inv(9) carrier per couple. Methods: Trophectoderm biopsies underwent next-generation sequencing (NGS). Standard whole-genome PGT-A detected aneuploidies across all chromosomes, while targeted high-resolution structural analysis of chromosome 9 was added to identify subtle inversion-associated imbalances beyond standard PGT-A resolution. Results: Mean maternal age was 36.8 ± 4.7 years. Aneuploidy was detected in 24 embryos (38.7%), all involving chromosomes other than chromosome 9. High-resolution analysis revealed no structural imbalances affecting chromosome 9. Marked variability between couples was observed, with some showing notably high aneuploidy rates, suggesting possible individual or gametogenesis-related effects. Given the limited sample size, findings should be interpreted cautiously. Study limitations: Retrospective design and small sample size limit definitive conclusions. Larger multicenter studies are needed, particularly to assess breakpoint-specific risks. Clinical implications: Combining whole-genome PGT-A with targeted structural analysis may improve IVF counseling and individualized risk assessment for common chromosomal variants such as inv(9).

C3.6.P

The presence of a dual paternal genome and absence of a maternal genome in analysis of chorionic villus sample with features of a complete hydatidiform mole.

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Słowa kluczowe: Complete hydatidiform moles (CHM), miscarriage, chorionic villus sample, array CGH, MLPA, QF-PCR, genomic imprinting effects

Complete hydatidiform mole (CHM) is a non-neoplastic proliferation of placental tissues characterized by swelling of the chorionic villi and the formation of grape-like vesicles. It typically develops when a sperm fertilizes an oocyte devoid of its own genetic material. The sperm then duplicates its material, resulting in all chromosomes being of paternal origin (46,XX karyotype), which precludes fetal development and leads to abnormal trophoblast (future placenta) proliferation. Fetal-placental developmental abnormalities in CHM cases are not associated with numerical chromosomal aberrations but essentially arise from aberrant genomic imprinting effects. Miscarriages associated with partial hydatidiform mole are frequently linked to the identification of triploidy. In QF-PCR testing, this manifests as the presence of informative autosomal STR markers that display triallelic patterns with relative peak area ratios of 1:2, 2:1, or 1:1:1. In the case of CHM, however, result interpretation is not so obvious due to the presence of single (homozygous) alleles observed across all autosomal STR markers. Furthermore, the application of a cytogenetic microarray: aCGH 8x60k will also fail to provide any significantly informative data to facilitate CHM identification, owing to the presence of a diploid genome in the tested sample. The aim of this study is to present the diagnostic challenges in examining chorionic villi with CHM features using routine methods applied in miscarriage tissue testing (QF-PCR, aCGH), as well as the possibility of unquestionable confirmation of genomic imprinting disorders resulting from the presence of a dual paternal genome in the tested sample through the application of the MLPA technique.

C3.7.P

Superiority of TP-PCR method over PCR screening in FMR1 gene analysis in patients with suspected FXPOI

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Słowa kluczowe: TP-PCR, PCR screening, Fragile X syndrome, FXPOI, FMR1-dependent diseases

Fragile X syndrome is one of the leading genetic causes of intellectual disability (ID) and autism spectrum behavioural disorders (ASD). Molecular testing to identify a dynamic mutation (CGG trinucleotide repeat >200) in the FMR1 gene is one of the first diagnostic tests for cases of developmental delay or behavioral disorders. Individuals who carry the premutation (CGG repeat count: 55-200) in the FMR1 gene do not experience the above symptoms, but they are at risk of developing other FMR1-dependent diseases such: Fragile X-associated primary ovarian insufficiency (FXPOI) or Fragile X-associated tremor/ataxia syndrome (FXTAS). For the diagnosis of patients with suspected Fragile X syndrome, a screening test based on the PCR technique often provides a fast, cost-effective, and, above all, reliable solution that guides clinicians towards a diagnosis or the need for further evaluation. However, in an era of increased interest in FMR1 gene testing among patients with suspected FXPOI, choosing a screening test is often a misguided decision due to the limitations of the method. The screening test does not allow for the identification of alleles within the premutation range. Furthermore, it does not allow for an unambiguous interpretation of results in females who are homozygous for normal-range alleles. Consequently, choosing a screening test in FXPOI diagnostics often requires additional testing using the TP-PCR technique, which increases overall testing costs and prolongs the turnaround time for the final result. In this study, we aimed to present statistical data from a 5-year period of FMR1 gene testing using the TP-PCR technique. These data clearly confirm the superiority of this technique over the screening test and establish TP-PCR as the method of choice in the diagnosis of patients with suspected FXPOI.

C3.8.P

Mosaic karyotype as a factor in pregnancy failure.

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Słowa kluczowe: mosaic karyotype, chromosomal mosaicism; 45,X/47,XX,+18, recurrent pregnancy loss, fetal aneuploidy, prenatal diagnosis, cytogenetic analysis

Background: Constitutional mosaicism in the form of aneuploidy involving two different chromosomes is an extremely rare in live-born children. A rarely observed type of mosaicism in patients is the karyotype $\text{mos } 45,X/47,XX,+18$, which is characterized by a variable phenotype representing a combination of features of Turner syndrome (associated with monosomy X) and trisomy 18 and preserved fertility. Particular attention should also be given to the impact of this karyotype on fertility and reproductive outcomes, including pregnancy failure, spontaneous miscarriages, and genetic abnormalities in the fetus. **Material and Methods:** The study material consisted of clinical and cytogenetic data from a family of five individuals in whom the mosaic karyotype $\text{mos } 45,X/47,XX,+18$ was identified, as well as data from five additional families with the same mosaic karyotype reported in the literature. Cytogenetic analysis of chromosome preparations revealed the presence of mosaic karyotype $\text{mos } 45,X/47,XX,+18$ in the proband from the investigated family. **Results:** Family studies of the patient's pregnancies, performed on amniocytes obtained from amniotic fluid during the patient's second pregnancy, revealed trisomy 18 ($47,XX,+18$). Similarly, prenatal testing carried out during the third pregnancy demonstrated the presence of an additional chromosome 18 in the fetus ($47,XY,+18$). **Conclusion:** Mosaic karyotype $\text{mos } 45,X/47,XX,+18$ is a rare finding associated with significant reproductive risk, including recurrent pregnancy loss and fetal aneuploidy. These observations highlight the importance of cytogenetic diagnostics and genetic counseling in affected patients.

C3.9.P

Genetic abnormalities and azoospermia - what are the chances of a positive testicular biopsy result?

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Słowa kluczowe: Azoospermia; EAU/EAA - European Association of Urology/European Academy of Andrology; karyotype, KS - Klinefelter Syndrome; CFTR-mutation; Y-chromosome microdeletion; TESA - testicular sperm aspiration; m-TESE - microdissection testicular sperm extraction; SSR - successful sperm retrieval; IVF-ICSI - in-vitro-fertilization – intracytoplasmic sperm injection

Introduction: Patients with azoospermia should undergo genetic verification prior to testicular biopsy with the intention of collecting sperm for the IVF-ICSI procedure (EAU/EAA Guidelines). Abnormal karyotype, CFTR gene mutations, and Y chromosome deletions in the AZF region are associated with a negative testicular biopsy result, indicating disqualification from IVF-ICSI. **Objectives:** SRR of testicular biopsies in azoospermic patients and genetic abnormalities: percutaneous 16-gauge needle TESA versus open microscopic procedure m-TESE. **Materials and methods:** 2021-2026: 517 testicular biopsies in men with azoospermia (230 TESA, 287 m-TESE). Patients with genetic abnormalities 99/517 (19,2%). TESA 24/230 (10,4%): CFTR-mutation 20 [IVS8-5T variants - (8); p.Phe508del/- (5); p.Phe508del/IVS8-5T(1); p.Phe508del/D1152H(1), p.Tyr515His/- (1); p.Ser519Gly/- (1), rare(3)]; Y-chromosome microdeletion 3 [B incomplete(1), C(2)]; chromosomal abnormalities 1 (46,XY[50]/47,XXY[1]). m-TESE 75/287 (26,1%): KS(32) [47,XXY(28); XXY/46,XY(1); 47,XXY+p.Phe508del/- (1); 47,XXY+IVS8(TG)13(T)5/- (1); 47,XXY[23]/46,XX[9](1)]; CFTR-mutation 16 [IVS8-5T variants/- (12); p.Phe508del/- (1); p.Ser519Gly/- (1); Tyr515His/- (1); Glu528(1)]; Y-chromosome microdeletion 19 [B incomplete(2), B incomplete/C(3), C(12)]; chromosomal abnormalities 8 (46,XY,t(2;20)(q31;q13.3); 46,XY,inv(9)(p12q13); 46,XY, 15ps+; 46,XY,22s+; 46,XY,t(1;15)(q10;q10); 46,XY,t(15;20)(p11.2;q11.2); 46,X + mar.FISH der (Y) (DYZ3+); Ring chromosome Y 46,r(Y)(p11.3q12[17] / 45,X[13]). **Procedures** - general anaesthesia; TESA outpatient, Menghini G16 needle; m-TESE one-day-surgery, Leica M8602x2 microscope, 25x magnification. **Results:** SRR 33/99 (33%). TESA 17/24 (70,8%); CFTR-mutation 14/20 (70%), the most common variant IVS8-5T(5); Y-chromosome microdeletion C

2/3(66,7%); Chromosomal abnormalities 1/1(46,XY[50]/47,XXY[1]).m-TESE 16/75(16%): CFTR-mutation 7/16(43,8%), the most common variant IVS8-5T(6); Y-chromosome microdeletion C 5/19(17,6%); KS 3/32(10%); Chromosomal abnormalities 1/8(12,5%)46,XY,t(1;15)(q10;q10). Conclusions:1. Genetic abnormalities are a very important factors predicting the chances of a positive results of testicular biopsies in patients with azoospermia, but they are not a reason for disqualification "a priori".2. The results of m-TESE SRR outcomes are worser compared to TESA and are associated with patients with more severe gonadal damage.3. Patients with mutations in the CFTR gene (dominant IVS8-5T variants) have the best chance for sperm obtaining in both the TESA and m-TESE biopsy groups.

C3.10.P

A variant of the NANOS1 RNA-binding protein disrupts pluripotency and Wnt signalling, leading to the absence of primordial germ cells

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Słowa kluczowe: Human infertility; Germ cells; RBPS, RNA-binding proteins; hESCs, human embryonic stem cells.

Infertility is increasingly recognised as a global health issue, often linked to genetic factors. NANOS1 is an RNA-binding protein crucial for germ cell development in *Drosophila*, regulating post-transcriptional mechanisms mediated by the 3'UTR of target mRNAs. We identified a NANOS1 variant (v-NANOS1) carrying the p.[Pro34Thr; Ser78del] mutation associated with the absence of germ cells in the seminiferous tubules of infertile patients. Preliminary data from the TCam-2 cell line, which mimics primordial germ cells (PGCs), revealed a proapoptotic switch for v-NANOS1, in contrast to the wild-type (wt) counterpart's antiapoptotic role. To understand the molecular basis of this phenotype, we aimed to identify v-NANOS1-bound RNAs and compare them with those bound by wt-NANOS1. To achieve this, we utilised a human embryonic stem cell (hESC) line expressing the NANOS3-tdTomato

reporter, a marker of PGC specification, as a model of human PGC differentiation in vitro. We generated doxycycline-inducible, stable, PiggyBac-transgenic cell lines expressing wt- and v-NANOS1. Expression of v-NANOS1 was studied in two stages: pre-mesendodermal (preME) and PGCs. The v-NANOS1 expression caused a significant reduction and, in some instances, a complete absence of PGCs. Using eCLIP/RNA-seq analysis, we found significantly more RNAs bound by v-NANOS1 than by wt-NANOS1, indicating a gain-of-function for v-NANOS1. At the preME stage, v-NANOS1 expression downregulated competence and pluripotency markers. It also downregulated canonical Wnt signalling targets, leading to their aberrant upregulation in PGCs. Inhibition of Wnt signalling at the PGC stage rescued PGC numbers, underscoring the crucial role of Wnt signalling in PGC development. This study underscores the substantial impact of the p.[Pro34Thr; Ser78del] mutation on v-NANOS1 gain-of-function, resulting in reduced PGCs during early germ cell development, potentially elucidating the observed infertility phenotype in patients. This research was supported by a grant from the National Science Centre, Poland, OPUS 2019/35/B/NZ1/01665 to JJ.

C3.11.P

Generation of an in vitro model for studying NANOS3-related human infertility

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Słowa kluczowe: infertility, primordial germ cells, NANOS3, CRISPR-Cas9

Infertility is a common disorder, with approximately 50% of cases attributed to genetic factors, including genes expressed in primordial germ cells (PGCs) during their specification and differentiation, such as NANOS3. NANOS3 encodes a conserved RNA-binding protein that serves as a marker of PGCs and functions as a post-transcriptional regulator of gene expression. Variants in NANOS3 have been associated with Primary Ovarian Insufficiency (POI) in women. Among the reported variants, the missense mutation c.358G>A (p.E120K), identified in a homozygous state in two sisters with POI, is particularly compelling. This variant results in an amino acid substitution at a conserved residue within the RNA-binding zinc-finger domain. To date, the effects of the E120K substitution on post-transcriptional gene regulation have not been investigated in any experimental model. Human PGC development can be modelled in vitro by differentiating human embryonic stem cells (hESCs) into human primordial germ cell-like cells (hPGCLCs), which recapitulate key molecular features of early PGCs. Here, we aim to generate hESC lines that will serve as an in vitro model to investigate the transcriptomic, translational, and cellular consequences of the infertility-associated NANOS3 E120K variant in hPGCLCs. To achieve this aim, we are engineering the H1 hESC line using a two-step CRISPR-Cas9 strategy: (1) insertion of a GFP reporter co-expressed with NANOS3 (NANOS3-GFP cell line), and (2) introduction of the E120K variant into the endogenous NANOS3 locus (E120K-NANOS3-GFP cell line). Generation of the cell lines is currently underway. The resulting model will enable comparative analyses of wild-type and E120K NANOS3-expressing cells, allowing characterization of the molecular and cellular consequences of the variant. These studies will provide insight into the mechanisms by which altered NANOS3 function may disrupt germ cell development and contribute to infertility. This research was supported by a grant from the National Science Centre, Poland, OPUS 2021/43/B/NZ3/01635 to KK-Z.

C3.12.P

Combined HAND2 and GATA4 Variants Identified in a Patient With 46,XY Gonadal Dysgenesis Impair NR5A1-Mediated SOX9 Activation

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Słowa kluczowe: testis determination; 46,XY gonadal dysgenesis; HAND2; GATA4; SOX9 regulation; oligogenic inheritance

SOX9 expression is essential for male gonadal sex determination, directing bipotential gonads toward testis development during early embryogenesis. SOX9 transcription is driven by NR5A1, whose activity is modulated by the cofactor GATA4. Pathogenic variants in SOX9, NR5A1, or GATA4 can disrupt testis development and cause 46,XY gonadal dysgenesis, in which individuals with a 46,XY karyotype develop female or ambiguous phenotypes. Beyond gonadal development, GATA4 also plays a critical role in cardiac development through cooperation with HAND2. Although HAND2 is expressed in developing gonads, its role in testis determination has not been characterized. Using whole-exome sequencing in a patient with 46,XY gonadal dysgenesis, we identified rare heterozygous variants in both GATA4 and HAND2. We therefore aimed to investigate the role of HAND2 in NR5A1-mediated SOX9 activation and to assess the functional impact of the patient-specific GATA4 and HAND2 variants. Luciferase reporter assays using a testis-specific SOX9 enhancer revealed that HAND2 significantly enhances NR5A1-dependent SOX9 transcriptional activation. Notably, the HAND2 variant identified in the patient abolished this stimulatory effect, supporting its pathogenic potential. While the GATA4 variant alone did not alter SOX9 activation, co-expression of the GATA4 and HAND2 variants in a heterozygous context resulted in a significant reduction in SOX9 activation. These findings identify HAND2 as a novel candidate regulator of SOX9 activation during testis development and support an oligogenic model in which combined HAND2 and GATA4 variants contribute to the pathogenesis of 46,XY gonadal dysgenesis.

C3.13.P

Preimplantation Genetic Testing from a Clinical Geneticist's Perspective

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Słowa kluczowe: Preimplantation genetic testing (PGT), In vitro fertilization (IVF), Aneuploidy screening, Genetic counselling

With the introduction of the government-funded Infertility Treatment Program in June 2024, a greater number of couples have gained access to this treatment option, which has also contributed to increased interest in preimplantation genetic testing of embryos. The aim of this study is to present the results of 131 PGT analyses performed between September 2024 and June 2026 in patients treated at a fertility clinic, with particular emphasis on the characteristics of the study population and the indications for testing. Overall, preimplantation genetic testing results were obtained for 298 embryos from 103 patients. The majority of tests performed consisted of preimplantation genetic testing for aneuploidy (PGT-A), five PGT-SR analyses were also performed in carriers of chromosomal translocations, as well as two PGT-M analyses for monogenic disorders. In the presented cohort, a total of 87% patients attended a genetic counseling consultation prior to qualification for PGT. The mean age of patients undergoing PGT-A was 37 years (range: 27 to 47 years). The primary indication for PGT-A was advanced maternal age (≥ 37). Among younger patients, the most common indication for PGT-A was recurrent implantation failure (RIF) (21/124; 17%), followed by recurrent pregnancy loss (RPL) (12/124; 10%). In total, 140 embryos were tested in women older than 36 years, of which 80 yielded an abnormal PGT-A result (80/140; 57%). Chromosomal abnormalities were least frequently identified in trophoctoderm biopsies from embryos of patients undergoing PGT-A due to RIF (18/47; 38%). PGT-SR was performed on twelve embryos, and only two of them yielded a normal result in the analyzed trophoctoderm samples. The available literature clearly demonstrates that the greatest benefit from PGT-A is observed in women over 36 years of age and in patients who are carriers of chromosomal translocations, which is consistent with the results presented in our study.

C3.14.P

Two Siblings, Two De Novo Disorders: TNRC6B-Related Neurodevelopmental Disorder and 8q22.1 Duplication–Associated Arthrogryposis

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Słowa kluczowe: quad Whole-Exome Sequencing (quad WES), 8q22.1 microduplication, TNRC6B, de novo mutation, arthrogryposis, neurodevelopmental disorder

Background Diagnostic evaluation of siblings with different clinical manifestations may be challenging, as a shared genetic etiology is often assumed. However, genomic studies have shown that independent de novo pathogenic variants may occur within a single family, resulting in distinct disorders. We report two siblings with different phenotypes carrying independent de novo genetic alterations identified by quad whole-exome sequencing (WES). **Case presentation** The younger sibling, a 1.5-year-old boy with no relevant family history, presented with motor developmental delay, generalized hypotonia and facial dysmorphism. His 5-year-old sister exhibited arthrogryposis without neurological abnormalities. **Results** Quad WES performed in both siblings and their unaffected parents identified distinct de novo genetic alterations, with no evidence of the variants in parental blood-derived DNA, although germline mosaicism cannot be excluded. In the boy, a heterozygous, novel c.3642del (p.Val1215CysfsTer4) variant in TNRC6B was detected, consistent with TNRC6B-related neurodevelopmental disorder. In his sister, WES identified a microduplication at 8q22.1, encompassing GDF6, UQCRB, MTERF3, PTDSS1, SDC2, CPQ, and TSPYL5. The duplication is considered clinically significant and correlated with symptoms of Leri pleonosteosis, mainly because of the role of GDF6 in skeletal development and joint formation. Similar duplications have been reported in ClinVar as either pathogenic or variants of uncertain significance, reflecting uncertainty regarding penetrance and phenotypic spectrum; however, the girl presents with clinical features. The occurrence of two independent de novo molecular diagnoses in siblings is rare and may complicate clinical. No likely pathogenic variants were identified in the parents to explain an increased de novo mutation burden, although genetic factors predisposing to germline mutagenesis cannot be excluded. **Conclusions** This case highlights the importance of comprehensive genomic testing in families with multiple affected children, particularly when clinical presentations differ substantially. Independent de novo molecular diagnoses may coexist within a single family, emphasizing the utility of quad WES for accurate diagnosis and genetic counseling.

C3.15.P

Genetic etiology of hydrocephalus – clinical, cytogenetic and molecular analysis of 11 patients

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Słowa kluczowe: hydrocephalus, ventriculomegaly, chromosomal aberrations, monogenic syndromes with hydrocephalus

Introduction:Hydrocephalus (HC) refers to the excessive accumulation of cerebrospinal fluid in the brain's ventricles, which can result from overproduction of fluid, impaired absorption or impaired circulation. Occurs in 4-8/10,000 of all births and is one of the most common disorders of brain development. Apart from external and internal factors, genetic factors play role in about 20-40%. Recently was identified more than 100 genes and several signaling pathways associated with hydrocephalus (WNT pathway, dystroglycanopathy, ciliopathy, RAS-MAPK, PI3K-AKT-mTOR) as well as chromosomal aberration (dupXp22.33, dup6p25.3, dup7p21.3, dup12q24 and del12q22q23.1). Patients and methods: We present clinical evaluation of 11 patients with HC (8pts) or ventriculomegaly (3pts), from prenatal diagnosis to 16th y: 5 with the chromosomal aberrations identified in aCGH, 1 patient with chromosomal aberration and gene variant identified by exom sequencing (WES) and 5 patients with gene variant identified by panel or exom sequencing.Results: 10 patients had DD, macrocephaly, delayed speech and dysmorphic features, 1 had vertriculomegaly and omphalocele and variant in PTCH1 gene, 1 HC and phocomelia, del1q21.1 and variant in RBM8A was diagnosed prenatally as TAR syndrome. Congenital heart defect (PS) and optic nerve hypoplasia were found in 1 patient, 2 cousins with HC had variant in L1CAM, 1 patient had variant in PTCH1, 1 patient in TRAF7 and 1 patient in NFIA gene. ACGH revealed aberrations: del2q34(214491708_214997351), del5p15.33, mosaic del9q34.3(139388124_139412705), dup6q15q16.1(91391803_94526635) and dup22q11.21(18842715_21457610), del15q11.2.Conclusions:5 our patients presented with congenital HC and rare chromosomal aberrations that were not yet connected with HC. From 6 patients with monogenic defects in 4 HC was never (TAR s., CAFDADD s.) or rarely (Gorlin s., NFIA related disorder) described.We hope that our investigations may offer an insight into the pathogenesis of HC and may have the impact on future therapy. In diagnostic guidelines for HC, WES should be included.

C3.16.P

A case of Treacher Collins syndrome due to a balanced translocation between 5q32 and 22q12 disrupting TCOF1 gene

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Słowa kluczowe: Treacher Collins syndrome, TCOF1, translocation

Treacher Collins syndrome (TCF, ORPHA:861) is a rare genetic mandibulofacial dysostosis characterized by malar hypoplasia, lower eyelid abnormalities, micro- or retrognathia and external ear anomalies leading to conductive hearing loss. The disorder results from bilateral hypoplasia of the zygomatic bones, maxilla, and mandible and is typically not associated with growth impairment, intellectual disability or other major congenital anomalies. The diagnosis is based on characteristic clinical features and the identification of pathogenic variants in the TCOF1, POLR1B, POLR1C, POLR1D or POLR1E genes, which account for the vast majority of cases. However, the molecular cause remains unknown in approximately 3% of affected individuals. A one-year-old patient was referred to our clinic because of facial dysmorphism. Clinical examination revealed features highly suggestive of TCS. Initial molecular testing, including next-generation sequencing (NGS) of the TCOF1 gene and array comparative genomic hybridization (array-CGH), yielded negative results. Diagnostic investigations were subsequently expanded to whole-exome sequencing (WES), encompassing all known TCS-associated genes and other genes of interest; however, no pathogenic variants were identified. Further evaluation included conventional karyotyping and whole-genome sequencing (WGS). These analyses revealed a balanced translocation, t(5;22)(q32;q12), disrupting the TCOF1 gene located at 5q32. Performed analyses showed that no other genes were disrupted by the chromosomal rearrangement, which occurred de novo. This report is of interest because it describes a rare and, to the best of our knowledge, previously unreported disease mechanism in TCS. The findings broaden the spectrum of pathogenic alterations affecting TCOF1 and suggest that chromosomal rearrangements disrupting this gene may account for a small subset of molecularly unresolved cases.

C3.17.P

Amino acid substitutions as the molecular basis for primary ciliary dyskinesia (PCD): Influence of E.coli strain on DNAI1 and DNAI2 production

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Słowa kluczowe: PCD, DNAI1, Recombinant protein

Introduction: Primary ciliary dyskinesia (PCD) is a rare hereditary condition impacting the physiological function of motile cilia, leading to chronic bronchitis, bronchiectasis, male sterility, and mirrored internal organ arrangement. One of the most frequent molecular defects in PCD involve outer dynein arms, elements of the ciliary ultrastructure responsible for generating ciliary movement. DNAI1 and its primary interactor, DNAI2, are essential for the proper structure and function of ODA complex. Among PCD-related genes, DNAI1 exhibits the highest frequency of amino acid substitutions. These pathogenic variants cluster within its WD40 repeat (WDR) domain known to be involved in protein-protein interactions. The presence of flexible protein-binding regions, IDRs, in both DNAI1 and DNAI2 proteins makes in vitro production of these proteins difficult. The aim of the study: This study aims to analyze the effects of different E. coli strains on the efficiency of DNAI1 and DNAI2 production, solubility, and folding. **Materials and Methods:** Three expression vectors: His-tag-TEV-DNAI1, His-tag-TEV-DNAI2, and DNAI2-TEV-His-tag were transformed into three E. coli strains: BL21(DE3)pLysS, Origami B (DE3), or ArcticExpress (DE3). Overexpressed proteins were purified from bacterial lysates using HisPur Cobalt resin. Insoluble protein fractions were first dissolved in 8M urea and later refolded on Amicon centrifugal filters. The amount of produced proteins and their solubility were analyzed using SDS-PAGE and Western Blot analysis. **Results:** Recombinant DNAI1 and DNAI2 proteins were found only in their insoluble form, requiring further refolding. The efficiency of production was the highest in the ArcticExpress(DE3) strain. **Conclusions:** IDRs present in DNAI1 and DNAI2 may cause the proteins to aggregate and form inclusion bodies. Specific modifications in ArcticExpress(DE3) and Origami B (DE3) allow for better folding of hard to express proteins. The use of those strains allowed for a better efficiency of recombinant protein production, resulting in a significantly higher yield of DNAI1 and DNAI2. This study was financed by National Science Centre (NCN) grant: 2023/49/B/NZ2/01356

C3.18.P

Intriguing OFD1 protein – at a crossroads of cell division and cilia formation

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Słowa kluczowe: OFD1, PCD, ciliopathies, cell cycle, proliferation

The OFD1 protein (oral-facial-digital syndrome 1) is required for control of centriole length and distal structure, cell cycle progression and cilia formation. Pathogenic variants in OFD1 cause defects of primary and motile cilia (ciliopathies). However the involvement of OFD1 C-terminus in the different OFD1 functions is poorly understood. This study investigated how OFD1 truncation at exon 16 and 21 (found in patients with primary or motile ciliopathies, respectively) affect proliferation, cell cycle and primary ciliogenesis. HEK293T cells carrying FLAG-tagged wild-type (WT) OFD1 were created using CRISPR-Cas9-HDR, truncations at exon 16 (ex16) or 21 (ex21) of OFD1 were created by CRISPR-Cas9. The cell cycle was studied in synchronized clonal lines by flow cytometry (FC) using 7-AAD staining. Long-term proliferation was assessed by FC and Cell Trace stain dilution. Ciliogenesis was evaluated using immunofluorescence. In all experiments, Flag-tagged truncation mutants were compared with FLAG-WT cells. Both truncation mutants demonstrated a significantly longer S-phase (128-130%) and an increased percentage of G1 cells after 24hrs compared to FLAG-WT OFD1, indicating elongated DNA synthesis. However, the ex21 clone displayed faster S-phase entry, a reduced percentage of G2/M after 24hrs and faster fluorescence decay in the CellTrace experiment, indicating faster proliferation compared to control, along with only slightly decreased cilia length and numbers. In contrast, ex16 clone displayed a lower percentage of cells at the S-phase peak (fewer actively replicating cells), a reduced number of cell generations by day 4 (reduced replicative capacity), and a total lack of cilia. Our results indicate that the OFD1 C-terminus is involved in the regulation of S-phase length and cell proliferation, likely through pRB-E2F-cell cycle pathway. A planned mitomycin C experiment will verify the contribution of DNA replication stress to these disruptions. Funded by National Science Centre, Poland [OPUS 2022/45/B/NZ4/00927].

C3.19.P

Regular triploidy in a three-year-old girl with normal aCGH and WES results – a case report

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Słowa kluczowe: triploidy, array CGH, whole exome sequencing, conventional karyotyping, Quantitative Fluorescent Polymerase Chain Reaction

Triploidy is a rare chromosomal abnormality characterized by the presence of 69 chromosomes instead of the normal 46. It is most commonly identified in spontaneous miscarriages or intrauterine fetal demise, usually during the first trimester. The incidence among live-born children is estimated at approximately 1:50,000. Only isolated cases of live-born children with complete (regular) triploidy have been reported, whereas mosaic triploidy is described more frequently. According to the literature, the oldest reported patient with regular triploidy survived less than one year. We present a 3-year-old girl with regular diandric triploidy. She was born at 38 weeks of gestation with multiple congenital anomalies, including hydrocephalus, syndactyly, craniosynostosis, and renal cysts. During follow-up, hearing impairment, epilepsy, and profound global developmental delay were diagnosed. Initial genetic testing, including single whole exome sequencing (WES) and chromosomal microarray analysis (CMA/aCGH), revealed no abnormalities. Repeat trio WES raised suspicion of triploidy, which was subsequently confirmed by conventional karyotyping from peripheral blood lymphocytes and QF-PCR analysis from buccal epithelial cells. Both tests confirmed triploidy in all analyzed cells. The patient died shortly after her third birthday. To the best of our knowledge, the patient presented here is the longest-living child with regular triploidy reported to date. The course of her diagnostic evaluation demonstrates the high level of vigilance required in the assessment of patients with multiple congenital anomalies. Due to the complex phenotype, genetic testing in this patient was initiated with high-throughput methods, and triploidy was not initially considered in the differential diagnosis because of the patient's age. Nearly one year elapsed between the patient's first consultation at the Genetic Clinic and the establishment of the diagnosis of triploidy.

C3.20.P

Capillary Malformation–Arteriovenous Malformation Syndrome: A Case Series of Genetically Confirmed CM-AVM1 and CM-AVM2

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Słowa kluczowe: Capillary malformation–arteriovenous malformation syndrome; RASA1; EPHB4; vascular anomalies; capillary malformations.

Capillary malformation–arteriovenous malformation syndrome (CM-AVM) is a rare autosomal dominant vascular disorder associated with pathogenic variants in the RASA1 (CM-AVM1) and EPHB4 (CM-AVM2) genes [1,2]. Because of its rarity and highly variable clinical presentation, the condition is frequently overlooked in childhood, which may delay genetic diagnosis, appropriate clinical follow-up, and family screening. In this study, we retrospectively reviewed a cohort of 25 patients with molecularly confirmed CM-AVM syndrome who were evaluated and managed at our center. Clinical characteristics, vascular anomalies, imaging findings, family history, and genetic results were analyzed, with particular attention to differences between CM-AVM1 and CM-AVM2. The clinical manifestations varied considerably, not only between unrelated families but also among affected members of the same family. Multiple capillary malformations were identified in all patients and constituted the most consistent feature of the syndrome, although their morphology and distribution differed substantially. Most individuals experienced a relatively mild disease course; however, some developed severe AVMs of the central nervous system, leading to significant neurological complications and requiring surgical treatment. Patients carrying EPHB4 variants shared many cutaneous features with those with RASA1 variants but demonstrated a somewhat different spectrum of associated vascular abnormalities. These findings illustrate the broad clinical spectrum of CM-AVM syndrome and suggest possible phenotypic differences between CM-AVM1 and CM-AVM2. Capillary malformations are often the earliest and most recognizable sign of the disorder and should raise suspicion of CM-AVM syndrome, particularly when multiple lesions are present. Although many affected individuals remain only mildly symptomatic, careful assessment and long-term follow-up are important because potentially serious vascular anomalies may occur in the central nervous system and other organs [1,2]. Early recognition of the syndrome is important because it helps identify affected relatives and allows appropriate monitoring and treatment of clinically significant vascular lesions.

C3.21.P

Genetic Basis of Congenital Hypothyroidism in Newborns Diagnosed Through Neonatal Screening

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Słowa kluczowe: congenital hypothyroidism; thyroid dysgenesis; thyroid dysmorphogenesis

Background: Congenital hypothyroidism (CH) is a group of clinical symptoms caused by the deficiency of thyroid hormones, with an estimated incidence of 1:3000 – 1:4000 live births. In 80 – 85% of cases, the cause of permanent congenital hypothyroidism is thyroid dysgenesis, while the remaining 10 – 15% result from thyroid dysmorphogenesis. In Poland, nationwide newborn screening for CH, based on TSH measurement, has been implemented in 1994. **Materials and Methods:** The study cohort included a total of 72 children with thyroid dysgenesis (47 patients: 42 athyreosis, 5 hypoplasia), thyroid gland in situ (GIS – 21 patients) and additional 4 children without ultrasound examination. Next-generation sequencing was carried out using a targeted panel including TSHR, PAX8, FOXE1, NKX2-1, NKX2-5 and DUOX2 genes. Library preparation was performed with the KAPA HyperCap Workflow v3.0 (Roche). Differences in TSH concentrations between the analyzed groups were assessed using the Mann–Whitney U test. **Results:** In a group of 72 patients analyzed with targeted NGS, variants requiring further interpretation were identified in six individuals (8.3%). In total, eight rare variants were identified in genes associated with thyroid development and function: DUOX2, NKX2-1, TSHR, and PAX8. Three pathogenic variants in the TSHR gene were identified in two CH patients, and one patient carried a likely pathogenic variant in the PAX8 gene. In two patients, VUS were detected in the NKX2-1 gene, while one patient had a VUS in DUOX2, and another carried a VUS in PAX8. No statistically significant difference in TSH levels was found between the group of patients with agenesis and the group of patients with a normal thyroid. **Conclusions:** The results indicate that the presence of pathogenic variants in analyzed genes is not very common in patients with congenital hypothyroidism. There is a need of the broader analysis that will include other genes related to CH.

C3.22.P

Monogenic Diabetes in Pregnancy: Between Genetics and Clinical Practice – A Guideline Gap Analysis

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Słowa kluczowe: monogenic, diabetes, pregnancy, guidelines

Monogenic diabetes comprises a heterogeneous group of single-gene disorders affecting pancreatic β -cell function. Pregnancy represents a unique clinical context in which maternal glycaemia and fetal genotype interact, particularly in GCK-, HNF1A-, and HNF4A-MODY. This review evaluates international guidelines and real-world clinical practice to identify inconsistencies and evidence gaps in management strategies. **Methods:** A narrative review of major international guidelines (ADA, EASD, ISPAD, NICE, and PTD) was performed, supplemented by analysis of published expert consensus statements and reports describing clinical practice patterns in the United Kingdom, United States, and Europe. Key domains included diagnostic strategies, pregnancy-specific treatment algorithms, fetal growth assessment, and genetic testing use. **Results:** Marked heterogeneity exists across guidelines. ADA and EASD focus on classification and recognition of monogenic diabetes without dedicated pregnancy algorithms. ISPAD emphasizes early identification and genetic counselling but provides limited obstetric guidance. PTD similarly lacks subtype-specific pregnancy recommendations. In contrast, NICE provides the most clinically operational framework, particularly for GCK-MODY, integrating fetal growth dynamics as a surrogate marker of fetal genotype to guide insulin initiation. However, this strategy remains largely restricted to GCK-MODY and is not extended to other subtypes. Real-world practice shows substantial international variability. In the United Kingdom, care is structured and protocol-driven, with fetal growth-based decision-making embedded in clinical pathways. In the United States, management is highly individualized and specialist-centred, with heterogeneous use of genetic testing and no unified national algorithm. In most European countries, including Poland, pregnancy care relies mainly on general diabetes-in-pregnancy protocols, with limited integration of MODY subtype-specific approaches. **Conclusions:** There is a clear discrepancy between advances in genetic understanding and their translation into clinical guidelines. NICE provides the most applicable framework for GCK-MODY; however, no guideline comprehensively addresses the full spectrum of monogenic diabetes in pregnancy. International harmonisation of genotype-informed pregnancy algorithms is needed. The presentation will be supplemented with clinical cases.

C3.23.P

A familial Xp22.33 duplication encompassing SHOX and its regulatory domain is associated with severe mesomelic and acromelic limb defects

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Słowa kluczowe: SHOX haploinsufficiency, Xp22.33 duplication, short stature, mesomelic and acromelic limb defects, whole exome sequencing, topologically associating domain

Background: SHOX haploinsufficiency is associated with a broad spectrum of skeletal phenotypes, ranging from idiopathic familial short stature and Léri–Weill dyschondrosteosis to the more severe Langer mesomelic dysplasia. In addition to coding variants and deletions involving regulatory elements, functional SHOX deficiency may result from duplications that alter the spatial organization of its regulatory domain and impair long-range enhancer–promoter interactions. Here, we describe a family with short stature and severe bilateral mesomelic and acromelic limb reduction defects associated with a 1.06 Mb Xp22.33 duplication encompassing the SHOX locus. Methods: Whole-exome sequencing (WES) was performed in a female proband, her sister, maternal half-brother, and their mother, all of whom presented with short stature and severe bilateral reduction defects of the upper and lower limbs. Segregation analysis was performed, and the identified variant was evaluated in relation to the SHOX regulatory landscape and the local organization of topologically associating domains. Results: Clinical examination of the affected family members revealed bilateral radial, ulnar, fibular and tibial hypoplasia, stiff elbow and ankle joints, Achilles tendon contractures, and foot deformities. Considerable intrafamilial variability was observed, with the proband showing the most severe phenotype. WES identified an approximately 1.06 Mb copy-number gain at Xp22.33, spanning chrX:284,187–1,345,434 (GRCh38), in all affected family members. The duplication cosegregated with the phenotype and encompassed SHOX together with its upstream and downstream enhancers and the predicted downstream boundary of the SHOX topologically associating domain (SHOX TAD). Conclusions: The severe limb abnormalities observed in the affected family overlap with the phenotypic spectrum of SHOX deficiency syndromes, although their severity exceeds that typically reported. We hypothesize that the duplication causes functional SHOX haploinsufficiency through a position effect involving disruption of local chromatin architecture, altered enhancer–promoter communication, and reduced effective SHOX expression. This proposed mechanism requires validation through functional studies of long-range chromatin interactions.

C3.24.P

From fraction to territory: effects of high-quality sperm selection on chromosome positioning within the human sperm nucleus in men with chromosomal aberrations

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Słowa kluczowe: sperm chromosomes; sperm fractions; reciprocal chromosomal translocations; sperm nucleus; male infertility

Sperm fractionation is routinely utilized in the infertility treatment for in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures. Some of the well-known methods are: swim-up (SU) fractionation or density-gradient centrifugation (DGC). Swim-up technique allows to separate motile fraction of spermatozoa from seminal plasma, while density-gradient centrifugation leads to separation of morphologically normal and motile spermatozoa. The selection of high-quality spermatozoon has a significant impact on ICSI outcomes, similarly as the chromosomal organization of spermatozoa in the initial interaction with the ooplasm, and then expression of the genes critical at the earliest stages of embryogenesis. The aim of this study was to answer the question whether fractionation of spermatozoa using SU or Percoll density gradient (90/47%), can select spermatozoa with enhanced chromatin integrity (protamination, DNA fragmentation) and remodelled chromosome positioning pattern of chromosomes 4/7/8/9/13/18/21/X/Y (fluorescence in situ hybridization (FISH)), for the first time evaluated in 5 carriers of reciprocal chromosomal translocations (RCT), a group of patients characterized by high heterogeneity in spatial nuclear arrangement, distinct from healthy controls with normal karyotype. Sperm fractionation improved selection of spermatozoa with good chromatin protamination and reduced DNA fragmentation in most cases, although the extent of improvement revealed diversity among RCT cases. Chromosome repositioning was observed in all RCT carriers – fractionation partially restored control-like chromosome positioning in selected cases, particularly after SU fractionation, indicating the link between sperm motility and chromosomal context for the first time. These findings denote that sperm selection techniques may improve nuclear organisation in so specific group as RCT carriers; however, the effectiveness of desirable control-like repositioning depends from the geometry of the chromosomal rearrangements. Funding: National Science Centre, Poland 2020/38/E/NZ2/00134 (MO)

C3.25.P

From Copy Number Change to Fully Resolved Pathogenic Allele: Long-Read Sequencing in Structural Variant Characterization

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Słowa kluczowe: TGM1, CNV, long read sequencing, ichthyosis

Structural variants (SVs) remain challenging to resolve using conventional short-read sequencing, particularly when precise breakpoint characterization and determination of rearrangement architecture are required. Long-read sequencing has emerged as a powerful approach for comprehensive analysis of clinically relevant genomic rearrangements. We present the application of Oxford Nanopore long-read whole-genome sequencing for the characterization of a recurrent pathogenic structural variant in the TGM1 gene, one of the most common causes of autosomal recessive congenital ichthyosis (ARCI). Copy number analysis of NGS data identified a recurrent duplication involving TGM1 exons 10–14 in 11 apparently unrelated Polish patients. While CNV analysis indicated the presence of a duplication, its exact structure, genomic location, and breakpoint architecture remained unresolved. To validate and fully characterize the variant, we applied long-read whole-genome sequencing. Breakpoint-spanning reads enabled complete reconstruction of the rearrangement, revealing a 4,010-bp tandem duplication encompassing exons 10–14 and a 13-bp microduplication at the breakpoint. Nucleotide-level breakpoint resolution provided insights into the molecular mechanism underlying the event and established this variant as the first recurrent, fully characterized large genomic rearrangement reported in TGM1. Importantly, breakpoint information generated by long-read sequencing enabled the design of a simple PCR assay for rapid and cost-effective detection of the duplication in routine diagnostics. This study demonstrates how long-read sequencing can transform an initially detected copy number change into a fully resolved pathogenic allele, providing information that is difficult or impossible to obtain using standard short-read approaches. Our findings highlight the value of long-read sequencing for precise structural variant resolution and support its broader implementation in rare disease diagnostics, particularly for CNVs and complex genomic rearrangements requiring breakpoint-level characterization.

C3.26.P

Functional assessment of non-canonical splice-site variants identified during molecular diagnostics of selected skin fragility disorders

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Słowa kluczowe: non-canonical splice-site variants, splicing defects, skin fragility disorders

Non-canonical splice-site variants represent a significant challenge in genomic diagnostics, as their impact on pre-mRNA processing is often difficult to predict using bioinformatic tools alone. During routine molecular diagnostics of inherited skin fragility disorders, we identified several non-canonical splice-site variants in TGM5 and COL7A1, including TGM5 c.1346-7A>G and COL7A1 c.1241-5G>A, c.5124+3A>C, c.85+5G>A, and c.5532+6T>C. Although in silico analyses predicted deleterious effects on splicing, computational predictions alone were insufficient to establish pathogenicity. Functional RNA studies were therefore undertaken to determine the actual transcript-level consequences of three selected variants. Patients were diagnosed with acral peeling skin syndrome (APSS) caused by TGM5 defects or dystrophic epidermolysis bullosa (DEB) associated with COL7A1. Genetic testing was initially performed using Sanger sequencing. Following the identification of variants outside canonical splice junctions, patient-derived keratinocytes and fibroblasts were used for transcript analysis. Functional studies were performed for TGM5 c.1346-7A>G and COL7A1 variants c.1241-5G>A and c.5124+3A>C. RNA analysis demonstrated that c.1241-5G>A activates a cryptic acceptor splice site at c.1258, resulting in a 17-nucleotide deletion within exon 10, a frameshift, and a premature termination codon. The c.5124+3A>C variant caused complete skipping of exon 57 while maintaining the reading frame. As exon 57 lies within the triple-helical collagenous domain of type VII collagen, its deletion is predicted to alter protein structure and function. Functional analysis of the previously unreported TGM5 variant c.1346-7A>G demonstrated inclusion of a six-nucleotide intronic sequence (CTTCAG) into the mature transcript, resulting in the insertion of two amino acids without disruption of the reading frame. These findings demonstrate that non-canonical splice-site variants can lead to diverse pathogenic mechanisms, including cryptic splice-site activation, exon skipping, and partial intron retention. Our study highlights the key role of functional RNA analysis in confirming pathogenicity and improving variant classification, as DNA-based predictions alone are often insufficient, and supports the integration of RNA studies into the diagnostic workflow for selected non-canonical splice-site variants.

C3.27.P

Application of Whole Exome Sequencing (WES) in the “double trouble” diagnosis of a 5-month-old boy: co-occurrence of Pearson syndrome and Xq22.3 region deletion

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Słowa kluczowe: Whole Exome Sequencing, mtDNA deletion, Pearson syndrome, IRS4, Xq22.3, dual diagnosis

Introduction The diagnosis of infants with multiple congenital anomalies and hematological symptoms poses a major clinical challenge. The traditional, targeted molecular approach is sometimes insufficient in patients with a complex phenotype whose symptoms are caused by a dual genetic diagnosis (so-called “double trouble”). **Aim** To present the case of a male patient in whom WES identified two independent genomic events: a pathogenic mtDNA deletion and a large X chromosome microdeletion. **Materials and Methods:** A 5-month-old boy diagnosed with anemia, thrombocytopenia, central congenital hypothyroidism without goiter, and dysmorphism (macroglossia, small ears, upslanting palpebral fissures, umbilical hernia). WES was performed with an extended evaluation of structural variants (CNVs) and an analysis of off-target reads mapping to mtDNA. **Results** WES detected a hemizygous deletion in the Xq22.3(108045131-108736344) region of 691.2 kb, encompassing genes including VSIG1, PSMD10, ATG4A, COL4A6, COL4A5, and IRS4. Furthermore, the analysis of mtDNA off-target reads revealed a decrease in the number of reads suggesting a deletion in the m.11150-14000 region, which confirmed Pearson syndrome. Both variants were independently confirmed by another method in an independent diagnostic center. The endocrine symptoms like congenital hypothyroidism and the resulting macroglossia and umbilical hernia are a direct consequence of the loss of the IRS4 gene. The hematological problems are a consequence of the mtDNA deletion, while the dysmorphic features are associated with the loss of the PSMD10 gene. **Conclusions** This case perfectly illustrates the high analytical value of the integrated assessment of off-target data in standard WES testing. The unambiguous confirmation of the co-occurrence of two independent diseases is of fundamental importance for the correct targeting of the patient's treatment and reliable genetic counseling for the family.

C3.28.P

Challenges in Interpreting Unexpected Genomic Findings in Craniosynostosis: A Case of a Novel EMD Variant

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Słowa kluczowe: Craniosynostosis, Whole-exome sequencing, EMD, Emery–Dreifuss muscular dystrophy, Neuromuscular disorders

Background: Craniosynostosis is a clinically and genetically heterogeneous condition. Although numerous causative genes have been identified, the molecular etiology remains unresolved in many patients. The increasing use of whole-exome sequencing has improved diagnostic yield but may also uncover unexpected variants of uncertain relevance to the presenting phenotype. **Materials and Methods:** A 4-year-old boy with sagittal-frontal craniosynostosis was referred for genetic evaluation. At the age of 23 months, he was noted to have postural abnormalities and gait disturbances, including excessive internal rotation of the hip joints, hyperlordosis, and bilateral forefoot adduction, more pronounced on the left side. During assessment in the genetics clinic, a positive Gowers' sign was noted. Clinical examination revealed short feet, valgus foot positioning with forefoot adduction, and a prominent sandal gap. Whole-exome sequencing was performed, followed by Sanger sequencing for segregation analysis. **Results:** Whole-exome sequencing revealed a novel hemizygous EMD variant, c.326_344del; p.(Glu109Glyfs*7), in a gene associated with Emery–Dreifuss muscular dystrophy. The variant was absent from gnomAD, classified as likely pathogenic according to ACMG criteria (PVS1, PM2), and inherited from the unaffected mother. Comprehensive WES analysis did not identify additional pathogenic variants or phenotype-relevant VUS, including in candidate genes associated with craniosynostosis or neuromuscular disorders. **Conclusions:** This case illustrates the challenges of interpreting unexpected findings identified through exome sequencing in patients with craniosynostosis. Although a novel likely pathogenic EMD variant was identified, its contribution to the craniosynostosis phenotype remains uncertain. The occurrence of variants in genes associated with neuromuscular disorders in additional patients from our craniosynostosis cohort, together with previous reports of craniosynostosis in neuromuscular conditions, raises the possibility that neuromuscular factors may influence cranial suture development. Notably, reduced fetal movement has been proposed as a potential mechanism linking neuromuscular disorders with craniofacial abnormalities, including craniosynostosis. Further studies are required to explore this potential association.

C3.29.P

The frequency and parental origin of recurrent copy-number variations at 15q11.2 in Polish pediatric patients: a single-centre study.

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Słowa kluczowe: 15q11.2 microdeletion, 15q11.2 microduplication, copy number variation, parental origin, pediatric genetics, array comparative genomic hybridization (aCGH)

The chromosomal region 15q11.2, contain four non-imprinted genes: NIPA1, NIPA2, CYFIP1, TUGCP5, is a hot-spot of recurrent copy number variations (CNVs). Deletions and duplications within this region are associated with developmental delay, autism spectrum disorder and dysmorphic features and are also present in nonsyndromic individuals. CNVs in this region are present in 0.5–1.5% of the population, thus constituting the most prevalent genomic alterations in humans. Their interpretation remains challenging due to its equivocal clinical status. The aim of the study was to determine the frequency, clinical significance and inheritance patterns of recurrent CNVs in the 15q11.2 region. The study included pediatric patients and their biological parents who attend the Genetics Clinic at the University Children's Hospital in Krakow between 2015 and 2025. aCGH technique was used as a first-line diagnostic tool. Among 4,221 children examined, 45 deletions (1.0%) and 34 duplications (0.8%) of 15q11.2 were identified. The higher frequency of deletions compared with duplications contrasts with findings reported in previous studies. Nearly all CNVs, except for one case, were inherited from asymptomatic parents. Deletions were inherited predominantly from fathers (31%) rather than mothers (18%), whereas duplications were more often maternally inherited (32%) than paternally inherited (18%). De novo events were rare. Interpretation of inheritance patterns was limited by the fact that almost half of the parents declined genetic testing. Maternal inheritance of

deletions was slightly more frequent in boys, while paternally inherited deletions were more common in girls. Duplications occurred more frequently in boys regardless of parental origin. Additionally, a recurrent nucleotide interval encompassing both deletions and duplications was identified in unrelated patients. The clinical significance of 15q11.2 CNVs still remains unclear. Given the current evidence supporting both pathogenic and benign interpretations, individualized assessment by experienced laboratory diagnosticians and clinical geneticists remains essential.

C4.1.P

When the Gold Standard fails: Overcoming diagnostic challenges in patients with BMFs.

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Słowa kluczowe: Sanger sequencing, BMF, pseudogenes, splicing aberrations

Despite the dominance of Next-Generation Sequencing (NGS), Sanger sequencing remains the gold standard for variant verification and targeted genetic diagnostics. However, genomic complexities such as highly homologous regions, tandem repeats and unpredictable splicing dynamics pose significant diagnostic challenges, requiring customized analytical approaches. To address these challenges, this study develops and applies specialized molecular workflows for highly problematic loci. We specifically address pseudogene-mediated masking in PMS2 and SBDS, alongside elusive splicing aberrations in GF11B and WAS from patients with bone marrow failure (BMFs). We analyzed four distinct clinical cases involving challenging gene loci. For PMS2 and SBDS, a two-stage PCR strategy was developed: an initial Long-Range PCR (LR-PCR) was used to selectively amplify a large genomic segment excluding pseudogene sequences, followed by a secondary, locus-specific PCR targeting the region of interest. To investigate suspected splicing defects in the remaining cases, an integrated approach combining RNA analysis with functional protein validation using flow cytometry was applied. The implementation of a two-stage protocol successfully eliminated pseudogene contamination and allowed for the definitive identification of nucleotide variants that were masked or misinterpreted in standard approaches. RNA analysis of GF11B and WAS combined with functional studies revealed highly atypical transcript profiles with unexpected amplification patterns, obscuring the true nature of the underlying alterations. Moving beyond standard protocols is crucial to avoid diagnostic traps in Sanger sequencing. While LR-PCR effectively isolates functional genes from pseudogenes, resolving complex cDNA profiles requires rigorous trace analysis to accurately characterize splicing aberrations.

C4.2.P

Non-canonical FLT3 variants in acute myeloid leukemia: limitations of standard diagnostic approaches and the value of extended NGS profiling

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Słowa kluczowe: Acute myeloid leukemia (AML), FLT3, next-generation sequencing (NGS), non-canonical FLT3 variants, molecular diagnostics, targeted therapy

Background: FLT3 alterations are among the most clinically relevant molecular abnormalities in acute myeloid leukemia (AML), affecting prognosis and selection for FLT3 inhibitor therapy. Standard diagnostics primarily rely on fragment analysis to detect internal tandem duplications (FLT3-ITD) and selected tyrosine kinase domain (FLT3-TKD) variants. However, next-generation sequencing (NGS) has revealed a broader spectrum of non-canonical FLT3 variants whose clinical significance remains incompletely understood. **Objective:** Characterization of the spectrum of non-canonical FLT3 variants detected in AML using NGS and evaluation of the limitations of conventional FLT3 testing. **Materials and methods:** NGS-based molecular profiling was performed in a cohort of AML patients. Detected FLT3 variants were evaluated according to variant type, domain localization, and detectability by standard diagnostic methods. **Results:** NGS identified multiple FLT3 variants that would not be detected by standard fragment analysis. Most alterations were found in the tyrosine kinase domain, although some non-ITD variants were also detected in the juxtamembrane domain. The detected abnormalities included both single-nucleotide variants and complex insertion/deletion events. Several variants affected regions involved in receptor activation, suggesting a potential role in constitutive FLT3 signaling similar to that of canonical FLT3-ITD and FLT3-TKD variants. These findings indicate that conventional diagnostic algorithms may underestimate the prevalence and diversity of FLT3 alterations in AML. **Conclusions:** NGS enables detection of a wider spectrum of FLT3 variants than standard fragment analysis. Comprehensive FLT3 profiling may improve molecular characterization of AML, support more accurate risk stratification, and contribute to future optimization of targeted therapy selection.

C4.3.P

Therapy related chronic myeloid leukemia in a patient with a history of follicular lymphoma – case report

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Słowa kluczowe: CML, Philadelphia chromosome, variant translocation

Introduction:Secondary hematological malignancies such as MDS and AML developing in patients previously treated with chemotherapy and/or radiotherapy are well documented in the literature. In contrast, therapy-related CML is exceedingly rare and has been reported almost exclusively in the form of individual case reports. **Case presentation:**A 73-year-old woman with a previous diagnosis of FL was admitted to the Department of Hematology. Following completion of the full course of treatment, the patient achieved complete remission and subsequently attended regular follow-up visits. Two years after the end of therapy, a routine PET scan revealed increased metabolic activity within the bone marrow accompanied by mild leukocytosis. Due to the suspicion of CML, a bone marrow sample was referred for cytogenetic and molecular studies. Conventional cytogenetic analysis demonstrated a rare variant Philadelphia translocation, t(9;22;22)(q34;q11;q13), involving both chromosome 22 homologs and resulting in the formation of a single BCR::ABL1 fusion gene located on the derivative chromosome 22. Importantly, the second chromosome 22 homolog was not involved at the BCR locus; the breakpoint was located more distally, most likely within a subtelomeric region. Notably, this was not the only chromosomal abnormality detected. All analyzed metaphases additionally harbored a balanced reciprocal translocation, t(14;16)(q24;p12). Given the possibility that this aberration may represent a constitutional rearrangement, peripheral blood lymphocyte karyotyping was planned as a subsequent diagnostic step. The patient is currently receiving tyrosine kinase inhibitor (TKI) therapy and remains under hematological follow-up. **Conclusion:**To the best of our knowledge, no case of a variant t(9;22) involving both homologous chromosomes 22 has been reported in the literature to date. In the present case, further investigation is required to clarify the nature of the t(14;16)(q24;p12) translocation. If this aberration proves to be acquired rather than constitutional, it may potentially influence the biological behavior of the disease and the response to therapy.

C4.4.P

The RAD51 rs1801321 variant may influence XRCC2 expression and the risk of multiple myeloma but is not associated with chromosomal changes.

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Słowa kluczowe: multiple myeloma; DNA repair system; homologous recombination; genetic variants

Introduction: Multiple myeloma (MM) is a hematological malignancy characterized by genomic instability, which often leads to the development of chromosomal translocations and deletions in the genome of myeloma cells. These abnormalities result from double-stranded DNA breaks. The RAD51 and XRCC2 gene products are homologous recombination proteins that play a key role in double-stranded break repair. **Aim:** In this study, we investigated the role of selected genetic variants in the pathogenesis and progression of MM. We analyzed two RAD51 polymorphisms (rs1801321 – 172G>T and rs1801320 – 135G>C) and one in XRCC2 (rs3218536 – Arg188His), which may influence protein function and repair efficiency. The aim of the study was to assess the association between these variants and chromosomal aberrations, the risk of developing MM, and treatment response. **Patients and Methods:** Genomic DNA extracted from 117 patients with newly diagnosed MM and 187 healthy blood donors was used to analyze RAD51 and XRCC2 variants. Genotypes were determined by real-time PCR. Chromosomal aberrations were analyzed using clg-FISH. **Results:** The T allele of the RAD51 rs1801321 gene was associated with a significantly reduced risk of MM (OR = 0.52, p < 0.001). Nevertheless, RAD51 rs1801320 and XRCC2 rs3218536 were not associated with MM susceptibility. Neither of the variants tested correlated with chromosomal aberrations or overall survival. However, the T allele of the rs1801321 gene and the GG genotype of the RAD51 rs1801320 gene were associated with increased XRCC2 expression. **Conclusions:** Our results suggest that the RAD51 rs1801321 variant may influence the risk of MM and XRCC2 expression. However, the RAD51 rs1801320 and XRCC2 rs3218536 variants do not appear to have a significant impact on chromosomal aberrations, disease progression, or treatment response.

C4.5.P

Single-cell discovery of candidate TCR clonotypes across distinct AML-associated antigens

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Słowa kluczowe: acute myeloid leukaemia; TCR-T cell therapy; single-cell sequencing; antigen priming; HLA-A*02:01

Background: Engineered TCR-T therapy is an attractive complement to CAR-T in acute myeloid leukaemia (AML). TCRs recognise peptides presented on HLA, so they can reach the entire tumour proteome, including intracellular antigens beyond the reach of surface-directed CAR-T - an advantage in AML, where tumour-restricted surface targets are scarce and shared myeloid antigens risk on-target myelotoxicity. The practical bottleneck is the identification of TCR sequences directed at AML-associated antigens, which remains slow and empirical. **Methods:** We developed an ex vivo priming system in which autologous monocyte-derived dendritic cells (Mo-DCs) were pulsed with peptides from four AML-associated antigens (CLL-1/CLEC12A, PRAME, TERT, WT-1) and co-cultured with autologous CD3⁺ T cells for 10 days. Primed cultures were profiled by paired single-cell RNA and V(D)J TCR sequencing (10x Genomics). Material came from HLA-A*02:01⁺ healthy donors and an AML patient. Libraries were processed with Cell Ranger Multi; analysis used Scanpy and Scirpy, with pseudobulk differential expression (PyDESeq2) and GSEA comparing stimulated and control conditions. **Results:** A single workflow jointly resolved the activated T-cell landscape and the antigen-presenting DC compartment from the same cultures. Leiden clustering separated several transcriptional states, including activated cycling T cells, effector-memory subsets, TEMRA, and mature activated DCs. Pseudobulk GSEA was consistent with antigen-driven activation in the candidate clusters. Clonotype analysis nominated TCR clonotypes preferentially enriched under antigen stimulation across the CLL-1, PRAME, and TERT conditions, whereas no comparable enrichment was observed for WT-1. TRAV/TRBV gene usage is reported for the candidate clonotypes. **Conclusion:** Coupling DC-based ex vivo priming with single-cell multi-omics provides a workable route to nominate candidate TCRs across mechanistically distinct AML antigens within a single experiment. The platform is a discovery step prioritising clonotypes for subsequent specificity and functional testing, and may be particularly useful for targets where CAR-T is limited by on-target myelotoxicity. Supported by KPOD.07-07-IW.07-0111/24 grant.

C4.6.P

Germline inv(16)(p13q22) Pseudorearrangement: A Rare Cytogenetic Mimic of AML in a CLL Patient

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Słowa kluczowe: constitutional inv(16), cytogenetic analysis, acute myeloid leukemia, chronic lymphocytic leukemia, pseudorearrangement, CBFB::MYH fusion

In a patient with previously diagnosed chronic lymphocytic leukemia (CLL), progression to acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) was suspected. Cytogenetic evaluation was performed using classical karyotyping and fluorescence in situ hybridization (FISH), including a CBFB::MYH11 dual-fusion probe. Bone marrow karyotyping revealed inv(16)(p13q22) in all analyzed metaphases. Initial FISH analysis appeared to confirm this finding, demonstrating CBFB::MYH11 rearrangement in 100% of cells, which tentatively suggested a diagnosis of CBFB::MYH11-rearranged AML. However, molecular testing for the fusion transcript was negative. Despite this discrepancy, subsequent follow-up bone marrow aspirates consistently demonstrated the inversion in all analyzed cells. Given the complete absence of clinical symptoms and lack of bone marrow blasts, a constitutional karyotype from peripheral blood lymphocytes was performed. This analysis revealed an identical pericentric inversion of chromosome 16 in all metaphases, strongly indicating a constitutional (germline) variant. Furthermore, a verification FISH study using a CBFB break-apart probe definitively excluded CBFB gene rearrangement in both bone marrow and peripheral blood cells. The comprehensive cytogenetic and molecular profile confirmed a diagnosis of constitutional inv(16)(p13q22), which closely mimicked the acquired somatic aberration characteristic of AML but lacked a functional fusion transcript. This case illustrates the critical role of molecular diagnostics in establishing a definitive leukemia diagnosis based on the detection of an active transcript. It highlights the essential importance of integrating complex cytogenetic and molecular methodologies to differentiate constitutional variants from acquired chromosomal abnormalities. Such a comprehensive approach is vital to prevent misdiagnosing AML and initiating unnecessary, toxic treatment. Constitutional inv(16)(p13q22) is an extremely rare, benign germline variant that morphologically mimics the somatic anomaly characteristic of AML, but does not disrupt the CBFB gene or increase cancer risk. While carriers are typically healthy, this inversion is incidentally discovered during hematologic investigations or reproductive counseling.

C5.1.P

Beyond CHIP Drivers: Loss of Y Chromosome and somatic clonal alterations in Alzheimer's Disease

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Słowa kluczowe: Clonal Hematopoiesis (CH), Loss of Y Chromosome (LOY), Retention of Y Chromosome (ROY), Clonal Hematopoiesis of Indeterminate Potential (CHIP), Somatic Mosaicism

Aging is associated with the accumulation of post-zygotic mutations in hematopoietic stem cells (HSCs). When mutated HSCs gain a selective advantage and expand, the process is termed clonal hematopoiesis (CH), or clonal hematopoiesis of indeterminate potential (CHIP) when myeloid malignancy-associated genes are involved. Loss of Y chromosome (LOY) is the most common post-zygotic aberration, occurring in normal hematopoietic cells and exhibiting temporal dynamics. Both LOY and CHIP are age-related events with implications for aging and Alzheimer disease (AD). LOY is linked to increased AD risk, whereas CHIP may be protective, and their co-occurrence remains unclear. We conducted whole-exome sequencing of CD4+ T cells, NK cells, and myeloid cells from 66 AD patients and 52 controls exhibiting LOY or retention of Y chromosome (ROY). We identified 39 variants in known myeloid and lymphoid driver genes, with up to 35% co-occurring with LOY in the same clone. In addition, we detected 192 unknown drivers of clonal hematopoiesis (UD-CH), enriched in AD-LOY individuals (odds ratio 4.8, $P = 0.041$). In myeloid cells, total driver burden correlated with LOY ($\rho = 0.52$, $P = 0.00041$). These results indicate that LOY is a primary driver of clonal hematopoiesis in AD, seeding myeloid clones that accumulate unknown driver variants, whereas most canonical CHIP mutations arise independently. The lack of gene-level recurrence, particularly among UD-CHs, highlights the heterogeneous nature of CH and suggests that rare, possibly individual-specific, mutations may contribute to clonal expansion. However, the functional relevance of many detected variants remains uncertain. Distinguishing true drivers from passengers remains a major challenge and requires larger cohorts and extensive functional validation.

C5.2.P

When ALSP mimics multiple sclerosis: a novel CSF1R c.2549C>T variant expanding the genotype–phenotype spectrum

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Słowa kluczowe: ALSP, leukoencephalopathy, MS mimic, CSF1R, novel variant, microglia

AbstractBackground: Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rapidly progressive white matter disorder caused by pathogenic variants in the CSF1R gene. CSF1R plays a critical role in the development, maintenance, and survival of microglia. Loss of functional CSF1R leads to a near-complete depletion of microglia in the brain, highlighting its fundamental importance for microglial homeostasis within the central nervous system. Despite increasing awareness, hereditary leukoencephalopathies remain diagnostically challenging. Case Presentation: We present the case of a 51-year-old male with rapidly progressive neurological deterioration, cognitive decline, gait impairment, and multifocal white matter lesions on brain MRI. The patient initially fulfilled diagnostic criteria for multiple sclerosis and was treated with disease-modifying therapy without clinical benefit, followed by further neurological progression. Subsequent neuroimaging demonstrated enlargement of white matter lesions without characteristic inflammatory activity. Results: Genetic evaluation performed due to the aggressive course of MS-like demyelinating disease, revealed a previously unreported variant in the CSF1R gene (c.2549C>T; p.Ser850Leu), located within the critical tyrosine kinase domain of the receptor. The variant was absent from available population databases and affected a highly conserved region of the protein. Bioinformatic analyses combined with genotype–phenotype correlation supported the pathogenic effect of the variant, which was classified as likely pathogenic. Conclusion: This case highlights the importance of considering ALSP in the differential diagnosis of atypical demyelinating disorders, particularly in patients presenting with progressive neurological decline and inconclusive features of multiple sclerosis. Identification and interpretation of novel CSF1R variants remain crucial in rapidly progressive neurological diseases and may significantly improve diagnostic accuracy in patients with atypical white matter pathology.

C5.3.P

Assessment of the incidence of selected microdeletions in patients diagnosed with schizophrenia

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Słowa kluczowe: CNV, schizophrenia, genetic testing, 22q11.2

Schizophrenia is a psychiatric disorder with a multifactorial etiology involving both genetic and environmental factors. Genetic alterations, including copy number variations (CNVs), have been widely implicated in neurodevelopmental disorders, including schizophrenia. The aim of this study was to analyze the incidence of selected CNVs in patients diagnosed with schizophrenia. The study focused on regions within chromosome 22q11.2 previously described as important for neurodevelopment and synaptic regulation, particularly those implicated in increased susceptibility to schizophrenia. Genomic DNA isolated from peripheral blood leukocytes of 69 patients with schizophrenia and 49 healthy controls was analyzed using multiplex ligation-dependent probe amplification (MLPA) with the SALSA® MLPA® Probemix P-250-B2 DiGeorge kit. Microaberrations covering the TOP3B gene on chromosome 22 were identified in two patients (one deletion and one duplication) and controls (three duplications). No other alterations were found. These findings suggest that variation in this region may represent a common polymorphism without clinical significance. This interpretation is supported by the high polymorphic variability of the genomic region encompassing the TOP3B. Further validation using techniques such as MLPA or array comparative genomic hybridization (aCGH) is warranted to confirm these findings, particularly given that CNVs within highly polymorphic genomic regions should be interpreted with caution.

C5.4.P

Integrative Genotype–Phenotype Analysis in Patients with Cardiomyopathy Based on Next-Generation Sequencing Data

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Słowa kluczowe: NGS, cardiomyopathy

Background: Cardiomyopathies represent a heterogeneous group of disorders with a significant genetic component. Although routine genetic testing is primarily focused on the identification and classification of pathogenic variants, broader interrogation of sequencing datasets may provide additional insights into the genetic basis of disease heterogeneity and clinical variability. **Aim:** To investigate genotype–phenotype relationships and explore genetic patterns associated with cardiomyopathy using an integrated analysis of next-generation sequencing (NGS) data and clinical characteristics. **Methods:** The study included 100 patients with clinically confirmed cardiomyopathy who underwent NGS-based panel testing of genes associated with inherited cardiovascular diseases. In addition to standard variant interpretation, extended analyses of sequencing data were performed, including assessment of cumulative genetic variation, gene-level analyses, and evaluation of associations between genetic findings and clinical features. Statistical genetics methods and exploratory data analysis techniques were applied to identify patterns of genetic variation within the cohort and to investigate relationships that may not be captured by conventional variant-focused approaches. Clinical and demographic variables were incorporated into the analyses to enable multidimensional characterization of the study population. **Results:** The applied analytical framework enabled the identification of genetic patterns extending beyond routinely reported diagnostic findings and revealed potential associations between genetic profiles and clinical characteristics. The analyses highlighted the value of integrating sequencing data with phenotypic information for the investigation of disease heterogeneity. **Conclusions:** Comprehensive analysis of NGS datasets using statistical genetics and data-driven approaches may provide additional insights into the genetic architecture of cardiomyopathies. Integration of molecular and clinical data has the potential to complement conventional diagnostic workflows and improve the understanding of genotype–phenotype relationships in inherited cardiovascular disease.

C5.5.P

Pathogenic variants in genes encoding proteins of the RAS/MAPK and PI3K/AKT/mTOR pathways as a cause of vascular malformations.

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Słowa kluczowe: vascular malformations, hypertrophy, PIK3CA, RASA1

Introduction: Vascular malformations are congenital abnormalities of blood and/or lymphatic vessel development resulting from disturbances in vascular embryogenesis. They are characterized by abnormal vessel structure and organization. Somatic or germline mutations in genes regulating angiogenesis and cellular signalling pathways, particularly the PI3K/AKT/mTOR and RAS/MAPK pathways, play an important role in their pathogenesis. The most commonly identified variants involve the PIK3CA, TEK (TIE2), RASA1, EPHB4, and GNAQ genes, leading to uncontrolled growth and abnormal differentiation of endothelial cells. **Aim:** The aim of the study was to identify the molecular basis of vascular malformations in a group of patients with vascular malformations. **Methods:** Thirty-two patients were included in the study and their DNA was extracted from peripheral blood or affected tissue. The KAPA Hyper Choice panel (Roche) was used to enrich patients' DNA for exonic sequences of a gene panel developed at the Department of Medical Genetics, Institute of Mother and Child. The resulting DNA libraries were sequenced using next-generation sequencing (NGS) on the NovaSeq 6000 platform (Illumina). Variant analysis was performed using the IMiD data processing pipeline, based on the human genome reference version GRCh38/hg38. **Results:** Twelve (12) pathogenic or likely pathogenic missense variants were identified, particularly in the PIK3CA gene (10). Single germline pathogenic variants in RASA1 and GNA11 genes have been detected in 2 patients. In remaining 20 patients, no variants associated with the vascular malformation pathogenesis were detected. **Conclusion:** Targeted next generation sequencing enables effective identification of genetic changes underlying vascular malformations and allows for making an appropriate clinical diagnosis.

C5.6.P

Role of genetic testing in cardiomyopathy.

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Słowa kluczowe: cardiomyopathies, genetic testing, NGS

Introduction: Cardiomyopathies are structural and functional disorders of the heart muscle. They cause mechanical or electrical dysfunction and are often genetic. Heart becomes enlarged, thick, or rigid. This remodeling causes the heart to contract and pump blood improperly, which can ultimately lead to heart failure or dangerous arrhythmias. The use of next-generation sequencing (NGS) has significantly improved diagnostic performance in cohorts of cardiac patients. Next-generation sequencing was used to simultaneously test for both sequence and exon-level copy number variants. Results: Whole exome sequencing (WES) analysis was performed in 62 patients with a suspected genetic cardiomyopathy. A positive result (molecular diagnosis) was confirmed in 19 of 62 patients. Age at genetic testing was 45 ($\pm$ 13 years). A pathogenic/likely pathogenic heterozygous variant, including 19 different variants of which 5 novel variants previously not reported in ClinVar or in The Human Gene Mutation Database (HGMD) were detected in 31% of total. These included variants within the MYBPC3, DSP, TTN, MYH7, FHOD3, RYR2, PKP2, TBX5, PRDM16, TNNI3K, FKTN genes. Within the full cohort, 34% of patients had 1 or more variant of uncertain significance in the absence of any pathogenic/likely pathogenic variant within tested genes. It highlights the need to resolve variants of uncertain significance (VUS). In these cases, clinical assessment and assessment of variants of uncertain significance in family members can promote definitive classification by clarifying the variant's association with disease. **Conclusions:** Genetic testing is a valuable tool for managing cardiomyopathy in patients and families, including hypertrophic, dilated, and arrhythmogenic cardiomyopathies. It provides a foundation for precise and individualized management. In addition, comprehensive testing provided diagnostic and prognostic information that could have potentially changed management and monitoring strategies for patients. By distinguishing phenotypic subgroups, identifying disease mechanisms, and gene-based diagnosis improve management. Also our results suggest that comprehensive testing reveals crucial prognostic information despite a higher rate of VUS.

C5.7.P

Metabolic signature of prevalent cardiovascular diseases

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Słowa kluczowe: Cardiovascular diseases; Hypertension; Linoleic acid; Omega-6 fatty acids; Metabolomics; Mendelian randomization

Purpose: We aimed to define metabolic signatures of 74 prevalent cardiovascular diseases (CVDs) by assessing associations and potential disease-to-metabolite effects on 25 metabolites.

Methods: Cross-sectional analyses used UK Biobank data (n=231,053). General linear models (GLMs) related 25 NMR-quantified metabolites to 74 ICD-10-defined prevalent CVDs, adjusting for age, sex and BMI; sensitivity analyses adjusted for smoking status and alcohol intake and/or excluded self-reported cases. Five externally trained PRSs were tested with adjustment for age, sex, genotyping chip and genetic ancestry. Two-sample Mendelian randomization (MR) used independent GWAS instruments for selected CVDs and blood pressure traits, with estimates obtained using inverse-variance-weighted (IVW), weighted-median and MR-Egger methods. GLM Bonferroni threshold was $p < 0.05 / (74 \times 25)$. Published MR estimates from a UK Biobank NMR metabolome–phenome atlas (You et al., Nat Metab 2025;7:2366–2384) were used to assess genetically proxied linoleic acid (LA) and total omega-6 fatty acids against ICD-10 cardiovascular phenotypes.

Results: GLMs identified eight CVDs with prevalence $> 0.1\%$, each associated with > 12 metabolites after Bonferroni correction. Most associations indicated lower fatty-acid levels, with the strongest reductions for LA in myocardial infarction and coronary artery disease. PRS analyses supported an inverse association between hypertension (HTN) liability and omega-6/LA levels ($p < 2 \times 10^{-6}$), which were highly correlated ($R = 0.95$). In IVW MR, HTN ($B = -0.07$ SD/ $\log[OR]$, $SE = 0.01$, $p = 6.5 \times 10^{-7}$) and coronary artery disease (CAD; $B = -0.08$ SD/ $\log[OR]$, $SE = 0.01$, $p = 6.7 \times 10^{-10}$) were associated with lower LA. Similar results were observed for omega-6 fatty acids and when systolic and diastolic blood pressure were modelled as continuous exposures. Atlas estimates linked genetically proxied LA/omega-6 fatty acids to higher odds of major coronary heart disease events and myocardial infarction and lower odds of non-ischaemic cardiomyopathy.

Conclusions: Circulating omega-6 fatty acids, particularly LA, are lower in prevalent CVD and appear to be modulated by HTN and CAD. These findings may inform diagnostic strategies and research on CVD progression.

C5.8.P

Regulatory QTL annotation of genetic variants associated with drug-induced QT prolongation

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Słowa kluczowe: drug-induced QT prolongation; pharmacogenomics; DNA methylation; quantitative trait locus; drug safety; regulatory genomics

Introduction: Drug-induced QT prolongation (diQTP) is a relevant drug safety problem and may predispose patients to torsade de pointes and sudden cardiac death. Previous pharmacogenomic studies have reported numerous genetic variants associated with diQTP. However, the biological relevance of many reported variants remains uncertain. Quantitative trait locus (QTL) annotation may help address this gap by linking genetic variants to molecular traits such as variation in DNA methylation, gene expression, and splicing. This study annotates diQTP-associated genetic variants using regulatory QTL resources. **Methods:** We included 129 common diQTP-associated variants with rsID annotation, selected from a systematic review. The variants were queried against eight whole-blood methylation QTL resources (Aberdeen, ARIES, GoDMC, iPSYCH, LBCBSGS, UCL, UKHLS, GenI), as well as expression and splicing QTL datasets from whole blood and heart tissues (GTEx Consortium). We applied source-specific significance thresholds as recommended by each QTL resource to report significant association between diQTP risk variants and downstream epigenetic and gene expression changes. **Results:** Among 129 common diQTP-associated variants, 115 had at least one QTL association, mainly mQTLs (111 variants), followed by eQTLs (64) and sQTLs (14). Cardiac GTEx signals included eQTL associations for 22 variants and sQTL associations for 8 variants in both atrial and ventricular tissue. Genes with multiple QTL-associated variants included several cardiac ion-channel genes, such as KCNQ1, KCNH2, SCN5A, and CACNA1C. **Conclusion:** Common diQTP-associated variants showed regulatory signals in blood and cardiac tissues. These signals may help prioritize variants for functional studies of cardiac repolarization.

C5.9.P

Surprising and unsurprising genetic findings in patients with restrictive and hypertrophic cardiomyopathy

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Słowa kluczowe: restrictive cardiomyopathy, hypertrophic cardiomyopathy

Background: Restrictive cardiomyopathy (RCM) is the rarest form of cardiomyopathy, and its genetic background remains poorly defined. In Europe, light chain amyloidosis (AL) is the most common cause of RCM. Purpose: To evaluate the genetic spectrum of RCM and hypertrophic cardiomyopathy with restrictive pathophysiology (rHCM).

Methods: Thirty-nine unrelated patients (median age 58 years; 18 males) were enrolled. Patients with AL or monoclonal gammopathy of unknown significance (MGUS) were not excluded from genetic testing if they had a positive family history of sudden cardiac death or cardiac imaging that suggested co-existing amyloidosis and hypertrophic cardiomyopathy. Patients with transthyretin amyloidosis were not enrolled in the study. Next-generation sequencing (NGS) has been performed in 21 individuals, including 20 using a 135-gene custom cardio panel and one by whole-exome sequencing. Variant interpretation followed American College of Medical Genetics and Genomics (ACMG) guidelines.

Results: Diagnoses included AL (n=16), MGUS with rHCM (n=6), rHCM (n=10), and non-amyloid RCM (n=7). Pathogenic or likely pathogenic variants were identified in 8 patients (38%). Unsurprisingly most variants affected sarcomeric genes, including MYBPC3, MYH7,

TNNC1, and FLNC. Three patients carried multiple pathogenic variants (MYH7 combined with TTN truncating variant, two variants in CAPN3 in a patient with limb-girdle muscular dystrophy and rHCM, or biallelic TNNC1 in early onset RCM female patient). Two patients with MYBPC3 mutations also had MGUS. A novel likely pathogenic truncating variant in FHL1 was identified in a patient with non-amyloid RCM, in the absence of prior clinical manifestations or a documented diagnosis of neuromuscular disease. Additionally, two pathogenic CAPN3 variants were found in a patient with limb-girdle muscular dystrophy and rHCM.

Conclusions: These findings underscore the clinical utility of genetic testing in RCM and rHCM, including in patients with acquired conditions such as AL or MGUS, highlighting the coexistence of inherited and acquired mechanisms influencing disease phenotype.

C5.10.P

Impact of TERT Gene Polymorphisms on Metabolic Profile and Neuropathy Risk in Patients with Type 2 Diabetes

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Słowa kluczowe: type 2 diabetes, TERT polymorphism, diabetic neuropathy

Background: Type 2 diabetes (T2D) is a highly prevalent metabolic disorder frequently accompanied by chronic complications, highlighting the need to identify genetic factors contributing to its clinical heterogeneity. The telomerase reverse transcriptase (TERT) gene is primarily involved in telomere maintenance; however, emerging evidence suggests that it may also influence metabolic regulation and oxidative stress through telomere-independent mechanisms. **Aim:** The present study investigated whether selected TERT promoter variants (rs2735940, rs7712562, rs2853669) are associated with relative telomere length (RTL), metabolic parameters, and the occurrence of diabetic complications in individuals with T2D.

Methods: The study cohort included 147 patients with T2D. Genomic DNA was extracted from whole blood, and genotyping was performed using TaqMan™ SNP Genotyping Assays. RTL was determined by qPCR using the standard curve quantitation method. Statistical analyses, including ANCOVA, independent t-tests, and binomial logistic regression, were conducted.

Results: No significant association was observed between the studied SNPs and RTL. However, specific TERT variants were significantly associated with the metabolic clinical phenotype. Certain genotypes were linked to unfavorable lipid profiles and altered anthropometric markers. Notably, multivariate logistic regression analysis identified a specific TERT polymorphism as a significant independent predictor of neuropathy risk, even after accounting for various clinical covariates.

Conclusion: The findings indicate that TERT promoter polymorphisms may contribute to the variability of the T2D phenotype, particularly with respect to lipid metabolism and susceptibility to neuropathy. These findings suggest that TERT gene variations may modulate the risk of diabetic complications through mechanisms beyond telomere shortening. Further studies are warranted to clarify the underlying biological mechanisms and to assess the potential clinical utility of these variants in personalized diabetes management. This study was funded from the budget allocated for statutory research at the Institute of Rural Health in Lublin (No. 25040).

C5.11.P

Ehlers–Danlos Syndrome: Identification of the Genetic Cause of Clinical Manifestations in Patients with Negative Targeted Gene Panel Sequencing Findings

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AbstractIntroduction: Ehlers–Danlos syndrome (EDS) comprises a heterogeneous group of inherited connective tissue disorders. In a proportion of EDS patients, the underlying molecular cause remains unidentified despite targeted gene panel sequencing. This study aimed to investigate the genetic basis of EDS features in patients with negative results of targeted sequencing and test other molecular methods and bioinformatic approaches in increasing diagnostic yield.

Materials and Methods: The study included 120 EDS patients and negative targeted sequencing results. First, Ximmer and FixItFelix tools were used to detect copy number variants (CNV) and pathogenic variants in CBS and TNXB. In six patients with a single variant in PLOD1 MLPA analysis was performed. Fifteen patients with a single TNXB variant underwent MLPA analysis and Sanger sequencing of exons 35–44. In addition, whole-exome sequencing (WES) was performed in 10 patients. Results: Two patients were found to carry pathogenic duplication involving exons 10–16 of PLOD1. In one patient, reanalysis using FixItFelix enabled the detection of two CBS variants in trans: c.1105C>T (p.Arg369Cys) and c.832_833insCTGG GGTGGATCATCCAGGTGGGGCTTTTCTGGGCTTGAGCCCTGAAGCCGCGCCCTCTGCAGATCA (p.Ile278ThrfsTer16). In another patient, MLPA revealed a duplication of exon 35 of TNXB, co-occurring with a previously identified sequence variant. This duplication was not detected by CNV prediction tools due to the presence of a TNXB pseudogene. In two patients with a single TNXB variant, Sanger sequencing revealed the presence of a second variant, c.12547G>A, p.Ala4183Thr (classified as a VUS). Exome sequencing identified variants in two further patients, particularly a duplication involving B3GALT6 gene in one individual and c.1045G>T (p.Val349Phe) in COL1A1 and c.2818G>A (p.Val940Met) in ADAMTS2 in the other. Conclusions: The use of complementary diagnostic approaches, including CNV analysis, MLPA, targeted Sanger sequencing, WES and advanced bioinformatic tools, improves the detection of genetic alterations in EDS patients with negative results of targeted NGS.

C5.12.P

Genotype–phenotype profiling in dentinogenesis imperfecta type II: clinical and molecular study.

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Słowa kluczowe: DSPP, dentinogenesis imperfecta, genotype–phenotype correlation, hereditary dentin disorders, molecular diagnostics, genetic counseling

Dentinogenesis imperfecta type II (DGI-II) is a rare autosomal dominant disorder of dentin formation, most commonly associated with pathogenic variants in the DSPP gene. Clinically, it is characterized by opalescent discoloration of the teeth, accelerated tooth wear, and obliteration of the pulp chambers and root canals. Although the molecular background of DGI-II is increasingly recognized, the spectrum of DSPP variants and their relationship with clinical presentation remain insufficiently characterized, particularly in Polish patients. The aim of this project is to perform a detailed genotype-phenotype analysis in a multigenerational family with a marked accumulation of DGI-II cases. Molecular testing will include sequencing of the DSPP gene in affected individuals and, when feasible, in presymptomatic children from affected families. Genomic DNA will be isolated from peripheral blood samples, and identified variants will be interpreted using available population databases and diagnostic resources. Clinical findings will be correlated with molecular data in order to assess whether the type of DSPP alteration influences disease severity and presentation. This study is expected to clarify the genetic basis of DGI-II in the investigated family, expand current knowledge on DSPP variant diversity in the European population, and provide novel data on genotype-phenotype correlations. The results may support earlier diagnosis, improve clinical management, and strengthen genetic counseling for families affected by DGI-II. In a broader perspective, the project may contribute to the development of more effective diagnostic strategies for hereditary dentin disorders.

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C5.13.P

PLA2G6-associated neurodegeneration (PLAN): analysis of potential pathogenetic pathways

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Słowa kluczowe: NBIA; pathogenesis; PLA2G6; iron; lipid metabolism; autophagy

PLA2G6-associated neurodegeneration (PLAN) is a rare, progressive neurodegenerative disorder belonging to the group of neurodegeneration with brain iron accumulation (NBIA) disorders and is associated with pathogenic variants in the PLA2G6 gene. Although PLAN is one of the most common forms of NBIA, the molecular mechanisms underlying disease development remain poorly understood, and the sequence of events leading to neurodegeneration has not yet been clearly established. The PLA2G6 gene encodes calcium-independent phospholipase A2, an enzyme involved in membrane phospholipid remodeling and the maintenance of the structural integrity of cellular and organellar membranes. Therefore, PLAN may be regarded not only as a disorder characterized by brain iron accumulation but also as a model of disrupted lipid homeostasis, in which dysfunction of multiple interconnected cellular processes occurs as a secondary consequence. The aim of this study is to comprehensively investigate molecular abnormalities in PLAN models by analyzing processes related to lipid metabolism, oxidative stress, ferroptosis, autophagy, and iron homeostasis. Particular emphasis is placed on determining whether these abnormalities constitute a common pathogenic cascade linking membrane dysfunction to enhanced oxidative damage, impaired cellular degradation, and disturbances in iron homeostasis. Preliminary results indicate that the cellular phenotype of PLAN is multidimensional and involves alterations in pathways responsible for membrane lipid homeostasis, regulation of oxidative stress, and the proper functioning of the autophagy–lysosomal system. The presence of abnormalities associated with markers of ferroptosis and iron homeostasis suggests that iron accumulation may represent part of a broader cellular response to primary defects in lipid metabolism rather than the sole initiating factor of neurodegeneration. These findings support the concept of PLAN as a disorder with a complex molecular basis, in which disturbances in lipid metabolism, oxidative stress, cellular degradation, and iron homeostasis are closely interconnected. This perspective may facilitate the identification of novel therapeutic targets that extend beyond strategies aimed solely at reducing iron accumulation.

C5.14.P

Iron Accumulation in NBIA: Cause or Consequence? The Role of Ferroptosis and VLCFA Metabolism.

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Słowa kluczowe: NBIA, ferroptosis, iron, peroxisomes, VLCFA

Neurodegeneration with brain iron accumulation (NBIA) comprises a group of rare neurodegenerative disorders characterized by excessive iron deposition in the basal ganglia. Although iron accumulation is a hallmark of NBIA, it remains unclear whether it represents a primary cause of neurodegeneration or rather a secondary consequence of other cellular abnormalities. Increasing evidence suggests that disturbances in lipid metabolism, oxidative stress, mitochondrial and peroxisomal dysfunction, and impaired inter-organelle communication may contribute significantly to NBIA pathogenesis. In the present study, alterations associated with ferroptosis and the metabolism of very-long-chain fatty acids (VLCFAs) were investigated in fibroblasts derived from patients with NBIA. Markers related to lipid peroxidation, peroxisomal function, VLCFA metabolism, and the cellular response to iron-related stress were analysed. Preliminary results indicate the presence of abnormalities in pathways associated with VLCFA and iron metabolism. The findings support the hypothesis that iron accumulation in NBIA is not the primary factor driving neurodegeneration, but may instead represent one component of a broader pathological cascade involving disrupted lipid metabolism, oxidative stress, and other cellular processes. A better understanding of the roles of ferroptosis and VLCFA metabolism may contribute to the identification of novel therapeutic targets aimed at protecting membrane lipids, supporting peroxisomal function, and reducing lipid peroxidation in NBIA.

C5.15.P

When Genotype Fails to Predict Phenotype: A Neurologically Unaffected Adult Male with a Pathogenic MECP2 Variant.

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Słowa kluczowe: MECP2, phenotypic resilience, genotype–phenotype discordance, neurodevelopmental disorders

Background: Pathogenic variants in MECP2 are a well-established cause of Rett syndrome and related neurodevelopmental disorders. In hemizygous males, disease-causing MECP2 variants are typically associated with severe neurological phenotypes, including neonatal encephalopathy, intellectual disability, epilepsy, and progressive neurodevelopmental impairment. The identification of a pathogenic MECP2 variant in a neurologically normal adult male is therefore unexpected and may provide insights into mechanisms underlying phenotypic resilience. **Case Presentation:** During preconception genetic screening, whole-exome sequencing (WES) with copy number variant (CNV) analysis identified the pathogenic MECP2 variant NM_004992.4.1155_1200del (p.Leu386AlafsTer8; rs267608329) in a 35-year-old male. The finding was independently confirmed by Sanger sequencing using a newly collected sample. The individual had normal cognitive and neurological development, completed education, and reported no history of developmental delay, intellectual disability, epilepsy, psychiatric disease, or other neurological manifestations. CNV analysis revealed no clinically significant genomic imbalances.

Discussion: The absence of an expected phenotype despite a known pathogenic MECP2 variant highlights the complexity of genotype–phenotype correlations. Tissue-specific mosaicism remains a plausible explanation, as the variant was identified in peripheral blood and its distribution in ectoderm-derived tissues has not been assessed. Evaluation of mosaicism and maternal segregation analysis is planned. Alternatively, protective genetic modifiers within epigenetic or neurodevelopmental regulatory networks may attenuate the pathogenic effects of MECP2 dysfunction. Evidence from other monogenic disorders, including cystic fibrosis, spinal muscular atrophy, inherited cardiomyopathies, and hereditary neuropathies, demonstrates that genetic modifiers can substantially influence disease severity and, in some cases, confer marked phenotypic resilience. Although variable expressivity and mosaicism have been described in MECP2-related conditions, fully asymptomatic adult males with a confirmed pathogenic hemizygous variant have not been convincingly reported.

Conclusions: This case expands the phenotypic spectrum associated with pathogenic MECP2 variants and underscores the importance of considering both mosaicism and genetic modifiers in variant interpretation. Further studies may elucidate mechanisms buffering MECP2 dysfunction and improve understanding of genetic resilience in neurodevelopmental disease.

C5.16.P

May the presence of GBA1 gene variants influence on the Parkinson's disease treatment with deep brain stimulation?

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Słowa kluczowe: GBA1, Parkinson disease, deep brain stimulation

Title: May the presence of GBA1 gene variants influence on the Parkinson's disease treatment with deep brain stimulation?

Background: Deep brain stimulation to subthalamic nucleus (DBS-STN) is one of the treatments for advanced Parkinson's disease. Variants in the GBA1 gene, encoding lysosomal enzyme glucocerebrosidase (GCase), represent the most common genetic risk factor for PD. Despite achieving all clinical criteria for the surgical treatment, not all patients respond to DBS-STN. The aim of this study was to determine frequency of GBA1 variants in DBS-STN patients and correlates them with treatment response.

Methods: The study included 91 patients diagnosed with PD qualified according CAPSIT-PD criteria and treated with DBS-STN. All were tested for the presence of GBA1 point variants (Sanger sequencing) and rearrangements (MLPA). The GBA1 locus exhibits complex genomic architecture because of pseudogene GBAP1. High sequence homology between them predisposes to gene conversion/rearrangements complicating molecular analysis, so the development of specific analysis strategy was necessary to avoid this. Results: Pathogenic GBA1 variants were identified in 13,8% of the patients. In the majority of cases SNVs were identified, and the rearrangements, excluded. Additionally in couple cases, the evidence suggesting GBA1–GBAP1 conversion we observed. Preliminary clinical data on the response to DBS indicate a variable pattern of treatment outcomes

Conclusion: The prevalence of GBA1 variants in the studied cohort of PD patients confirms the significant role of this gene as a major risk factor for the disease. The relationship between the status of GBA1 variant's carrier and the response to DBS requires further analysis and may be an important part of the stratification of patients eligible for this treatment.

C6.1.P

Prediction and detection of off-target mutations caused by CRISPR/Cas9 base editors

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Słowa kluczowe: Base editors, off-target mutations, gene editing, off-target prediction

CRISPR/Cas9 base editors enable precise nucleotide conversions without introducing double-strand DNA breaks, providing a powerful approach for correcting pathogenic variants. Their therapeutic potential has led to rapid development of treatments for inherited conditions and cancers, with several base editing based therapies already entering clinical trials. However, unintended off-target mutations remain insufficiently characterized and may carry serious consequences, including disruption of tumor suppressors or activation of oncogenes. Because unbiased genome-wide detection approaches are technically demanding and difficult to scale, regulatory agencies recommend the use of multiple orthogonal prediction methods to maximize sensitivity and ensure comprehensive assessment of genome editing specificity. Consequently, accurate prediction of candidate off-target loci represents a critical first step in safety evaluation. To maximize off-target site discovery, we combined three complementary prediction approaches: an in silico workflow integrating Cas-OFFinder and BEDeepoff, the in vitro Nano-OTS method, and the hybrid CRISPRroots approach, which incorporates transcriptomic data from edited cells to refine computational predictions. Together, these methods identified more than 6,000 putative off-target sites. To efficiently interrogate such a large number of loci, we employed adaptive sampling using nanopore sequencing technology. This targeted enrichment strategy enabled the detection of 25 verified off-target mutations, predominantly occurring at sites with four to five mismatches relative to the guide RNA sequence. Several off-target events were observed at frequencies comparable to on-target editing efficiency. Notably, one mutation was identified within an intronic region of the RNF43 proto-oncogene, although no alterations in gene expression were detected. As base editing technologies advance toward clinical implementation, comprehensive characterization of off-target mutagenesis is essential to ensure therapeutic safety. Our results demonstrate that clinically relevant off-target events can occur at frequencies comparable to on-target editing and underscore the need for integrated prediction and detection workflows tailored specifically to base editors.

C6.2.P

Temporal miRNA dynamics and functional characterization of miR-92b-3p during human airway epithelial differentiation

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Słowa kluczowe: airway epithelial differentiation, multiciliated cells, miRNA-92b-3p, miRNA-mRNA regulatory network, post-transcriptional regulation

Human airway epithelium (AE) differentiation is a multistep process, whose transcriptional and post-transcriptional regulation is not fully understood. Recent studies identified members of the miRNA-34/449 family as key regulators of multiciliated cell (MCC) differentiation. However, the temporal dynamics of miRNA expression and the mechanisms by which they influence MCC differentiation remain unclear. This study aimed to characterize miRNA expression profiles and miRNA-mRNA regulatory networks during the in vitro differentiation of human AE cells. Primary human AE cells from healthy donors were differentiated in air-liquid interface (ALI) cultures and collected at various stages (days 0, 3, 6, 12, and 21). Expression profiles of miRNAs were identified using small RNA sequencing and integrated with previously generated, matched RNA-seq datasets. Putative miRNA targets were predicted in silico and verified in HEK293 cells using dual luciferase reporter assays. The functional role of identified miRNAs was investigated through lentiviral miRNA silencing (miRZip) in AE cultures. The most strongly altered miRNAs during AE differentiation included members of the known miR-34/449 family as well as hsa-miR-92b-3p, which has not previously been studied in the context of AE differentiation. We confirmed that AURKA, DKK3, and CDKN1C are targets of hsa-miR-92b-3p. Silencing of hsa-miR-92b-3p in AE cultures did not stop AE differentiation in vitro. Further analyses of AE differentiation at transcript and protein levels is ongoing. In conclusion, this study delineates the temporal dynamics of miRNAs during human AE differentiation. Identification of hsa-miR-92b-3p targets provides new insights into the regulatory networks underlying AE development. The established lentiviral modulation platform provides a robust foundation for further dissecting the complex miRNA-dependent mechanisms that govern AE homeostasis and differentiation.

C6.3.P

Blood transcriptome profiling in endometriosis reveals immune escape, inflammatory activation, and mitochondrial dysfunction

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Słowa kluczowe: endometriosis, transcriptomics, gene expression, immune regulation, mitochondrial dysfunction

Background: Endometriosis is a chronic estrogen-dependent inflammatory disease affecting 6–15% of women of reproductive age and is a major cause of pelvic pain and infertility. Despite its high prevalence, the molecular mechanisms promoting ectopic endometrial cell survival, immune evasion, and lesion persistence remain incompletely understood. Reliable non-invasive biomarkers are also lacking. This study aimed to characterize peripheral blood transcriptomic alterations in women with endometriosis and to identify dysregulated biological pathways potentially involved in disease pathogenesis. **Methods:** Peripheral blood samples were collected from women with surgically and histologically confirmed endometriosis (n=24) and endometriosis-free controls (n=24). Genome-wide gene expression profiling was performed using Agilent SurePrint G3 Human Gene Expression 8×60K v2 microarrays. Differentially expressed genes were identified using standard bioinformatic pipelines. Functional enrichment analyses were performed using Gene Ontology, KEGG, and Reactome databases. **Results:** Up-regulated genes were enriched in pathways associated with chronic inflammation, including CCL13 and IL17A, angiogenesis, including VEGF and FGF19, extracellular matrix remodeling and tissue invasion, including MMPs and COL3A1, hormonal signaling, including ESR1 and PGR, and enhanced cell survival, including BCL2 and BCL10. Conversely, down-regulated genes were predominantly involved in immune regulation and antigen presentation, including HLA-DMA and CIITA, cell cycle control and apoptosis, including EGR1 and KLF4, mitochondrial metabolism and oxidative stress response, including COX10, NDUFA1, and SOD1, cell adhesion, including ITGA3 and ITGB1, and hormonal responsiveness, including ESRRB and ID4. **Conclusion:** Endometriosis is characterized by a coordinated blood transcriptomic signature combining activation of inflammatory, angiogenic, invasive, and pro-survival pathways with suppression of immune surveillance, mitochondrial function, apoptotic regulation, and hormonal responsiveness. These alterations may facilitate immune escape, persistence, and invasiveness of ectopic endometrial lesions. Importantly, this systemic molecular profile may reflect disease-associated biological processes beyond the local pelvic environment. The identified pathways may support future development of non-invasive biomarkers and targeted therapeutic strategies.

C6.4.P

RNA modifications as a potential post-transcriptional regulatory layer in endometriosis: insights from Oxford Nanopore direct RNA sequencing

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Słowa kluczowe: endometriosis; RNA modifications; epitranscriptomics; m6A; m5C; direct RNA sequencing; oxford nanopore technologies

Endometriosis is a chronic gynecological disease characterized by the presence of endometrial-like lesions outside the uterine cavity. Ectopic endometrial cells display disease-associated features such as altered adhesion, migration, extracellular matrix remodeling, angiogenesis and changes in stromal cell behavior. Although transcriptional dysregulation in endometriosis has been widely investigated, the contribution of post-transcriptional RNA regulation remains less understood. In this study, we explored RNA modifications as a potential additional layer of gene expression regulation in endometriosis. Patient-derived primary stromal cells from eutopic and ectopic endometrial tissue were analyzed using Oxford Nanopore direct RNA sequencing, enabling transcriptome profiling and RNA modification-oriented analysis from native RNA molecules. Candidate RNA modification signals, including m6A- and m5C-associated sites, were identified and interpreted in the context of differential gene expression and functional enrichment. Functional analysis of m6A-associated transcripts detected in ectopic stromal cells revealed enrichment of biological processes related to cell migration, adhesion, cytoskeletal organization, extracellular matrix-associated functions and blood vessel formation. Importantly, these processes correspond to known cellular properties of ectopic endometrial lesions. The overlap between m6A-associated transcripts and differentially expressed genes obtained from the same sequencing experiment further supports the biological relevance of these modification-associated signals. In parallel, selected candidate m5C-modified transcripts linked to cytoskeletal dynamics, adhesion, matrix remodeling and cellular signaling point to additional disease-relevant regulatory candidates. Together, these results suggest that RNA modifications may contribute to the post-transcriptional organization of cellular programs associated with the ectopic endometrial phenotype. Oxford Nanopore direct RNA sequencing provides a valuable approach for exploring native RNA modification landscapes in clinically relevant cellular models of endometriosis.

C6.5.P

Functional analyses of the SLC19A1 promoter variants

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Słowa kluczowe: One-carbon metabolism, Functional studies, Reduced Folate Carrier, SLC19A1

Folate (vitamin B9) is an essential component of one-carbon metabolism. Naturally occurring folates, such as 5-methyltetrahydrofolate (5-mTHF), are transported into cells via the reduced folate carrier (RFC1), encoded by the SLC19A1 gene. Variants of SLC19A1 have been associated with susceptibility to several human pathologies, such as NTDs, cardiovascular disease and cancer. This study aimed to determine whether selected SLC19A1 polymorphisms are inherited as a haplotype, and whether its promoter variants are functional. The study included 604 individuals from Northern Poland. Genotyping was performed using TaqMan assays for selected SLC19A1 variants: the 1R/2R 61-bp VNTR, c.-43T>C (rs1131596), c.80G>A (rs1051266), and c.696C>T (rs12659). Next, haplotype analysis was performed. Promoter regions containing different SLC19A1 variants were cloned into a promoter-less reporter vector (pNL1.2, Promega). Several cell lines were co-transfected with new constructs and a control vector (pGL4.54). Promoter activity was assessed using the Dual-Luciferase Reporter Assay System (Promega). This study identified common SLC19A1 haplotypes. Moreover, functional analyses suggest that promoter variants may influence gene expression and that folic acid concentrations modulate SLC19A1 promoter activity. These findings improve our understanding of inter-individual differences in folate metabolism and susceptibility to folate-related diseases.

C6.6.P

Single-cell transcriptomic profiling reveals nanoplastic-induced stress responses and mitochondrial dysfunction in human PBMCs

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Słowa kluczowe: nanoplastic, PBMC, single-cell transcriptomic

Background: Nanoplastics (NPs) are emerging environmental contaminants capable of crossing biological barriers and accumulating in human tissues, including the circulatory system. Although increasing evidence suggests that NPs may affect immune-cell homeostasis, the cellular mechanisms underlying NP-induced toxicity remain insufficiently understood. The aim of this study was to investigate the effects of polystyrene nanoplastics on human peripheral blood mononuclear cells (PBMCs), focusing on cellular uptake, biophysical alterations, mitochondrial function, and transcriptional responses.

Methods: Human PBMCs were exposed to 25-nm fluorescent polystyrene nanoplastics and analyzed using complementary imaging, metabolic, and transcriptomic approaches. Confocal microscopy and optical diffraction tomography were used to evaluate NP internalization and changes in cellular morphology and biophysical parameters. Mitochondrial function was assessed using Seahorse extracellular flux analysis. In parallel, single-cell RNA sequencing (scRNA-seq) was performed to characterize NP-induced transcriptional alterations across immune-cell populations.

Results: Nanoplastics were efficiently internalized by PBMCs and induced rapid cellular remodeling, including reduced cell volume and dry mass together with increased intracellular density. NP exposure significantly impaired mitochondrial respiration, resulting in decreased basal respiration, ATP production, maximal respiration, and spare respiratory capacity, indicating pronounced mitochondrial dysfunction. Transcriptomic profiling further revealed activation of stress-response pathways associated with proteostasis, heat-shock signaling, and metabolic dysregulation. Single-cell analysis identified a distinct stress-associated cell population characterized by increased expression of stress-response genes and reduced mitochondrial transcript abundance. These alterations were accompanied by broad changes in immune-cell metabolic programs, suggesting impaired cellular homeostasis following NP exposure.

Conclusion: Our findings demonstrate that polystyrene nanoplastics induce profound metabolic, biophysical, and transcriptional alterations in human immune cells. The integration of functional assays with single-cell transcriptomics provides novel insight into the mechanisms of NP-induced immune-cell stress and highlights mitochondrial dysfunction as a key component of nanoplastic toxicity. These results contribute to a better understanding of the potential health risks associated with environmental nanoplastic exposure.

C6.7.P

From keratoconus-specific spatial organization to altered cell-cell interactions

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Słowa kluczowe: cornea, keratoconus, spatial transcriptomics, deconvolution, interactions

Background/objectives: Keratoconus (KTCN) is a degenerative eye disease in which the thinned cornea acquires a cone shape. The human cornea is a complex tissue consisting of five layers. In our previous study (PMID: 41931003), we discovered that the interaction patterns between corneal spatial clusters were altered in the KTCN cornea. Here, we delve into this aspect by comparing the interaction patterns between deconvolved spots, which allows insight into interactions at a level closer to cellular interactions while maintaining spatial localization.

Methods: The ST data, from 8 KTCN and 5 non-KTCN corneas, were organized in five spatial clusters and annotated to particular corneal layers. The spot's deconvolution was performed using the STdeconvolve R software package. Each spot is represented by a pie chart visualizing the proportion of cell types, i.e., the topics. The transcriptionally distinct topics were recognized based on the data generated in the Loupe Browser software. The CellChat package was used for the cell-cell interaction visualization.

Results: Different interaction numbers and patterns between cell-types/topics in KTCN compared to control corneas were found. The altered interactions in the pathways, especially the collagen pathway, are associated with the characteristic composition of topics within the KTCN cornea.

Conclusion: The altered cellular composition of the KTCN cornea influences the intercellular interactions, pointing to the need for further research into the contribution of individual corneal layers to the KTCN cone-formation mechanism. These results are useful for further integration with scRNA-seq outputs from KTCN corneas. Grants: Supported by the National Science Centre in Poland, grants no. 2021/41/B/NZ5/02245 and no. 2025/57/N/NZ2/04337

C6.8.P

Genistein-mediated modulation of mitochondrial and lysosomal pathways in CRISPR/Cas9-engineered cellular models of Huntington's disease

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Słowa kluczowe: Huntington's disease; genistein; mitochondrial dysfunction; autophagy; TFEB

Background: Huntington's disease (HD) is a progressive neurodegenerative disorder caused by CAG repeat expansion in the HTT gene. Besides protein aggregation, increasing evidence indicates that disturbances in mitochondrial homeostasis and lysosomal function contribute significantly to disease progression. Modulation of cellular quality control pathways has therefore emerged as a promising therapeutic strategy. Genistein, a natural isoflavone known to activate lysosomal biogenesis and autophagy, may represent a potential candidate for targeting these processes.

Methods: CRISPR/Cas9-engineered HEK293 cell lines carrying 41, 53 and 84 CAG repeats in the endogenous HTT locus were treated with genistein (50 μ M). Mitochondrial function was assessed by measurements of mitochondrial membrane potential using JC-1 and Mito-Tracker assays together with evaluation of respiratory chain complexes II, III and V activities. Lysosomal pathways were investigated using fluorescence colocalization analyses involving TFEB, LAMP2 and lysosomal markers. Transcriptomic profiling was additionally used to identify mitochondrial gene dysregulation associated with CAG expansion.

Results: Expanded CAG repeats were associated with progressive mitochondrial impairment, including reduced mitochondrial membrane potential and altered respiratory chain activity. Transcriptomic analyses revealed increasing numbers of dysregulated mitochondrial genes with disease severity. Genistein treatment significantly improved mitochondrial membrane potential and restored complex III activity in mutant cell lines. Furthermore, increased colocalization between TFEB, LAMP2 and lysosomal markers suggested activation of lysosomal biogenesis and cellular quality control pathways. Changes in apoptosis-associated markers indicated that cellular remodeling induced by genistein may involve both adaptive and elimination mechanisms.

Conclusions: Our findings indicate that genistein modulates mitochondrial and lysosomal homeostasis in CRISPR/Cas9-generated cellular models of Huntington's disease. Targeting the mitochondria-lysosome axis may therefore constitute a promising therapeutic strategy in HD.

C7.1.P

Expanding the dominant RP1 truncation spectrum in autosomal dominant retinitis pigmentosa

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Słowa kluczowe: RP1 gene, autosomal dominant retinitis pigmentosa, truncating variants

Background: The RP1 gene encodes a photoreceptor-specific microtubule-associated protein essential for photoreceptor outer segment organization and stability. Truncating variants in RP1 show a distinctive genotype–inheritance correlation. Heterozygous truncating variants affecting residues 661–917 are typically associated with autosomal dominant retinitis pigmentosa, whereas biallelic truncating variants in the N- and C-terminal regions, encompassing residues 1–613 and 939–2157, are predominantly associated with autosomal recessive disease. The intervening regions, p.614–p.660 and p.918–p.938, remain insufficiently characterized and are considered grey zones. **Materials and methods:** We investigated a 58-year-old man presenting with nyctalopia and impaired vision, without extraocular manifestations. His 80-year-old mother had decreased visual acuity and retinal pigmentary changes. Next-generation sequencing was performed in the proband, followed by familial segregation analysis.

Results: A heterozygous truncating variant in RP1, NM_006269.2.2756G>A, p.(Trp919*), was identified in the proband. The variant introduces a premature termination codon at residue 919 and is predicted to result in a truncated RP1 protein. It is located within the poorly characterized region immediately downstream of the established dominant truncation interval. Segregation analysis confirmed its presence in the affected mother. The variant was classified as likely pathogenic according to ACMG/AMP criteria. Its presence in two affected individuals from successive generations supports an association with autosomal dominant RP1-related retinopathy.

Conclusions: Familial segregation of the RP1 p.(Trp919*) variant provides additional evidence that the spectrum of dominant truncating variants may extend beyond residue 917. This observation contributes to refinement of the boundary between dominant and recessive RP1-associated regions. Further reports in unrelated families, supported by detailed ophthalmological phenotyping and functional studies, are required to define these boundaries more precisely and improve prediction of the inheritance pattern associated with individual truncating variants.

C7.2.P

Functional assessment of EYA1 and CHD7 splicing variants by minigene assay in branchiootorenal spectrum disorder and CHARGE syndrome

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Słowa kluczowe: EYA1, CHD7, minigene, functional assays, NGS, splicing

Introduction: Next-generation sequencing (NGS) is increasingly used in clinical practice, particularly in genetically heterogeneous disorders such as hereditary hearing loss (HL). However, the large number of variants identified by NGS creates challenges in assessing their clinical significance. Transcript analysis, especially for variants predicted to affect splicing, can provide critical evidence supporting variant interpretation.

Methods: Seven variants detected using dedicated HL multi-gene panel (v1 - 237 genes, v2 - 263) in 5 patients with branchiootorenal spectrum disorder (BORSD) and two patients with CHARGE syndrome were selected for the minigene assay: EYA1 c.1200-1G>A, c.1360G>A, c.1360+5G>A, c.1475+1G>T, c.1698+1G>A, CHD7 c.4534-1G>A, c.5301-14A>G. Fragments of the genes of interest were cloned into the expression vector pDEST-pCl-Neo-RHO with use of the Gateway system. Generated vectors were transfected into HEK293T cell culture. After 48h incubation cell were lysed and RNA isolation was performed. Experimental transcripts were analyzed using RT-PCR and Sanger sequencing.

Results: All studied variants were shown to affect splicing. Most commonly observed consequences were exon shortening (3 /7) and exon skipping (3/7). In most cases, aberrant splicing resulted in the introduction of the premature termination codon.

Discussion: Minigene assays enabled precise characterization of the splicing defects caused by the analyzed variants and provided important evidence supporting their pathogenicity. Notably, EYA1 variants c.1360G>A and c.1360+5G>A, despite affecting the same splice site, resulted in different transcript patterns. This finding highlights the value of functional studies in variant interpretation, even when the predicted molecular consequence appears straightforward.

Grant: 2021/41/B/NZ5/04390, National Science Centre, Poland

C7.3.P

Dual genetic etiology of congenital aniridia in one pedigree: identification of a novel splice donor PAX6 variant and a 11p13 microdeletion.

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Słowa kluczowe: congenital aniridia, pax6

Background: Congenital aniridia is a rare bilateral developmental eye disorder characterized by partial or complete absence of the iris. Most cases are caused by pathogenic variants in PAX6, although mutations in FOXC1, PITX2, CYP1B1, FOXD3, and TRIM44 should also be considered in the differential diagnosis. The condition is frequently associated with progressive visual impairment and secondary complications, including glaucoma, cataracts, and keratopathy.

Case Presentation: We report a family with congenital aniridia affecting two sisters and their parents, in whom two independent genetic causes of the disease were identified. In the younger sister, chromosomal microarray analysis revealed a heterozygous ~703 kb deletion at 11p13, partially overlapping the WAGR syndrome critical region. The deletion encompassed four OMIM-listed protein-coding genes (PAX6, ELP4, IMMP1L, and exons 3–5 of DNAJC24) but did not include WT1. The same deletion was subsequently confirmed in the father, who had initially been suspected of having ophthalmic abnormalities secondary to congenital infection. MLPA analysis confirmed the deletion in the younger sister but excluded it in the mother and the older sister. Due to the unsolved genetic background of aniridia in part of the family, a targeted next-generation sequencing panel for microphthalmia and anterior segment dysgenesis genes was performed. This analysis identified a previously unreported potentially pathogenic splice donor variant, c.1032+2T>G, in PAX6. The same variant was subsequently detected in the mother.

Conclusions: This family illustrates an unusual occurrence of two independent PAX6-related molecular mechanisms causing congenital aniridia within a single pedigree. Comprehensive molecular testing, including copy number analysis and next-generation sequencing, is essential for accurate diagnosis, genetic counseling, and assessment of recurrence risk. The novel PAX6 splice donor variant c.1032+2T>G expands the mutational spectrum associated with congenital aniridia.

C7.4.P

Rare causes of autosomal dominant optic atrophy (ADOA) are quite frequent in Polish cohort of patients

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Słowa kluczowe: ADOA - Autosomal dominant optic atrophy, NGS – Next Generation Sequencing, ACO2, AFG3L2 (OPA12), SSBP1 (OPA13), WFS1 genes

Autosomal dominant optic atrophy (ADOA), alongside Leber Hereditary Optic Neuropathy (LHON), is the most common form of hereditary optic atrophy, with a prevalence of about 1/35 000 births. ADOA is a relatively mild, progressive visual loss that may manifest from early childhood, including visual field defects and tritanopia. Unlike the classic form of LHON, ADOA is caused by mutations in nuclear genes that encode mitochondrial proteins. About 60-75% of ADOA cases are caused by heterozygous OPA1 gene variants. Our study included a cohort of 64 patients with ADOA or ADOA plus syndrome who were referred to the genetic clinic between January 2020 and July 2025. Most patients underwent next-generation sequencing (NGS) of a panel encompassing genes associated with optic atrophy or targeted NGS of Inherited Retinal Dystrophies (IRD)-related genes and one patient underwent whole exome sequencing (WES). Potentially pathogenic OPA1 variants were detected in 45 families; however, this report focuses on 17 families, in which we identified 14 pathogenic or potentially pathogenic variants in four genes: ACO2, AFG3L2 (OPA12), SSBP1 (OPA13), and WFS1. Six new variants have been detected, broadening the spectrum of mutations in these genes. Pathogenic variants in the ACO2, AFG3L2 (OPA12), and SSBP1 (OPA13) genes have not been previously reported in Polish patients with ADOA. In contrast, single variants in the WFS1 gene have been identified in patients with Wolfram-like syndrome, a type of ADOA plus. Based on our study, we found that among rare causes of ADOA, the most common in Polish patients are pathogenic variants in ACO2 and AFG3L2 genes. Our findings confirmed the importance of applying next-generation sequencing methods in identifying rare causes of ADOA.

C7.5.P

Integrated methylation and transcriptomic signatures in keratoconus corneas

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Słowa kluczowe: keratoconus, DNA methylation, transcriptomics, cornea, corneal epithelium, topographic regions

Background: Keratoconus (KTCN) is a multifactorial corneal disease characterized by structural and histological alterations affecting distinct corneal layers and topographic regions (TRs) with variable severity. However, most molecular studies have been performed using full-thickness corneal tissue and have not addressed layer- or region-specific molecular alterations. This study aimed to integrate DNA methylation and transcriptomic data to identify regulatory mechanisms associated with KTCN.

Materials and methods: Previously generated reduced representation bisulfite sequencing (doi:10.1167/iovs.18-25916) and RNA sequencing datasets (doi:10.1038/ejhg.2017.4) from full-thickness KTCN (n=5) and non-ectatic control corneas (n=5) were reanalyzed in an integrative framework. Differentially methylated regions (DMRs) were prioritized when located within regulatory elements, including promoters, enhancers, and CpG islands. These results were further interpreted together with transcriptomic profiles from the central, middle, and peripheral TRs of corneal epithelium (CE) from KTCN (n=17) and non-ectatic control samples (n=5) (doi:10.1167/iovs.64.2.22), followed by pathway enrichment analysis. Results: Integrative analysis identified KTCN-associated DMRs within regulatory elements of PARVB, WNT5A, IQGAP2, SYNJ2, CYP1B1, and MYO1G. Among these candidates, PARVB showed a significant inverse correlation between CpG methylation and transcript abundance ($R = -0.6989$, $p < 0.0001$), supporting methylation-linked transcriptional regulation. The TR-specific transcriptomic profiles of CE demonstrated pronounced biological heterogeneity. The DNA methylation pathway was among the most enriched hallmarks in the central TR, with relative upregulation of EZH2 and UHRF1, while DNMT1, RBBP7, EZH2, GATAD2A, and UHRF1 were downregulated in the peripheral TR.

Conclusions: Integrated methylation and transcriptomic signatures revealed regulatory and topographic heterogeneity in KTCN corneas. The central TR of CE showed the strongest enrichment of DNA methylation-related factors, suggesting that epigenetic regulation may be particularly relevant for the cone formation process in KTCN. Funding: National Science Centre in Poland, 2022/45/N/NZ5/02989 (to KJR)

C7.6.P

The Role of microRNAs in the Pathogenesis of Retinal Detachment: Vitreous Humor Expression Analysis Using Next-Generation Sequencing (NGS)

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Słowa kluczowe: microRNA, Retinal detachment, Next-Generation Sequencing (NGS) epigenetics

Background: Retinal detachment (RD) is a severe vitreoretinal disorder leading to progressive photoreceptor dysfunction and irreversible visual impairment. Neurodegeneration, inflammation, and extracellular matrix remodeling play crucial roles in its pathogenesis. MicroRNAs (miRNAs), which regulate post-transcriptional gene expression, have emerged as important modulators of cellular proliferation, apoptosis, oxidative stress, and fibrosis. This study aimed to evaluate the vitreous miRNA expression profile in patients with RD compared with controls.

Materials and Methods: Twelve patients were enrolled in this study. The RD group included six patients undergoing vitrectomy for rhegmatogenous retinal detachment, while the control group consisted of six patients operated on for idiopathic macular hole. Vitreous samples were collected intraoperatively, and total RNA was isolated. miRNA concentration was determined fluorometrically. miRNA expression profiling was performed using next-generation sequencing (NGS) on the Illumina MiniSeq platform with libraries prepared using the Lexogen kit. Bioinformatic analysis was conducted using the Galaxy Europe platform.

Results: More than 200 miRNAs were detected in vitreous samples. Comparative analysis identified seven miRNAs with significantly altered expression between RD patients and controls: hsa-miR-10a-5p, hsa-miR-143-3p, hsa-miR-486-5p, hsa-miR-181a-5p, hsa-let-7f-5p, hsa-let-7a-5p, and hsa-let-7c-5p. Previous studies indicate that these miRNAs are involved in pathways regulating inflammatory responses, extracellular matrix remodeling, retinal cell apoptosis, oxidative stress, and activation of transforming growth factor-beta (TGF-β) and epithelial–mesenchymal transition (EMT) signaling.

Conclusions: Altered vitreous expression of selected miRNAs in RD patients suggests their potential involvement in the molecular mechanisms underlying disease pathogenesis. miR-143-3p and members of the let-7 family may be particularly relevant due to their association with fibrosis, tissue remodeling, and retinal neurodegeneration. These miRNAs may serve as promising diagnostic and prognostic biomarkers and potential therapeutic targets in vitreoretinal diseases.

C8.1.P

Loss of Y chromosome in peripheral blood immune cell subpopulations in prostate cancer patients.

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Słowa kluczowe: loss of Y chromosome; prostate cancer; regulatory T cells; monocytic myeloid-derived suppressor cells; immune subpopulations

Background: Loss of the Y chromosome (LOY) is the most common age-related somatic chromosomal alteration in men and has been linked to immune dysregulation and increased cancer risk. In prostate cancer, LOY may impair anti-tumor immunity; however, its distribution across immunosuppressive cell populations - regulatory T cells (Tregs) and monocytic myeloid-derived suppressor cells (M-MDSCs) - and its functional consequences remain poorly characterized.

Methods: Sixty-one prostate cancer patients undergoing radical prostatectomy were classified as LOY-positive (n = 31) or LOY-negative (ROY; n = 30) by droplet digital PCR (ddPCR). Seven peripheral blood immune subpopulations - Tregs, T helper cells (Th), cytotoxic T lymphocytes (CTL), natural killer cells (NK), monocytes, M-MDSCs, and granulocytes - were isolated by fluorescence-activated cell sorting, and LOY% was determined in each fraction. Selected M-MDSC and Treg samples additionally underwent bulk RNA sequencing. Differential expression and pathway enrichment analysis were performed to explore transcriptomic consequences of LOY. **Results:** LOY patients were significantly older than ROY patients (median 68.0 vs. 62.0 years; p = 0.0066). Among sorted subpopulations, Tregs displayed the highest LOY% (17.6%), followed by granulocytes (15.1%) and M-MDSCs (10.1%), while CTLs showed the lowest (2.4%). Transcriptomic analysis of M-MDSCs and Treg suggests that LOY may be associated with a shift towards a more immunoregulatory and immunosuppressive phenotype, reflected by downregulation of inflammatory response pathways. A partial concordance in transcriptional changes was observed between M-MDSCs and Tregs, supporting a coordinated immunosuppressive effect.

Conclusions: LOY in peripheral blood is non-uniformly distributed across immune cell subpopulations in prostate cancer patients. The preferential enrichment of LOY in immunosuppressive cells suggests a potential role for LOY-driven immune dysregulation in prostate cancer pathogenesis. These findings provide a basis for future functional and mechanistic studies examining the consequences of LOY in tumor-associated immune cells.

C8.2.P

Integrative Identification of Transcriptional Dependencies in Merkel Cell Carcinoma: Preliminary Data and Perspectives for the Use of Base Editors

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Słowa kluczowe: Merkel cell carcinoma, MCC, transcriptional addiction, hypertranscription, POLR2B, CHD8, TFDP1, base editors

Introduction: Merkel cell carcinoma (MCC) is a rare but highly aggressive neuroendocrine skin cancer. Despite distinct initiating mechanisms, MCPyV-positive and MCPyV-negative subtypes show similar transcriptional programs, suggesting the existence of shared molecular dependencies. The aim of this study was to identify transcription-associated genes that may represent potential therapeutic vulnerabilities in MCC.

Materials and Methods: We developed an integrative bioinformatic pipeline combining DepMap CRISPR dependency data, gene expression profiles, common-essential gene filtering, a healthy skin single-cell atlas, and two independent MCC single-cell RNA-seq datasets. Candidate genes were evaluated based on the strength of functional dependency, expression in tumor cells, reproducibility across datasets, and limited expression in normal skin.

Results: The analysis identified CHD8 and TFDP1 as high-confidence transcription-associated dependencies in MCC. CHD8, a regulator of chromatin accessibility, showed a median Chronos gene-effect score of -0.743 , whereas TFDP1, a key partner of E2F transcription factors, showed a score of -0.821 . Both genes were consistently expressed across independent MCC cohorts. POLR2B, encoding a core subunit of RNA polymerase II, showed the strongest dependency signal, with a median Chronos gene-effect score of -2.61 . However, due to its common-essential gene status, POLR2B was classified as a mechanistic comparator.

Conclusions: These preliminary data suggest that MCC may exhibit features of transcriptional addiction. CHD8, TFDP1, and POLR2B represent three complementary levels of transcriptional regulation and constitute rational targets for further functional validation. The planned use of cytosine base editors will enable precise introduction of premature stop codons without inducing double-strand DNA breaks, allowing assessment of how loss of function of these genes affects MCC cell survival and phenotype.

Keywords: Merkel cell carcinoma, MCC, transcriptional addiction, hypertranscription, POLR2B, CHD8, TFDP1, base editors

C8.3.P

Circular RNAs as potential regulators of alternative splicing in lung cancer cells

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Słowa kluczowe: circular RNA; circRNA; alternative splicing; lung cancer; long-read sequencing; nanopore sequencing; RNA isoforms

Background: Alternative splicing is extensively remodeled in cancer and can generate protein isoforms with distinct functional properties. Circular RNAs (circRNAs) are increasingly recognized as regulators of RNA metabolism, but the mechanisms by which they influence alternative splicing in lung cancer remain largely unexplored. We aim to determine whether selected circRNAs modulate splice-site choice and thereby alter the balance of functionally relevant protein isoforms.

Methods: A549 non-small cell lung cancer cells and MRC-5 lung fibroblasts were used as malignant and non-malignant models. Total RNA was subjected to ribosomal RNA depletion followed by RNase R treatment to enrich circular transcripts. The enriched RNA was reverse-transcribed and processed by SplintR-mediated ligation, rolling circle amplification, T7 endonuclease I treatment, and size selection. The resulting material is being prepared for Oxford Nanopore long-read sequencing to enable circRNA characterization and isoform-resolved transcript analysis. Candidate circRNA-splicing relationships will subsequently be validated by RT-qPCR and functional perturbation assays.

Results: A multistep workflow for circRNA enrichment and amplification was successfully implemented in A549 and MRC-5 cells, enabling progression from total RNA to material suitable for downstream isoform-resolved analysis. The initial experiments confirmed the feasibility of sequential rRNA depletion, RNase R treatment, reverse transcription, SplintR-mediated ligation and rolling circle amplification in both cellular models. Optimization efforts identified amplification efficiency and size selection as the main parameters requiring further refinement prior to long-read sequencing. The established workflow provides a methodological basis for subsequent identification and comparison of circRNA-associated alternative splicing events in malignant and non-malignant lung cells.

Conclusion: This ongoing study addresses an incompletely understood layer of post-transcriptional regulation in lung cancer. Defining circRNA-dependent changes in alternative splicing may reveal mechanisms that shift transcript isoform balance and identify RNA-based targets for restoring functionally favorable protein isoforms.

C8.4.P

Comprehensive cancer genomic profiling in clinical practice.

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Słowa kluczowe: Comprehensive genomic profiling, ovarian cancer, ERCC3 gene, hereditary cancer

Background: Comprehensive genomic profiling (CGP) is an advanced diagnostic approach that enables simultaneous analysis of a broad spectrum of germline and somatic genetic alterations. Integration of these data provides insights into cancer predisposition, potential therapeutic targets, and the clonality of distinct tumor lesions.

Case Presentation: A 26-year-old woman was diagnosed with a mucinous ovarian carcinoma of the right ovary, characterized by predominantly expansile invasion with focal microinvasion and marked cytological atypia. Immunohistochemical analysis demonstrated TP53 accumulation and strong diffuse HER2 overexpression. Treatment consisted of unilateral adnexectomy. Given the early age of onset, whole-exome sequencing (WES) was performed using blood DNA. A potentially pathogenic, heterozygous variant in ERCC3 gene c.799_802del p.(Thr267CysfsTer9) was identified. Allele segregation analysis confirmed the presence of the same ERCC3 variant in the patient's father. One year later, an identical tumor developed in the contralateral ovary and was surgically removed. Histopathological and immunohistochemical examination revealed the same expression profile as observed in the primary tumor. To assess clonality, both tumors underwent comprehensive somatic profiling using the TSO500 assay. An identical spectrum of genetic alterations was detected in both lesions, including pathogenic variants in TP53 c.581T>A p.(Leu194His) and KRAS c.35G>T p.(Gly12Val) and high-level of ERBB2 amplification. Both tumors exhibited a low tumor mutational burden and were microsatellite stable. The identical immunophenotype and shared mutational landscape strongly supported metastatic spread of the tumour rather than the development of an independent primary one. After 3.5 years of follow-up following the second surgery, the patient remains disease-free.

Conclusions: Comprehensive genomic analysis of the patient, encompassing both germline and somatic alterations, enabled the identification of a genetic predisposition to cancer, determination of the metastatic nature of the disease, and detection of predictive/prognostic biomarkers as well as potential therapeutic targets. This integrated approach facilitated optimization of the patient's clinical management and enabled genetic counseling and surveillance of at-risk family members.

C8.5.P

Evaluation of ctDNA Liquid Biopsy Efficiency and Alteration Profiles in Breast Cancer Patients: A Single-Center Experience

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Słowa kluczowe: liquid biopsy, breast cancer, targeted therapy

Background: Tissue core needle biopsy (CNB) remains the standard material for molecular profiling in breast cancer. However, spatial and temporal tumor heterogeneity, together with limitations related to tissue quality and availability, may prevent accurate representation of the current molecular landscape. Liquid biopsy using circulating tumor DNA (ctDNA) offers a minimally invasive approach to capture ongoing tumor evolution. This study evaluated the efficiency of ctDNA analysis and compared the detection of actionable mutations with results obtained from CNB tumor specimens.

Materials and Methods: We analyzed breast cancer patients who underwent molecular profiling. Although CNB and ctDNA samples originated from independent patient cohorts, we compared analytical success rates, mutation detection rates, and molecular profiles between tissue-based testing and a newly implemented ctDNA workflow. Our data included 17 CNB analyses, while ctDNA testing was performed on 5 plasma samples using a targeted next-generation sequencing (NGS) panel.

Results: Among 17 CNB analyses, 9 were non-diagnostic and variants were identified in only 5 samples. In contrast, all 5 ctDNA analyses were successful, and 4 revealed clinically relevant alterations involving key therapeutic targets and resistance mechanisms, including PIK3CA, AKT1, and ESR1. Although BRCA1/2-targeted treatment decisions require confirmation of germline status, these genes were included in the plasma panel to assess somatic tumor evolution and loss of heterozygosity. Compared with CNB data, ctDNA detected emerging resistance-associated mutations, such as ESR1, which may be underrepresented in primary tumor tissue or missed due to non-diagnostic samples.

Conclusion: ctDNA liquid biopsy demonstrated high efficiency and a substantially lower non-diagnostic rate than CNB-based testing. Even in this small cohort, it identified actionable therapeutic targets and resistance markers. Integration of ctDNA sequencing with conventional CNB analysis may improve detection of dynamically evolving tumor profiles. Larger studies are needed to validate these findings.

C8.6.P

PD-L1 Gene Copy Number and Protein Expression in Relation to the State of Immune System in Early-Stage NSCLC

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Słowa kluczowe: CNV, lung cancer, NSCLC, immunotherapy, PD-L1

Introduction: Testing PD-L1 expression on cancer cells enables the qualification of NSCLC patients for immunotherapy. A better understanding of the relationship between PD-L1 copy number, protein status, and the state of the immune system may help limit the use of immunotherapy in resistant patients. This study aimed to correlate copy number variation (CNV) in the CD274 gene, which encodes PD-L1, with clinical data from early-stage NSCLC patients that reflect immune system function.

Methods: The study group comprised 71 non-squamous NSCLC patients. In 38% (27/71) of patients, molecular aberrations in KRAS, ALK and ROS1 genes were detected. Formalin-fixed paraffin-embedded tissue collected before treatment implementation was used for evaluation of PD-L1 expression with immunohistochemistry and for DNA extraction. DNA was assessed by qPCR to evaluate PD-L1 CNV. Routine blood count measurements were performed before therapy implementation.

Results: The analysis showed a tendency towards statistical significance for a positive correlation between PD-L1 CNV and the percentage of tumor cells expressing PD-L1 in patients without ALK, ROS1 or KRAS lesions ($p=0.08$). Moreover, CD274 CNV showed a significant positive correlation with lymphocyte count in the blood of patients without the abovementioned driver lesions ($p=0.03$). Copy number of CD274 correlated with platelet count in blood in patients with PD-L1 expression on $\geq 50\%$ ($p=0.02$) and on $< 50\%$ ($p=0.008$) of tumor cells. These correlations were negative in the first group and positive in the second group.

Conclusions: Increased CD274 CNV, which enhanced PD-L1 expression, may reflect tumor adaptation to abundant lymphocyte infiltration in immunocompetent patients. This relationship may be less pronounced in patients with detectable driver mutations. Results indicate that patients without driver lesions have a distinct immunological profile, which may be reflected in treatment effectiveness.

C8.7.P

Fusion landscape in cancer patients using Next-Generation Sequencing (NGS) – 3 years' experience from The Prof. F. Łukaszczyk Oncology Center in Bydgoszcz.

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Słowa kluczowe: fusion, NGS, sequencing, FISH, Sanger, RNA

Introduction: Rapid advances in molecular diagnostics have enabled identification of predictive genetic alterations, including gene fusions targeted by modern molecularly targeted therapies. Molecular heterogeneity in cancer includes ALK, ROS1, RET, MET, and NTRK rearrangements, as well as rare fusion variants with potential clinical implications.

Aim of the study: Characterization of the molecular landscape of gene fusions detected in oncological patients with NGS, with a particular focus on individual cases exhibiting diverse rearrangement profiles of potential diagnostic and therapeutic significance. **Materials and methods:** Retrospective study of fusion positive cancer patients (with lung, colorectal, melanoma, breast or urothelial cancer) from F. Łukaszczyk Oncology Centre in Bydgoszcz (n = 43). The starting material was paraffin embedded formalin fixed (FFPE) tumour tissue. DNA and RNA simultaneous isolation with RNeasy Lysis Buffer, was followed by 3D Med OncoTM Core Tissue Kit library preparation (3D Med Diagnostics) that enables to detect single nucleotide variants (SNVs) and small indels in 27 genes and rearrangement in 15 genes. Libraries were sequenced on MiniSeq (Illumina). FASTQ files underwent bioinformatic analysis using TiNaiLab. If needed, fusions were confirmed by FISH or Sanger sequencing. **Results:** We have identified 56 fusion transcripts. Apart from most frequent fusion like EML4::ALK, ROS1 rearrangements, and METex14 skipping transcript, we have identified some rare and clinically relevant fusions as followed: CD74::NRG1; CUL1::BRAF; KIT::NTRK1; MAD1L1::BRAF. Interestingly, alternative fusion transcripts were observed predominantly in ROS1 fusion-positive cases. Thanks to simultaneously performance of DNA and RNA targeted analysis, we were able to identify additional potential molecular targets on DNA level, as well.

Conclusions: Comprehensive molecular analysis allows for a more accurate characterization of an individual tumour profile and supports patient selection for targeted therapies. Simultaneous detection of gene fusions and DNA alterations may complicate interpretation due to the absence of well-defined clinical guidelines and management strategies.

C8.8.P

miRNA-155-5p as a denominator communis in endometrial cancer

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Słowa kluczowe: endometrial cancer, microRNA, p53, MSI, dMMR

Background: Tests for microsatellite instability (MSI), mismatch repair deficiency (dMMR), p53 protein expression, and the POLE (DNA Polymerase Epsilon, Catalytic Subunit) variants are recommended in molecular classification of endometrial cancer. MicroRNAs are considered potential biomarkers and may also be useful in the clinical management of endometrial cancer.

Material and methods: The study included 64 patients diagnosed with endometrial cancer. Endometrioid adenocarcinoma was diagnosed in 46 (72%) patients, and another histological type in the remaining 18 (28%) patients. According to the FIGO classification, 37 (58%), 13 (20%), and 14 (22%) patients were in stages I, II, and III, respectively. All patients underwent diagnostic testing for POLE, MSI, dMMR, and p53. The expression of miRNA-155-5p was determined by qPCR in both formalin-fixed, paraffin-embedded (FFPE) tissues and patients' plasma.

Results: Abnormal p53 protein expression was observed in 29 patients (45%), and it was more frequent in stage G3 ($p=0.060$, $X^2=3.513$). MSI-high was observed in 14 (22%) patients and was significantly more frequent in patients with wild-type p53 ($p=0.036$, $X^2=4.379$). dMMR was detected in 23 (36%) patients and was significantly more frequent in patients with p53-WT ($p=0.02$, $X^2=5.35$). The expression of microRNA-155-5p in FFPE was significantly higher in patients with FIGO stage II than in those with stage I in the p53-abnormal group ($p=0.0008$). Furthermore, plasma expression of this miRNA was significantly higher in patients with p53-abnormal at FIGO stage II+III compared to FIGO stage I ($p=0.014$). Higher tissue expression of miRNA-155-5p (regardless of histological type) was observed in cases where any of the markers (dMMR, MSI, p53) were abnormal ($p=0.018$) compared to the group with no abnormalities. This association was maintained in endometrioid adenocarcinoma ($p=0.049$).

Conclusion: The expression of microRNA-155-5p may serve as an additional diagnostic parameter in patients with endometrial cancer. It may act as a marker indicating DNA and protein abnormalities associated with an aggressive endometrial cancer phenotype that requires intensified treatment.

C8.9.P

Molecular profiling of somatic variants and multiple classifiers in endometrial cancer

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Słowa kluczowe: Endometrial cancer, next generation sequencing, molecular classification

Background: Molecular classification of endometrial cancer (EC) has transformed clinical practice, yet implementation remains challenging. We aimed to characterize the molecular profiles of 96 EC patients using a comprehensive sequencing approach.

Methods: Samples were processed at Alab Laboratoria. Genomic DNA was extracted from FFPE tissues (tumor content $\geq 30\%$). Libraries were prepared using the a targeted next-generation sequencing (NGS) panel and sequenced on the Illumina NextSeq 550Dx. We evaluated somatic variants across multiple classifiers.

Results: The cohort comprised: NSMP (65.6%), TP53-mutant (20.8%), dMMR (8.3%), and POLE-mutant (5.2%). CTNNB1 mutations were highly frequent in NSMP (44.4%). Tumors driven by high-VAF ($>30\%$) TP53 or AKT1 clones exhibited significantly reduced genomic complexity (mean 1.9 vs. 4.5 mutations). We also identified rare cases of overlapping landscapes, such as a POLE-mutant tumor with MLH1 variant, illustrating the complexity of multi-classifier interpretation.

Conclusions: This study confirms distinct genomic architectures across EC subtypes. CTNNB1 clustering in NSMP highlights its role as a potential surrogate marker. High NSMP prevalence in our cohort likely reflects an underestimation of dMMR; consequently, patients were recommended for MSI analysis to refine molecular assignment. "Stand-alone" high-VAF AKT1 and TP53 variants mark distinct clonal drivers. Furthermore, MLH1 alterations within the POLE subtype underscore that relying solely on IHC may obscure the true driver phenotype. Our findings demonstrate that these complex cases require an integrated, multi-modal assessment combining IHC, MSI, and methylation analysis alongside high-coverage NGS for accurate risk-stratification and personalized patient management.

C8.10.P

Integrated NGS-Based CNV Detection for Molecular Glioma Classification

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Słowa kluczowe: Glioma, FISH, NGS, 1p19q, CDKN2A

Introduction: According to the 2021 WHO CNS5 guidelines, CNS tumor classification relies on molecular alterations, and glioma diagnosis requires a multistep workflow often limited by scarce tissue. These challenges underscore the need for comprehensive methods enabling simultaneous biomarker assessment. Although NGS is routinely used for single-nucleotide variants, small insertions/deletions and RNA alterations, copy number variations (CNVs) are still underutilized in routine FFPE-based diagnostics. Aim To evaluate the potential of NGS as a primary method for the molecular classification of gliomas, including the detection of copy number alterations such as 1p/19q codeletion and CDKN2A deletion. Methods Molecular analysis was performed in 29 patients with CNS tumors (surgically treated 2021–2025). NGS libraries were prepared based on DNA and RNA using Human Glioma 26 Gene Mutations Detection Kit (SpaceGen). FISH analysis was performed using Vysis 1p36/1q25 19q13/19p13 FISH Probe Kit and Vysis LSI CDKN2A/CEP9 FISH probe Kit (Abbott). Results The mean age at diagnosis was 51 years (range, 24–72). CDKN2A results obtained by both NGS and FISH were available in 27 cases, demonstrating a concordance rate of 93% (25/27). For 1p/19q status, NGS–FISH comparison was available in 25 cases, with concordant results observed in 96% of cases (24/25). NGS analysis was performed in all patients (n=29), whereas 6 cases (20.7%) were excluded from at least one FISH analysis due to insufficient tissue material. Conclusions NGS demonstrated analytical concordance rates of 93% and 96% with FISH for CDKN2A deletion and 1p/19q codeletion, respectively. Discrepancies between the methods were observed in three cases and may reflect technical and biological factors affecting NGS-based CNV analysis, including high-level amplifications (e.g., EGFR), whole-chromosome gains or losses involving chromosomes 1 and 19, and degradation of FFPE-derived nucleic acids. Furthermore, NGS analyzes DNA from multiple tissue sections encompassing thousands of cells, thereby reflecting tumor heterogeneity, whereas FISH evaluates approximately 100 cells from a single section.

C8.11.P

Donor-aware machine learning classification of breast-tissue-derived DNA using Oxford Nanopore methylation profiles

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Słowa kluczowe: DNA Methylation, breast cancer, Adaptive sampling ONT

Background: Distinguishing breast-tissue-derived DNA from background tissue DNA is critical for emerging applications in precision oncology, e.g. thin needle biopsy or cfDNA sequencing. Conventional bisulfite-based methods cannot distinguish 5-methylcytosine (5mC) from 5-hydroxymethylcytosine (5hmC) and rely on predefined CpG probes, limiting their resolution. We applied native long-read Oxford Nanopore (ONT) Sequencing with adaptive sampling to enable simultaneous, base-resolution detection of both modifications, and developed a donor-aware machine learning classifier discriminating breast-tissue-derived from background DNA.

Methods: Targeted ONT sequencing was performed on 37 samples from 10 breast cancer patients - primary tumor (T, n=10), uninvolved margin (U, n=17), skin (S, n=6), and blood (B, n=4) - using adaptive sampling enrichment of 41 breast-cancer-associated genes at 6× mean enrichment. Methylation was called from native long reads at base resolution across 5mC and 5hmC in sites showing consistent coverage $\geq 10\times$. A donor-aware logistic regression classifier was trained on the top 200 features ranked by a stability-adjusted score prioritizing large

effect size, broad coverage, and low within-class variability across donors. Performance was evaluated by leave-one-donor-out cross-validation to prevent donor leakage.

Results: The classifier discriminated breast-tissue-derived DNA (n=27) from background (n=10) with ROC AUC of 1.000, accuracy 94.6%, sensitivity 92.6%, and specificity 100%, with no background sample misclassified. Top discriminatory features were located in TBX3, ESR1, ANKRD30A, PTEN, and NF1. Directional concordance with publicly available array-based methylation data provided additional external support.

Conclusion: ONT-based methylation profiling combined with donor-aware machine learning provides a reproducible, biologically interpretable framework for classifying breast-tissue-derived DNA, establishing a foundation for biomarker prioritization and validation in expanded cohorts, with relevance to low-invasive diagnostic applications.

Funding: This research was supported by the “Young Creator of Science” grant (71-01427) within the Medical University of Gdańsk.

C8.12.P

ctRNA: a limitation or the future of molecular diagnostics in pediatric cancers?

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Słowa kluczowe: liquid biopsy, ctRNA, pediatric cancers, personalized medicine, digital PCR

Background: Liquid biopsy is a minimally invasive method increasingly used in personalized molecular diagnostics, enabling genetic testing and real-time disease monitoring. A key limitation is ctRNA isolation for detecting gene fusions, crucial in pediatric cancers such as sarcomas and CNS tumors. Despite reported challenges(1,2), our experience shows that standardized isolation allows obtaining ctRNA of sufficient quality and concentration for reliable gene fusion detection using digital PCR.

Material and methods: The study included 7 patients diagnosed with rhabdomyosarcoma, Ewing sarcoma and pilocytic astrocytoma, from whom liquid biopsy samples were collected. ctRNA was isolated according to an in-house protocol. In parallel, molecular diagnostics for gene fusions were performed on tissue samples using NGS. Digital PCR analysis was conducted for ctRNA obtained from liquid biopsy and RNA derived from tissue. In a subset of patients with sarcoma, follow-up analysis was performed during treatment.

Results: Digital PCR analysis of ctRNA from plasma and cerebrospinal fluid enabled detection of gene fusions with quantitative assessment (copies/ μ L). Results were consistent with NGS analysis of tumor tissue. In sarcoma during chemotherapy, no gene fusions were detected in plasma, indicating signals below the detection limit, while maintaining reference gene fluorescence.

Conclusion: A key element in the application of liquid biopsy remains the standardization of ctRNA isolation, enabling its use for sensitive detection of gene fusions by digitalPCR. Quantitative determination of copy/ μ L allows for dynamic patient monitoring over time, as reflected in clinical observations. Our pilot study provides preliminary evidence supporting the utility of this diagnostic approach. Further patient recruitment and expanded analyses will allow validation in a larger cohort and assessment of its prognostic and predictive value in pediatric cancer monitoring. The project was funded by The Children's Memorial Health Institute, Young Investigator projects M61/24 and M60/24 and MEDWAY (2024/ABM/02/00032-02) founded by the Medical Research Agency.EN and KK contributed equally to this work

C8.13.P

Unlocking single-cell transcriptomics and epigenomics in FFPE samples.

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Słowa kluczowe: single-cell, transcriptomics, epigenomics, multimodal profiling, FFPE

Understanding the complex cellular heterogeneity of the tumor microenvironment is essential for advancing targeted cancer therapies. Archival formalin-fixed paraffin-embedded (FFPE) tissues represent a vast, invaluable resource for retrospective oncology research, offering unique opportunities to study rare cancers and longitudinal tumor evolution. However, the profound nucleic acid degradation and fragmentation inherent to the FFPE process severely restrict the application of standard single-cell sequencing technologies. Current commercial methods are costly and rely on restricted targeted-probe sets, while existing non-commercial protocols suffer from critically low gene detection rates due to fragmented mRNA lacking 3' poly-A tails and highly fragmented genomic DNA in nucleosomes. To address these critical limitations, I will present a newly developed method in our lab for high-throughput, single-cell multimodal profiling that captures both the transcriptome (scRNA-seq) and chromatin accessibility (scATAC-seq) simultaneously from the exact same cell in FFPE samples. By successfully bridging the gap between archival clinical specimens and advanced single-cell multi-omics, this cost-effective approach provides a powerful new tool for dissecting tumor heterogeneity, immune responses, and therapeutic resistance.

C8.14.P

Telomere Length and Mitochondrial DNA Copy Number in Urinary Bladder Cancer Patients

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Słowa kluczowe: telomere length, mitochondrial DNA copy number, urinary bladder cancer, human studies

Urinary bladder cancer (UBC) is a significant clinical problem due to the increasing number of cases and mortality, especially in the male population. UBC is strongly associated with environmental exposure, however among the molecular-based alterations that are potentially associated with UBC, telomere length (TL) and mitochondrial DNA copy number (mtDNAcn) are considered particularly relevant. Repetitive DNA sequences at chromosome ends, known as telomeres, help maintain genomic stability; however, their shortening points to genomic instability. Alterations in mtDNA copy number might also indicate mitochondrial dysfunctions and disrupted cellular homeostasis. Given that both factors have been linked to carcinogenesis, assessing them might offer further understanding of molecular alterations associated with UBC. Most human cancer studies measure TL and mtDNAcn in peripheral blood as a surrogate tissue. To add, there is little data on these molecular indices in UBC. In this study, we examine tumor tissues, adjacent non-tumor tissues, and blood samples obtained from patients with UBC, together with blood specimens from urological and healthy controls (5 tissue types). Absolute TL and mtDNAcn were assessed using qPCR. Statistical analyses were conducted using the GraphPad Prism software. The outcomes showed molecular parameters variations in tumor versus non-tumor tissue, alongside distinctions in the analyzed blood samples. Overall, tumor tissues display decreased TL and mtDNAcn when compared to their corresponding non-tumor tissues. The same trend was noted in the analysed blood samples of UBC patients when compared to urological and healthy controls. There was observed variability in these parameters across patients, which suggests possible connections to individual clinical traits and risk factors. Our research indicates that TL and mtDNAcn might be linked to molecular changes associated with UBC and could contribute to a deeper understanding disease etiology. This work was supported by the OPUS research project no. 2020/37/B/NZ5/01684 funded by the National Science Center in Poland.

C8.15.P

Expanding the landscape of actionable and emerging molecular alterations in lung cancer using RNA-based NGS

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Słowa kluczowe: lung cancer, RNA-based NGS, molecular diagnostics, gene fusions, precision oncology, actionable biomarkers

Background: Precision oncology in lung cancer increasingly relies on biomarkers beyond established actionable alterations. High-throughput molecular profiling enables detection of rare, emerging, and uncharacterized variants with potential therapeutic relevance. We evaluated the clinical potential of a targeted RNA-based NGS assay in detecting established, rare, and emerging oncogenic alterations in a real-world lung cancer cohort.

Methods: Targeted RNA sequencing of 17 clinically relevant genes was performed on 2,198 lung cancer specimens. The cohort included adenocarcinomas (76%), neuroendocrine tumors (14%), and other non-squamous histologies (10%). Analysis comprised detection of oncogenic and pathogenic single nucleotide variants (SNVs), INDELs, exon-skipping events, and gene fusions.

Results: Molecular alterations were identified in 1,321 cases (60%), comprising 232 unique variants. Co-occurring alterations were detected in 119 tumors. Canonical actionable drivers accounted for ~43% of mutation-positive cases. Uncommon clinically actionable events, including BRAF V600E, non-canonical EGFR alterations, activating ERBB2 variants, and NTRK fusions, represented ~9%. Emerging and investigational biomarkers, including NRG1, FGFR, and MET fusions, non-V600E BRAF alterations, non-G12C KRAS codon 12 variants, and EGFR and MET kinase domain duplications, were observed in 33% of cases. Sporadic alterations with limited clinical evidence, such as KRAS codon 13/61 variants and PIK3CA mutations, accounted for ~15%. Notably, 39 previously unreported variants, including novel fusion partners/breakpoints, were identified. Over one-third of tumors harbored uncommon, emerging, or sporadic alterations, suggesting that conventional testing may overlook a clinically meaningful subset of patients with therapeutic opportunities.

Conclusion: Targeted RNA-based NGS substantially expands the detectable landscape of actionable and novel oncogenic alterations in lung cancer. Beyond established drivers, it identifies rare, emerging, investigational, and previously undescribed events, including novel gene fusions and co-occurring alterations that may influence therapeutic decisions. Integration of RNA-based profiling into routine diagnostics may improve molecular stratification, expand access to targeted therapies, and increase eligibility for biomarker-driven clinical trials.

Funding: IGIChP grants 3.13/25, 3.11/23, 3.3/22.

C8.16.P

MicroRNAs as potential liquid biopsy biomarkers for differentiating Pancreatic Ductal Adenocarcinoma (PDAC) from Pancreatic Neuroendocrine Tumors (PNETs)

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Słowa kluczowe: microRNA, liquid biopsy, PDAC, PNETs, neuroendocrine tumors

Introduction: Pancreatic ductal adenocarcinoma (PDAC) represents one of the most aggressive malignancies and is currently the seventh leading cause of cancer-related mortality worldwide. In contrast, Pancreatic neuroendocrine tumors (PNETs) constitute a rare neoplasms characterized by a less aggressive clinical behavior. Both, PNETs and PDAC frequently remain clinically silent for prolonged periods, resulting in diagnosis predominantly at advanced stages of disease progression.

Methodology: The study included patients with PDACs and PNETs hospitalized in Department of Gastroenterology (PUM, Szczecin). The study was conducted using serum samples obtained from patients diagnosed with PDAC (n = 15; median age: 61 years; male/female 8/7) and PNETs (n = 16; median age: 61.5 years; male/female 8/8), representing various stages of disease progression, and from healthy control subjects (n = 10; median age: 60 years; male/female 6/4). Total RNA of serum samples were analyzed using an Agilent miRNA microarray (2,549

human miRNA probes). The aim of the study was to identify microRNAs capable of differentiating PDAC from PNETs, which may contribute to the earlier and more accurate discrimination of pancreatic tumor subtypes. Furthermore, the study sought to provide deeper insight into the distinct pathogenesis underlying these neoplasms. Results We identified 71 microRNA that differentiated PDAC from PNETs (p -values $< 0,05$). Four microRNAs were identified as the most statistically significant- hsa-miR-3976 (p -value = 0,005, FC = 1,55, AUC = 0,801), hsa-miR-4688 (p -value = 0,006, FC = 3,55, AUC = 0,782), hsa-miR-509-3-5p (p -value = 0,005, FC = 1,33, AUC = 0,859), has- miR-4758-3p (p -value = 0,003, FC = 1,80, AUC = 0,821). Pathway enrichment analysis based on the KEGG database revealed that miRNAs significantly different between PDAC and PNET were associated with neurodegeneration-related pathways and the MAPK signaling pathway.

Conclusion: The findings suggest that serum-derived microRNAs may serve as minimally invasive liquid biopsy biomarkers for differentiating tumor types, thereby supporting differential diagnosis, facilitating earlier cancer detection and disease monitoring.

C8.17.P

Pathogenesis and Diagnostic Pathways for Pancreatic Cancer-Associated Cachexia from a Genetic Perspective

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Słowa kluczowe: cachexia, genetics, pancreatic cancer

Background/Objectives: Pancreatic cancer-associated cachexia (CAC) is a severe metabolic syndrome affecting approximately 80% of patients with pancreatic ductal adenocarcinoma (PDAC), directly accounting for ~30% of cancer-related deaths. While systemic inflammation and metabolic dysregulation are well recognized, an increasingly important dimension involves genetic and epigenetic alterations that drive the progression of cachexia. This review examines CAC from a genetic perspective.

Methods: Literature was searched in PubMed, Web of Science, Scopus in accordance with the PRISMA guidelines, and keywords related to pancreatic cancer cachexia and genetics. The search covers 2016-2026. Higher-evidential-value studies were prioritized.

Results: Recent genomic and transcriptomic analyses reveal that oncogenic mutations (e.g., KRAS, TP53) and epigenetic reprogramming contribute to molecular pathways associated with pro-cachectic factor expression. Furthermore, germline variants (e.g., in the IL6R gene) and metabolic gene regulation (e.g., NRK2 in NAD⁺ metabolism) may influence individual risk of developing cachexia. Recent studies have also linked the IRE1α/XBP1 signalling axis to muscle wasting in CAC. The emerging role of noncoding RNAs (e.g., miR-155, miR-373) and single-nucleus transcriptomics further highlights the multi-layered genetic architecture of this syndrome.

Conclusions: Understanding the genetic and molecular background of CAC may provide important insights into its pathogenesis in PDAC. Integrating genomic profiling, noncoding RNA biomarkers, and genetic polymorphism analysis with traditional diagnostic criteria may improve early detection. Targeted therapies addressing GDF-15, myostatin-activin signalling, and NAD⁺ metabolic failure represent promising genetically informed interventions to improve survival and quality of life.

C8.18.P

Automation of NGS in Oncogenetics for BRCA1 & BRCA2 Variant Assessment: Experiences of the Molecular Oncology and Genetics Department, Oncology Center in Bydgoszcz

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Słowa kluczowe: next-generation sequencing, automation, library preparation

Introduction: Molecular diagnostics of BRCA1/2 using next-generation sequencing (NGS) is essential for hereditary cancer risk assessment and targeted therapy selection. Germline and somatic testing is routinely used in breast, ovarian, pancreatic and prostate cancer. Increasing test volume requires workflow automation while maintaining expert interpretation.

Aim: To compare automated and semi-automated NGS workflows for germline and somatic BRCA1/2 testing in a single-center study.

Materials and Methods: A total of 61 DNA samples from patients with breast, ovarian, and prostate cancer were analyzed. Germline BRCA1/2 testing was performed in 27 samples using the Genexus System (ThermoFisher Scientific) or ANDIS500 (3DMedDiagnostics), followed by MiniSeq (Illumina) sequencing and TINaiLab or Genexus/IonTorrent analysis. Somatic BRCA1/2 testing was performed in 26 samples using either the BRCAaccuTest™(NGeneBio) or the BRCA1/2GeneMutation Detection Kit (3DMed Diagnostics) by MiniSeq sequencing. Additionally, 8 somatic samples were analyzed in parallel using the BRCA1/2 GeneMutations Detection Kit SpaceGen and BRCAaccuTest™(NGeneBio) assays. Results:Overall concordance between Genexus/IonTorrent and Illumina/TINaiLab workflows was 97%, with 3% discordant cases mainly due to false positives or QC failures, all resolved by Sanger sequencing. Somatic testing showed high concordance between workflows, lower QC metrics and a small number of non-diagnostic samples in the manual approach, while variant detection remained concordant in valid samples. The SpaceGen and NGeneBio assays showed 100% concordance in variant detection with minor QC differences.

Conclusions: Automated NGS workflows enable efficient and standardized BRCA1/2 diagnostics. Genexus and Illumina/TINaiLab showed high analytical concordance and are suitable for routine use. In somatic testing, both approaches provided comparable variant detection, while automation improved quality metrics. Further automation of library preparation using liquid handling systems such as Veya™ may enhance standardization and reproducibility. Expert review remains essential for final clinical interpretation.

C8.19.P

Homologous Recombination Deficiency Beyond Ovarian Cancer: Emerging Applications Across Solid Tumors from a Single Diagnostic Laboratory's Experience

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Słowa kluczowe: Homologous Recombination Deficiency, PARPi, HRD, cancer, solid tumors

Introduction: Homologous recombination deficiency (HRD) has become an established predictive biomarker in ovarian cancer, guiding the use of poly(ADP-ribose) polymerase inhibitors (PARPi) and shaping precision oncology strategies. Increasing evidence indicates that HRD extends beyond ovarian malignancies and represents a biologically relevant vulnerability across multiple solid tumors.

Methods: 4.505 FFPE tissue biopsies of patients with epithelial ovarian, fallopian tube, and primary peritoneal cancer (cumulatively OC) and 136 samples from early-stage non-small cell lung cancer (NSCLC) patients were referred to our laboratory for HRD testing, performed by AmoyDx HRD Focus and Complete Panels (Amoy Diagnostics).

Results: HRD positivity was identified in 43.7% OC, highlighting the substantial prevalence of HRD within the tested population. 89.5% of HRD-positive tumors were Genomic Scar Score positive (GSS+) vs 41.4% harboring BRCA mutations. In the NSCLC cohort, 28.7% of samples were GSS+, while variants in genes participating in homologous recombination repair (HRR) had a minor contribution towards HRD characterization. Interestingly, a high GSS score is significantly associated with pathogenic TP53 mutations.

Discussion: We present our significant diagnostic experience in HRD testing for OC patients and our research experience of HRD testing in NSCLC. Alterations in HRR genes are observed in diverse cancer types, although their prevalence, functional significance, and therapeutic implications vary considerably. In BRCA-related cancers, HRD has already demonstrated clinical utility, supporting the incorporation of PARP inhibitors into standard treatment paradigms for selected patient populations. More recently, genomic scar assays, mutational signatures, and comprehensive molecular profiling have identified HRD-associated phenotypes in additional malignancies, including endometrial, biliary tract, lung, and gastrointestinal cancers, stimulating growing interest in expanding HRD-directed therapeutic strategies beyond traditionally recognized indications. Despite this progress, significant challenges remain. The lack of standardized HRD assessment methods, tumor-specific differences in the biology of DNA damage repair, and uncertainty regarding optimal biomarker thresholds limit broader clinical implementation.

C8.20.P

Integrating Liquid Biopsy into Precision Oncology; real world data on ctDNA ESR1 Testing in Metastatic Breast Cancer as an example

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Słowa kluczowe: liquid biopsy; LBx; circulating tumor DNA; ctDNA; ESR1 testing, ddPCR, NGS

Introduction: Although tissue biopsy remains the gold standard for tumor profiling, its invasive nature and inability to capture temporal tumor heterogeneity and evolution represent important limitations. Liquid biopsy (LBx) is a minimally invasive alternative that enables analysis of tumor-derived material circulating in body fluids. Advances in highly sensitive molecular technologies, including next-generation sequencing (NGS) and digital PCR (ddPCR), facilitate the detection of clinically relevant genomic alterations, monitoring of treatment response, and identification of resistance mechanisms. As an example, we present real-world data on ESR1 mutation detection in estrogen receptor-positive metastatic breast cancer (ER+ mBC) using ddPCR hotspot testing and targeted NGS.

Methods: 495 women with mBC were referred to our laboratory for ESR1 LBx testing. 312 HR+/HER2– patients with disease progression following at least one line of therapy were analyzed using ddPCR (Bio-Rad). The remaining 153 patients, a more heterogeneous mBC cohort, were tested using a 34-gene targeted NGS panel (Pillar Biosciences). Results: ESR1 positivity rates were consistent with those reported in the literature (10–50% in mBC). ESR1 mutations were detected in 114/312 (36.5%) patients by ddPCR and in 32/153 (20.9%) patients by NGS, with variant allele frequencies ranging from 0.06%-23.48% and 0.06%-57.15%, respectively. In the NGS cohort, 101 patients harbored an additional 168 non-ESR1 Tier 1/2 variants, predominantly in the AKT pathway (51,2%), including PIK3CA (36,3%), PTEN (12,5%), and AKT1 (2,4%).

Conclusions: Both ddPCR and NGS sensitively detected ESR1 mutations in LBx samples from mBC patients. Clinical characteristics, cfDNA input, and assay design should be considered when comparing assay performance. Beyond ESR1, NGS enables comprehensive detection of actionable co-mutations, supporting its use for patients with complex genomic profiles. By moving beyond the limitations of tissue sampling, liquid biopsy has the potential to provide a more comprehensive and longitudinal view of tumor evolution, ultimately improving patient stratification, treatment selection, and clinical outcomes.

C8.21.P

Evaluation of the prognostic value of chromosomal rearrangement patterns in non-WNT/non-SHH medulloblastoma

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Słowa kluczowe: medulloblastoma, risk stratification, MLPA, chromosomal aberrations

Background: Medulloblastoma (MB) is the most common malignant central nervous system tumor in children. The non-WNT/non-SHH group, comprising molecular groups 3 (G3) and 4 (G4), is characterized by substantial biological and clinical heterogeneity, which complicates precise risk stratification. Recent studies have demonstrated that selected copy number variations (CNVs) and chromosomal rearrangement patterns may have significant prognostic value in this group of tumors.

Project Objective: The aim of this project was to determine the frequency and clinical significance of chromosomal aberrations involving chromosomes 7, 8, and 11 in patients with non-WNT/non-SHH medulloblastoma and to evaluate their potential utility as prognostic biomarkers.

Materials and Methods: The study included a cohort of 35 pediatric patients diagnosed with medulloblastoma classified into the non-WNT/non-SHH group and assigned to molecular Group 3 or Group 4 based on histopathological evaluation and DNA methylation profiling. Analyses were performed on DNA isolated from tumor tissue preserved by snap-freezing as well as from formalin-fixed paraffin-embedded (FFPE) specimens. Copy number alterations were assessed using Multiplex Ligation-dependent Probe Amplification (MLPA) with probe sets targeting regions on chromosomes 7, 8, and 11. The results obtained were correlated with patients' clinical data, including age, sex, disease stage, response to treatment, progression-free survival (PFS), and overall survival (OS).

Conclusions: This project enabled the characterization of CNV patterns affecting chromosomes 7, 8, and 11 in non-WNT/non-SHH medulloblastoma and assessment of their prognostic significance. The findings may contribute to the identification of novel prognostic biomarkers and support the development of cost-effective diagnostic tools for risk stratification and personalized treatment of patients with medulloblastoma. The project was funded by The Children's Memorial Health Institute, Young Investigator Project (M64/25), and Medical Research Agency, project MEDWAY (2024/ABM/02/00032) and BraimTOR (2021/ABM/01/00027).

C9.1.P

Integrated Clinical and Molecular Landscape of PURA-Related Neurodevelopmental Disorder: A Natural History Study

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Słowa kluczowe: Natural History Study, PURA Syndrome

Background: PURA-related neurodevelopmental disorder (PURA-NDD, OMIM:616158) is a recently identified disease associated with phenotypic features such as neonatal apnea, hypotonia, epilepsy and neurodevelopmental delay. Pur-alpha protein, encoded by PURA gene, is known to play an essential role in brain and hematopoietic development in mammals. Due to its DNA- and RNA-binding activity it acts as a transcription activator of several genes downstream relevant to neurodevelopment. A natural history study plays a crucial role in understanding the course of a disease, including its signs, symptoms, and time of onset, and provides a foundational control for the development of subsequent treatment research. To date all research performed on PURA-related neurodevelopmental disorder's natural history has been either performed as a single cross-sectional study or has been based on patient self-reported questionnaires, not validated by a clinician and without any additional molecular or imaging studies.

Patients and methods: Our study aimed to integrate the data from patients' past medical history with neurological examination, validated by a physician, as well as metabolomic, lipidomic, and RNA-sequencing data from blood samples. These analyses would give us a comprehensive view of the natural history of the PURA syndrome and allow identification of correlations between molecular pathomechanisms in Pur-alpha involved processes and phenotype. The knowledge about proteome-phenotype correlations is particularly crucial giving the fact that some patients respond to treatment with pyridostigmine and salbutamol, with improvement of motor function and hypotonia, whereas others either do not respond or even deteriorate during treatment. The knowledge of affected molecular pathways would be valuable for gaining insight into the role of Pur-alpha protein's role in neuromuscular junction physiology. Moreover, investigating the molecular mechanisms underlying this disease could potentially lead to identification of therapeutic targets or biomarkers that would enable more effective diagnosis and monitoring of the disease.

C9.2.P

Variable Expressivity of a KIF11 Mutation in a Four-Generation Family with Microcephaly

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Słowa kluczowe: KIF11 gene, microcephaly, MCLMR syndrome, variable expressivity, pedigree analysis, next-generation sequencing

Introduction: Pathogenic variants in the KIF11 gene, which encodes the Eg5 kinesin protein, disrupt mitotic spindle assembly, cell division, and lymphangiogenesis. These mutations typically cause Microcephaly, Lymphedema and Chorioretinopathy Syndrome (MCLMR, OMIM #152950). This autosomal dominant condition exhibits incomplete penetrance and highly variable phenotypic expression. To date, only 100–150 cases have been detailed in the medical literature. Case Report A 10-month-old male infant was referred to our Genetic Counseling Clinic presenting with severe microcephaly (<3rd percentile), global psychomotor delay, seizures, congenital lymphedema of the feet, an atrial septal defect, bilateral congenital cataracts and feeding difficulties. Next-Generation Sequencing (NGS) conducted at ICZMP identified a pathogenic heterozygous deletion of approximately 993 bp spanning exons 2–4 of the KIF11 gene: NC_000010.10:g.(94366012_94367005)del. Familial Segregation The identical KIF11 mutation was later confirmed in the patient's two-year-old sister. She exhibited a very mild phenotype, presenting solely with microcephaly (42.5 cm, <3rd percentile) and motor stereotypies. The children's mother had been followed since infancy for microcephaly, though historical cytogenetic testing reported a normal female karyotype. She returned for re-evaluation only after her children's molecular diagnosis. Her current phenotype includes microcephaly (48.5 cm, <3rd percentile), intellectual disability, and significant learning difficulties. Testing for the biological father was unavailable.

Pedigree and Conclusion: Pedigree analysis revealed the segregation of microcephaly across three generations on the maternal grandmother's side; targeted genetic screening for these family members is currently planned. This clinical case clearly underscores the remarkable phenotypic heterogeneity of KIF11 mutations, which can range from isolated microcephaly to complex syndromic MCLMR manifestations within a single family lineage.

C9.3.P

Preserved Expressive Speech in MECP2- and FOXG1-Related Rett Spectrum Disorders: Domain-Specific Genotype–Phenotype Correlations

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Słowa kluczowe: Rett syndrome spectrum; preserved speech; MECP2; FOXG1; functional domains

Introduction: Pathogenic or likely pathogenic variants of MECP2 and FOXG1 cause Rett syndrome-spectrum neurodevelopmental disorders through convergent mechanisms of action related to DNA methylation regulation. Mild phenotypes with preserved speech are very rare, particularly in disorders caused by FOXG1 gene variants.

Material/methods: We present two female patients of Polish origin with preserved expressive speech and complex neurodevelopmental disorders, in whom the presence of heterozygous pathogenic and likely pathogenic de novo variants in the MECP2 and FOXG1 genes was confirmed, respectively. We also reviewed the correlation between clinical presentation and functional MECP2 and FOXG1 domains in our patients and previously published patients with preserved expressive speech phenotypes.

Results: The first patient, a 11-year-old girl with dysarthric but sentence-level speech, mild truncal and gait ataxia, and focal seizures carried a previously reported heterozygous de novo start-loss variant MECP2 c.2T>C (p.Met?), with random X-inactivation. The variant affects the N-terminal domain (NTD), and missense variants in the NTD or transcriptional repression domain (TRD) have been most frequently published in patients with preserved expressive speech phenotypes. In a second patient, a 6-year-old girl with speech limited to simple sentences, dystonic gait disturbances, and generalized epileptiform activity, we identified a previously unpublished heterozygous de novo missense variant FOXG1 c.628T>C (p.Phe210Leu). This variant is located within a discrete segment of the FOXG1 forkhead domain (p.185–254), in which preserved-speech phenotypes have most often been reported in association with missense variants, as in our patient. In conclusion, known MECP2 and FOXG1 variants associated with preserved speech create some clusters in protein domains involved in transcriptional repression during CNS development. However, their functional structure appear to differ. The FOXG1 variants are rather associated with a localized conformational change within one highly conserved forkhead domain, whereas MECP2 variants can be related to broader, non-point-restricted modification of interactions between MECP2 functional domains and methylated DNA.

C9.4.P

Application of classical Sanger sequencing to confirm a rare disease diagnosis: a case of limb-girdle muscular dystrophy

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Słowa kluczowe: Sanger sequencing, NGS, limb-girdle muscular dystrophy, rare diseases

14.5-year-old female patient underwent diagnostic tests for different medical conditions due to persistent hypertransaminasemia and hyperCKemia. A stepwise diagnostic workup excluded Duchenne muscular dystrophy, Becker muscular dystrophy, Pompe disease, as well as a range of metabolic disorders. Next-generation sequencing (NGS) identified two variants in the CAPN3 gene (calpain-3, NM_000070.3): c.550del and c.1636C>T, both classified in genomic databases as pathogenic or likely pathogenic. Sanger sequencing confirmed the presence of both variants in the proband's sample and demonstrated that each parent carried one of the variants while remaining clinically unaffected. Segregation analysis established that the variants were located in trans (compound heterozygosity). This finding explains the patient's clinical manifestations and supports the clinical diagnosis. The molecular findings, together with the patient's phenotype and the absence of symptoms in the parents, confirmed the diagnosis of limb-girdle muscular dystrophy R1 calpain 3-related (LGMDR1), an autosomal recessive disorder. The hallmark of LGMDR1 is progressive weakness and atrophy of the pelvic and shoulder girdle muscles, as well as the proximal muscles of the limbs. Sanger sequencing remains the gold standard for confirming sequence variants detected by NGS. It is also a rapid and cost-effective method for evaluating familial segregation of variants and plays a crucial role in determining cis/trans configuration in autosomal recessive disorders, particularly given the limitations of short-read NGS technologies in resolving variant phasing. NGS is still regarded primarily as a screening tool in genetic diagnostics and - despite fast and continuous development of technology - it seems unreasonable to give up on Sanger sequencing in the near future.

C9.5.P

Molecular and Functional Characterization of ST3GAL5-Related GM3 Synthase Deficiency in Polish Patients

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Słowa kluczowe: ST3GAL5; GM3 synthase deficiency; gangliosides; neurodevelopmental disorder; epilepsy; induced pluripotent stem cells

Introduction: GM3 synthase deficiency (GM3D; OMIM #609056) is an ultra-rare autosomal recessive neurodevelopmental disorder caused by pathogenic variants in the ST3GAL5 gene, encoding lactosylceramide alpha-2,3-sialyltransferase. The disease is characterized by profound developmental delay, infantile-onset epilepsy, secondary microcephaly, failure to thrive, movement disorders, sensory impairment, and, in some patients, skin dyspigmentation. Despite the recognized clinical phenotype, the molecular mechanisms underlying neuronal dysfunction in GM3D remain poorly understood.

Aim: The aim of the study was to characterize the molecular basis of GM3D in Polish patients with ST3GAL5 variants and to develop a patient-specific cellular model for functional analyses.

Material and Methods: Clinical and molecular data from patients with biallelic ST3GAL5 variants were analyzed. Genetic diagnosis was performed using next-generation sequencing followed by Sanger confirmation and segregation analysis. Functional studies were designed using patient-derived fibroblasts. Planned analyses include RNA sequencing, quantitative real-time PCR, western blotting, and microscopy to assess the expression of genes and proteins that may be involved in disease development.

Results: Three patients from two unrelated families were found to carry the homozygous frameshift variant c.353del, p.(Lys118Argfs*70), in ST3GAL5. In an additional patient, compound heterozygosity for c.353del and c.703G>A, p.(Gly235Arg), was identified. The affected individuals presented with severe neurodevelopmental impairment consistent with GM3D. Preliminary functional observations suggest loss of function ST3GAL5 protein in patient-derived cells carrying the homozygous frameshift variant.

Conclusions: The identification of recurrent ST3GAL5 variants in Polish patients expands the mutational and clinical spectrum of GM3D. Patient-derived iPSC models combined with gene and protein-level analyses may provide insight into the disease mechanisms and help identify pathways relevant to neurodevelopmental dysfunction in GM3 synthase deficiency.

C9.6.P

Beyond Gene Panels: Molecular Diagnosis of DEEs After Negative Targeted NGS Testing

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Słowa kluczowe: Developmental and epileptic encephalopathies, Panel sequencing, Whole exome sequencing

Introduction: Developmental and epileptic encephalopathies (DEEs) are a heterogeneous group of severe, early onset, drug resistant epilepsy syndromes associated with neurodevelopmental impairment and poor prognosis. Clinical presentation is highly variable and often evolves over time, making accurate diagnosis particularly challenging, especially in the initial stages of disease. Early identification of the underlying etiology is important for therapeutic decision making and prognostic assessment. Consequently, genetic testing has become an essential component of the differential diagnosis of DEEs. Despite the use of advanced genomic technologies, more than 50% of patients with DEEs remain genetically undiagnosed. Purpose: To identify the molecular cause of DEEs in patients with well characterized clinical phenotypes who remained undiagnosed after targeted next generation sequencing (NGS) panel testing.

Materials and Methods: The study group included 68 patients with clinically diagnosed DEEs who had previously undergone targeted NGS panel testing of 49 DEE-associated genes without identification of a molecular cause. Whole exome sequencing (WES) was subsequently performed. Data analysis included the detection of single nucleotide variants (SNVs) and copy number variants (CNVs).

Results: WES identified variants in genes associated with DEEs as well as in genes linked to neurodevelopmental disorders with epilepsy. Pathogenic variants were found in a small number of DEE-related genes not included in the initial panel due to limited knowledge at the time of its design. However, most pathogenic variants were identified in genes associated with neurodevelopmental syndromes in which epilepsy is not the predominant feature and are therefore not covered by DEE-focused panels.

Conclusions: WES is a valuable complementary approach in patients who remain undiagnosed after targeted gene panel testing. Most additional diagnoses involve genes linked to neurodevelopmental disorders in which epilepsy is not the primary clinical manifestation, highlighting the limitations of targeted DEE panels. NGS-based methods remain essential for improving the molecular diagnosis of DEEs and other epilepsy syndromes.

C9.7.P

Whole-exome sequencing in symptomatic patients with FMR1 premutation: searching for additional genetic causes of FXS-like phenotypes

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Słowa kluczowe: fragile X syndrome, premutation, whole-exome sequencing, autism spectrum disorder

Introduction: Fragile X syndrome (FXS) is a major monogenic cause of autism spectrum disorder and intellectual disability, usually caused by CGG repeat expansion above 200 in FMR1. In patients with ASD features and only FMR1 premutation, additional genetic mechanisms should be considered.

Aim: The aim of the study was to evaluate the diagnostic utility of whole-exome sequencing in patients with predominant ASD features and an identified FMR1 premutation, as part of a statutory project investigating the clinical significance of FMR1 premutation alleles.

Material and Methods: The study included six male patients with FMR1 premutation and a neurodevelopmental phenotype dominated by ASD. The number of CGG repeats in FMR1 was determined using TP-PCR followed by fragment analysis. In selected patients, DNA from peripheral blood and buccal epithelial cells was analysed to assess potential size mosaicism. FMR1 methylation was evaluated using MS-MLPA. Whole-exome sequencing was performed using the Twist Human Core Exome Plus Kit on the NovaSeq 6000 platform. Variants were analysed using an in-house pipeline based on GRCh38 and interpreted with reference to ClinVar, HGMD Professional 2026.1, NCBI, OMIM and the internal IMiD variant database.

Results: The FMR1 analysis confirmed premutation-range alleles and did not identify a full mutation, size mosaicism or methylation in the analysed region. Whole-exome sequencing did not reveal pathogenic or likely pathogenic variants clearly explaining the ASD-dominant phenotype, however, variants of uncertain significance were detected in genes associated with neurodevelopmental disorders and/or ASD-related features.

Conclusions: Whole-exome sequencing may be useful in patients with ASD-dominant neurodevelopmental phenotypes and FMR1 premutation, particularly when full mutation, methylation and size mosaicism are excluded. Nevertheless, further studies, including transcriptome analysis and long-read sequencing, are needed to confirm or exclude the contribution of the FMR1 premutation to ASD-related features and to identify other potential molecular mechanisms.

C9.8.P

The use of combined methods whole exome sequencing analysis and aCGH as an example of complete diagnostics of autism spectrum disorder, intellectual disability and epilepsy in half-siblings and their mother - case report

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Słowa kluczowe: array CGH, whole exome sequencing, Sanger Sequencing, autism, epilepsy, CNV, SNP, autism spectrum disorder

Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders characterized by communication and impaired social interaction, repetitive and stereotyped behaviors and restricted interests and activities. The etiology of ASD disorders still remains largely unknown, but there is no doubt that genetic and environmental factors play a crucial role in determining of these disorders. Currently, ASD is defined as a complex disorder with a multifactorial basis. In the group of patients meeting clinical criteria for ASD, approximately 75% present isolated ASD, while the remaining 25% present complex and syndromic clinical phenotypes (10% of which have specific clinical phenotypes such as Fragile X syndrome, tuberous sclerosis complex, and Angelman or Rett syndrome). The use of genetic tests based on microarrays has led to a significant increase in the frequency of identified copy number variants (CNVs) in patients with autism spectrum disorders (ASD), making this genetic method one of the most valuable tools in clinical studies of ASD patients. However, the problem lies in the clinical interpretation of submicroscopic chromosomal rearrangements, often classified as variants of unknown significance due to inconsistent genotype-phenotype correlations. An explanation of these dependencies may be complex interactions of potentially pathogenic CNVs with additional (secondary) CNVs or single nucleotide variants (SNVs). The additive effect of CNVs, single nucleotide variants (SNVs) or other sequence variants for autism spectrum disorder risk should be taken into consideration. We present the family case of two half-brothers and their mother with varied clinical phenotypes of autism spectrum disorder, intellectual disability, dysmorphic features and epilepsy. Based on the results obtained using microarray and whole-exome sequencing (WES) technologies simultaneously, it can be said with certainty, that these methods are key molecular tools in the diagnostic process in the case of families with a complex spectrum of clinical symptoms.

C9.9.P

Clinical utility of Chromosomal Microarray Analysis (CMA) in the diagnosis of neurodevelopmental disorders

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Słowa kluczowe: autism, chromosomal microarray, neurodevelopmental disorders, intellectual disability

Background: Neurodevelopmental disorders (NDDs), including intellectual disability (ID), global developmental delay (GDD) and autism spectrum disorders (ASD), are a heterogeneous group of conditions with an important genetic basis. Chromosomal microarray analysis (CMA) is recommended as a first-tier genetic test for patients with unexplained developmental disorders due to its ability to detect genome-wide copy number variants (CNVs). **Methods:** This retrospective, single-center cohort study evaluated the diagnostic utility of CMA in 573 children diagnosed with ID, GDD and/or ASD. The clinical and genetic data were collected and analyzed to assess the effectiveness of CMA as a diagnostic tool and to characterize the spectrum and clinical relevance of the detected CNVs.

Results: CMA identified submicroscopic chromosomal abnormalities (microdeletions and microduplications), across multiple genomic regions. The overall diagnostic yield was approximately 29%. The detected CNVs demonstrated considerable genetic heterogeneity. Among all identified CNVs, 36% were classified as pathogenic or likely pathogenic, whereas 61% were categorized as variants of uncertain significance (VUS). Many of the detected CNVs exhibited variable expressivity and incomplete penetrance, posing challenges for clinical interpretation.

Conclusions: CMA is an effective first-tier diagnostic tool for patients with ID, GDD, and ASD. Its genome-wide approach enables the detection of a broad spectrum of clinically relevant genomic alterations. In the studied cohort, CMA enabled the identification of numerous well-established microdeletion and microduplication syndromes. In addition, several recurrent CNVs were identified in genomic regions of potential clinical relevance that may constitute novel susceptibility loci associated with NDDs. These findings highlight the genetic heterogeneity of neurodevelopmental disorders and demonstrate the value of CMA not only as a diagnostic tool but also as an approach that may contribute to the discovery and characterization of clinically relevant genomic regions. However, the high proportion of VUS underscores the need for cautious interpretation of CMA findings and continued efforts to improve CNV classification.

C9.10.P

Somatic mosaicism in DNM1 gene in a Patient with severe neurodevelopmental disorder: a case report.

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Słowa kluczowe: neurodevelopmental disorder, DEEs, DNM1, somatic mosaicism

Introduction: Developmental and epileptic encephalopathies (DEEs) are genetically heterogenous disorders with marked phenotypic variability in which many cases remain molecularly unresolved. Somatic mosaicism is increasingly recognized as an important genetic mechanism underlying DEEs and may account for currently undiagnosed cases. DNM1 is a well-established DEE-associated gene causing autosomal dominant (DEE31A) and autosomal recessive (DEE31B) disorders. While parental germline mosaicism has been reported, somatic mosaicism in patients with DNM1-related disease has not been documented. We report a patient with severe developmental disorder in whom a low-level somatic mosaic DNM1 missense variant p.Arg361Pro was identified as the likely disease cause.

Case description: The Patient is a 19-year-old-female with severe neurodevelopmental disorder of early childhood onset, characterized by developmental delay, profound intellectual disability, absent speech, hypotonia, autoaggression, stereotypic hand movements and epilepsy. Rett syndrome was initially suspected but molecularly excluded.

Results: Whole exome sequencing identified a mosaic DNM1 missense variant p.Arg361Pro as the only plausible molecular cause. The variant affects a highly conserved residue within the stalk domain of dynamin-1, a region enriched for pathogenic variants associated with severe DNM-1 related disorders. Mosaicism, with variant allele frequency <20% was confirmed in peripheral blood, buccal swabs and hair roots, suggesting an early postzygotic mutational event and possible presence in brain tissue.

Conclusion: This is the first report of a somatic mosaic pathogenic DNM1 variant associated with severe neurodevelopmental phenotype. Identification of p.Arg361Pro within a mutational hotspot supports its pathogenic role. The phenotype severity despite relatively low mosaicism suggests either higher mutant allele burden in clinically relevant tissues or particularly strong pathogenic effect. This case expands the molecular spectrum of DNM1-related disorders and highlights somatic mosaicism's potential contribution to unresolved DEE cases.

C9.11.P

Rare non-recurrent CNVs in epilepsy plus reveal convergent genetic architecture of neurodevelopmental dysfunction

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Słowa kluczowe: epilepsy plus, neurodevelopmental disorders, array CGH, copy number variations

Background: Epilepsy with additional neurodevelopmental or systemic features (epilepsy-plus) exhibits marked genomic heterogeneity. Although rare recurrent copy number variants (CNVs) are well-established contributors to epilepsy and neurodevelopmental disorders, the role of rare non-recurrent CNVs remains poorly understood. Patients with epilepsy-plus phenotypes frequently harbor heterogeneous genomic alterations that do not correspond to recognized microdeletion or microduplication syndromes. **Materials and methods:** We retrospectively analyzed cytogenetic and clinical data from 660 patients with preexisting array comparative genomic hybridization (aCGH) or CGH+SNP array-based CNV data retrieved from the Department of Medical Genetics database at the Children's Memorial Health Institute between 2013 and 2025. From this cohort, we selected 35 individuals harboring 36 rare non-recurrent pathogenic or likely pathogenic CNVs (28 deletions and 8 duplications) for further analysis, while recurrent genomic hotspot rearrangements associated with known microdeletion/microduplication syndromes were excluded. Clinical data, including epilepsy characteristics, neurodevelopmental abnormalities, and dysmorphic features, were systematically reviewed.

Results: The identified CNVs affected genes predominantly involved in three interconnected biological pathways. Genes associated with neurodevelopmental programming and chromatin/transcriptional regulation included CHD2, SMARCA2, MBD5, MECP2, SIN3A, and TCF4. Synaptic organization and neuronal connectivity pathways involved genes such as SHANK3, NRXN1, CNTNAP2, ARHGEF9, and L1CAM. A substantial proportion of CNVs also affected genes related to neuronal excitability and ion transport, including SCN2A, SCN3A, KCNQ2, HCN2, and CHRNA4. Despite marked genomic heterogeneity and the absence of recurrent hotspot rearrangements, the identified CNVs demonstrated functional convergence within pathways critical for neurodevelopment and synaptic function.

Conclusions: Our findings suggest that genetically heterogeneous rare CNVs associated with epilepsy-plus phenotypes may share a common molecular basis affecting the development and function of neuronal networks. Integrating detailed phenotypic characterization with pathway-oriented genomic interpretation may improve the clinical assessment of ultra-rare CNVs and broaden our understanding of the genetic architecture underlying complex epilepsy disorders.

C9.12.P

WES and WGS as a Tool for Detecting Dynamic Mutations

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Słowa kluczowe: Repeat expansion (RepExp), Dynamic mutations, Myotonic dystrophy type 1 (DM1), Myotonic dystrophy type 2 (DM2), DMPK, CNBP, Whole exome sequencing (WES), Whole genome sequencing (WGS), Neuromuscular disorders, Bioinformatic analysis

Dynamic mutations caused by short tandem repeat expansions are an important cause of neuromuscular disorders. Recent ACMG recommendations indicate that RepExp can be screened using WES and WGS data using dedicated bioinformatic tools, although positive findings obtained with short-read sequencing require confirmation by alternative methods. The aim of this study was to assess the usefulness of additional RepExp analysis performed on WES/WGS data as a tool supporting the diagnosis of myotonic dystrophy type 1 and type 2 (DM1 and DM2). The laboratory has implemented an additional RepExp, performed in parallel with routine interpretation of WES/WGS data. The analysis included loci associated with DM1 (CTG expansion in DMPK gene), and DM2 (CCTG expansion in CNBP gene). All abnormal RepExp results were verified using dedicated methods (TP-PCR and GeneScan analysis). Abnormal RepExp results were identified in 17 patients. In 8 patients, the result suggested DM1, in 8 patients DM2, and in 1 patient both DM1 and DM2-associated expansions. Confirmatory testing verified all DM1-associated findings, including the patient with suspected concomitant DM1 and DM2 expansions. Among patients with suspected DM2, TP-PCR/GeneScan analysis showed a repeat number at the upper values of the normal range in 2 individuals. In some patients, the detection of DM1- or DM2-associated expansions explained the clinical phenotype. Early identification of DM1 may help prevent severe cardiac complications. RepExp analysis performed as an additional component of WES/WGS data interpretation is an effective tool supporting the diagnosis of dynamic mutations, particularly in DM1 and DM2. The obtained results support the inclusion of RepExp analysis in routine bioinformatic workflows used in NGS-based diagnostics. The detection of concomitant DM1- and DM2-associated expansions in one individual illustrates the potential advantage of simultaneous multi-loci repeat expansion screening over targeted single-locus testing. Due to the limited number of patients with detected expansions and potential borderline or false-positive results, further data collection and results confirmation remain necessary.

C9.13.P

Developmental and Epileptic Encephalopathy Caused by a DNM1 Mutation – Polish cohort characterization

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Słowa kluczowe: DNM1, developmental and epileptic encephalopathy, synaptopathy, dynamin-1, pharmacoresistant epilepsy, pediatric neurology

Background: Developmental and epileptic encephalopathy (DEE) caused by pathogenic variants in DNM1 (DEE31A, OMIM #616346) is a rare synaptopathy resulting from dominant-negative dysfunction of dynamin-1, a presynaptic GTPase essential for synaptic vesicle endocytosis. Published data derive predominantly from multinational cohorts; geographically defined series remain scarce.

Methods: We conducted a retrospective observational study at the Department of Pediatric Neurology, Pediatrics, and Rare Diseases, Medical University of Warsaw. Six Polish children (3F/3M, aged 1.5–11 years) with molecularly confirmed DNM1-related DEE were included. Pathogenic variants were identified by whole-exome sequencing or targeted NGS epilepsy panels. Clinical, neurophysiological, neuroimaging, and treatment data were collected using standardized questionnaires and on-site EEG recordings.

Results: Pathogenic variants clustered in the GTPase domain (3/6), middle domain (1/6), and intronic regions (2/6). Seizure onset occurred within the first year of life in five patients (range: 2 months – 4 years), with semiology including epileptic spasms, tonic, focal, and generalized seizures. EEG demonstrated multifocal or generalized epileptiform activity in all cases; two patients displayed hypsarrhythmia. Despite complex multi-drug regimens including corticosteroids and ketogenic diet, seizure control was achieved in only one patient. Global developmental delay was universal; none acquired expressive language, and only one child achieved independent ambulation (at age six). Axial hypotonia, cortical visual impairment, strabismus, and feeding difficulties were prevalent. MRI revealed delayed myelination (3/6), corpus callosum hypoplasia (2/6), and microcephaly (4/6). Gastrointestinal dysmotility was present in four patients.

Conclusions: This first Polish cohort of DNM1-related DEE demonstrates a severe, largely homogeneous phenotype consistent with international series. The high rate of pharmacoresistant epilepsy and profound developmental impairment underscores the unmet therapeutic need. Early genetic diagnosis is essential for prognostication, family counseling, and future enrollment in targeted therapy trials.

C9.14.P

A novel leukodystrophy subtype characterized through the case of a patient presenting with an atypical magnetic resonance imaging brain phenotype

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Słowa kluczowe: Leukodystrophy, new genetic disease, genetic diagnostics

Leukodystrophies are a heterogeneous group of inherited disorders characterized by progressive degeneration of central nervous system white matter. These conditions arise from mutations affecting myelin formation, maintenance, or turnover, leading to severe neurological impairment. A patient with an atypical pattern of brain white matter is under the care of the Institute of Mother and Child. The unusual MRI findings, like cysts arising from the cortex and thickening of the olfactory nerves, have not been found in the available literature and made us think about a possible new disease entity or new features in the MLC spectrum. The absence, atrophy or infiltration of the olfactory nerves have been described in the literature, but not their thickening or the presence of cysts. Cortical cysts were described in mitochondrial diseases, mainly in MELAS, but in a completely different configuration. Contrast enhancement of the medulla and spinal cord has not been found in the available literature on MLC either - no descriptions of the spinal cord in this disease are available. Other diseases were also considered in differential diagnosis of MR appearance of the white and grey matter:- Canavan disease – cons: partially spared subcortical white matter, partially affected corpus callosum, spared globi pallidi- L-2-hydroxyglutaric aciduria – cons: spared basal ganglia, especially the globi pallidi- merosin-deficient congenital muscular dystrophy – cons: progression of changes on consecutive MRI studies- vanishing white matter – only the hypointense signal intensity of the white matter in the anterior parts of the brain on T2 FLAIR sequence ‘fits’.The patient underwent trio based whole genome sequencing using long read technology. Comprehensive bioinformatic processing, including variant calling, structural variant detection, and inheritance pattern analysis, did not reveal pathogenic or likely pathogenic alterations that could explain the clinical phenotype. Currently, transcriptome profiling is being performed to assess gene expression abnormalities and potential splicing defects.

C9.15.P

A male patient with a confirmed pathogenic mutation in the PASC1 gene, diagnosed with the Schuurs-Hoeijmakers syndrome (SHMS) - characteristics of the clinical picture, genotype-phenotype correlation.

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Słowa kluczowe: PASC1 gene, Schuurs-Hoeijmakers syndrome

Introduction: The Schuurs-Hoeijmakers syndrome, caused by a pathogenic mutation in the PACS1 gene, is a clinically recognizable intellectual disability syndrome characterized by multiple congenital developmental anomalies. These primarily include cardiac (45% of patients), cerebral (40%), ocular (40%), and renal abnormalities. Other common manifestations include cryptorchidism, feeding difficulties, delayed or absent speech development, and hyperacusis. Patients present with characteristic dysmorphic features. Mutations in the PACS1 gene exhibit autosomal dominant inheritance

Aim: Detailed clinical characteristics and genotype-phenotype correlation in a 17-year-old male with SHMS in the context of the whole exome analysis (WES) result. The prominent clinical features of the presented patient include severe intellectual disability, absent speech development, motor stereotypies, delayed CNS myelination, bilateral cryptorchidism, epilepsy, increased muscle tone, a history of delayed closure of the anterior fontanelle, weight and height deficits, relative macrocephaly, constipation, and facial dysmorphic features. **Material and methods:** The patient underwent Whole Exome Sequencing (WES) as well as Sanger sequencing analysis to confirm the identified variant. The patient's parents are undergoing Sanger sequencing analysis as well.

Results: The patient's Whole Exome Sequencing (WES) analysis identified a pathogenic variant (c.607C>T) in one allele of the PACS1 gene. Additionally other variants were revealed including the variant of uncertain significance (VUS) of the gene QRIC11.

Conclusions: The obtained tests results establishes the diagnosis of the Schuurs-Hoeijmakers syndrome. In most cases, as in the proband, this is a de novo mutation. SHMS is an extremely rare disorder; consequently, precisely predicting the patient's long-term developmental prognosis remains challenging.

C9.16.P

Diagnostics, therapy and family planning in Duchenne muscular Dystrophy - where are we now?

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Słowa kluczowe: Duchenne muscular dystrophy, gene therapy, diagnostics

Duchenne muscular dystrophy (DMD) occurs in 1 of 3-5k boys newborns. The disease's background is mutation of DMD gene on X chromosome leading to the severe dystrophin loss and premature death due to heart and respiratory muscles failure. DMD follows an X-linked recessive inheritance pattern. Females carrying a pathogenic DMD variant and a normal allele are usually asymptomatic; however, nearly 10% exhibit mild skeletal muscle or cardiac manifestations. For every pregnancy, a female carrier has a one-in-two chance of passing the mutated allele to her offspring; affected sons will develop the disease, whereas daughters inheriting the variant will typically be carriers. Important cause of condition are also de novo mutations occurring in one-in-three cases of DMD. Spontaneous replication errors during gametogenesis or early embryogenesis can give rise to de novo pathogenic variants in the DMD gene. At the beginning, diagnostic process require creatine kinase level testing and then further genetic workflow. Genetic testing is aimed at detecting the specific pathogenic variant in the DMD gene, as the mutation type has important implications for prognosis and therapeutic decision-making. For a long time disease was the object of study, leading to new therapies outcome. The FDA has approved eight therapeutic options for DMD, which differ in their mechanisms of action and are designed either to delay disease progression, enhance muscle performance, or address particular genetic defects. However, the spectrum of DMD patients is wide and investigation on gene therapies will be developed to find universal, applicable approaches or easy to modify mechanism for highly personalised medicine.

C9.17.P

Implementation of FGF14 GAA Repeat Expansion Testing for SCA27B in Poland: Results from a Clinically Characterized Cohort of Genetically Unresolved Late-onset Ataxia Patients

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Słowa kluczowe: Spinocerebellar ataxia type 27B, Late-onset Ataxia

Background: Spinocerebellar ataxia type 27B is a recently recognized autosomal dominant late-onset cerebellar ataxia caused by a heterozygous intronic GAA repeat expansion in FGF14.

Aim: To present diagnostic experience with targeted FGF14 GAA repeat-expansion testing for SCA27B in a clinically characterized Polish cohort of patients with genetically unresolved late-onset cerebellar ataxia (LOCA).

Materials and methods: We retrospectively analyzed 191 patients suspected of having LOCA. After excluding cases with alternative or known molecular diagnoses, 53 patients with genetically unresolved LOCA remained. In most patients, common repeat-expansion ataxias had previously been excluded, including SCA1, SCA2, SCA3, SCA6, SCA7, SCA12, SCA17, DRPLA, and FMR1-associated disorders. Each patient was examined by a neurologist during the genetic counseling visit, allowing direct clinical phenotyping. The intronic FGF14 GAA repeat was assessed using long-range PCR and repeat-primed PCR, followed by capillary electrophoresis.

Results: Pathogenic or likely pathogenic FGF14 GAA expansions ≥ 250 repeats were identified in 10/53 patients (18.9%, 95% CI: 10.6–31.4%). Three patients (5.7%) carried expansions in the pathogenic/full-penetrance range (>300 repeats), and seven patients (13.2%) carried expansions in the reduced-penetrance range (250–300 repeats). Intermediate alleles in the range of 200–249 repeats were detected in three patients (5.7%).

Conclusions: Our preliminary results indicate that FGF14 GAA repeat expansions represent a relevant cause of genetically unresolved LOCA in Polish patients. Detecting SCA27B should be considered after exclusion of common SCA repeat expansions. Intermediate alleles require careful genotype–phenotype correlation and may warrant further characterization using long-read sequencing.

C10.1.P

Tissue-specific expression regulation of full-length MBNL1 via intronic polyadenylation

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Słowa kluczowe: Muscleblind-like 1, intronic polyadenylation, expression regulation

Muscleblind-like (MBNL) proteins are key regulators of alternative splicing and alternative polyadenylation. They also play a central role in the pathomechanism of myotonic dystrophy type 1 and type 2. In these diseases, toxic RNA sequesters MBNL proteins, reducing their functional pool and leading to widespread splicing dysregulation. In addition to regulating numerous splicing targets, MBNL1 itself undergoes alternative splicing, suggesting that context-dependent mechanisms controlling its expression may provide deeper insight into DM pathogenesis. In immune system cells and secondary lymphoid organs, expression of a truncated MBNL1 isoform (MBNL1-IPA) has been observed. This isoform is produced through activation of intronic polyadenylation (IPA), coupled with inclusion of a cryptic exon. Notably, its expression is negatively correlated with a level of full-length MBNL1. In this study, we investigated the regulation of MBNL1-IPA expression. After confirming its presence in immune cells, we demonstrated that its production competes with canonical splicing. Knockout of the MBNL1-IPA isoform highlights its functional relevance, as it results in an increased level of full-length MBNL1. Furthermore, we analysed transcription kinetics using α -amanitin-mediated RNA polymerase II inhibition, as well as slow and wild-type polymerase variants. Our results indicate that reduced transcriptional elongation favors inclusion of the cryptic exon. To further elucidate the regulatory mechanisms governing MBNL1-IPA expression, we identified both trans-acting factors and cis-regulatory elements controlling production of the truncated isoform. Taken together, our findings reveal a tissue-specific regulatory mechanism involving intronic polyadenylation that modulates MBNL1 expression. This mechanism, in turn, affects downstream MBNL-dependent splicing events in a context-dependent manner.

C10.2.P

BK virus reactivation after allo-HSCT – a case report

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Słowa kluczowe: BK virus, allo-HSCT, hemorrhagic cystitis, hyperbaric chamber therapy

Introduction: BK virus reactivation is one of the leading causes of hemorrhagic cystitis in patients undergoing immunosuppression following allo-HSCT. Ineffective treatment and complications can lead to secondary bone marrow failure and, ultimately, death.

Case report: A 29-year-old patient diagnosed with ALL B-common Ph(+)-p190 received allo-HSCT from an unrelated, fully HLA-matched, with a high blood group incompatibility donor, at the Bone Marrow Transplant Center of the WIM-PIB. In the post-transplant period, immunosuppression with cyclosporine, methotrexate and antithymocyte globulin was used. On the 30th day after transplantation, recovery of the white blood cell count was observed. However, normalization of morphological parameters was not achieved. On day 33, the patient developed hematuria. qPCR BKV-DNA testing revealed a result exceeding 100 million copies/ml in the urine sample and 1,700 copies/ml in the plasma. Cidofovir was added to the treatment and immunosuppression was reduced. Additionally, IVIG was administered twice. The patient's condition continued to deteriorate. After two months, hyperbaric chamber therapy was initiated, requiring 29 sessions. After the treatment cycle, the hematuria resolved, and BKV-virusuria down to 1 million copies/ml. On day 37, graft failure was diagnosed, resulting in multiple transfusions of packed red blood cells and platelets. Tests showed a reduction in recipient chimerism to 0.08%. A bone marrow biopsy revealed no evidence of disease recurrence, and no residual disease was detected. It was decided to begin another donor search.

Summary: The primary recommendation for the treatment of infections associated with BKV reactivation is to reduce immunosuppression. There is no standard treatment protocol for hemorrhagic cystitis caused by BKV, and regimens using agents such as leflunomide, cidofovir, ciprofloxacin, or IVIG are based on case reports or studies with small numbers of patients. It seems realistic that the use of therapy in a hyperbaric chamber may become an effective method in the eradication of BKV.

C10.3.P

Red blood cell antigen genotyping – a new era in transfusion immunology

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Słowa kluczowe: Red blood cell antigen genotyping , personalized medicine, transfusion medicine

The growing popularity of genetic methods could make personalized medicine a reality in transfusion medicine. Red blood cell antigen genotyping can be used to precisely determine the recipient's and/or donor's RBC phenotype, allowing for safer selection of blood for subsequent transfusions or when serological techniques fail. The aim of the study was to determine whether serological procedures for blood selection could be shortened without harming the patient, and to assess whether the use of matched RBC could reduce the risk of alloimmunization. The RBC genotyping was performed a group of patients with difficulties in serological identification and selection of blood for transfusion: - patients with autoimmune hemolytic anemia; patients after stem cell allograft transplantation; patients with two red blood cell populations; patients with MDS. Method Genotype determination was performed using the ID CORE XT kit (Progenika Biopharma). The Lumineks device allows for the detection of 29 polymorphisms defining 37 antigens in 10 blood group systems. Results: In 28 patients, genotype-based phenotype determination was necessary due to the presence of autoantibodies and a positive BTA. In seven patients with co-occurring autoantibodies and alloantibodies, genetic testing allowed for the final identification of the alloantibody and the determination of the RBC phenotype for subsequent transfusions. In four patients with alloantibodies, genotyping was necessary to determine the phenotype due to recent transfusions. In eight patients, genetic testing was performed due to the poor specificity of the alloantibodies. In 18 patients with MDS, RBC transfusions were performed to reduce the risk of immunization. In total, RBC genotyping was performed in 67 patients. The introduction of molecular techniques shortened the time to identify the phenotype, made it less complex, and the results were significantly more reliable. Our own experience demonstrates that a personalized approach to transfusion can lead to more effective and safer blood component therapy.

C10.4.P

Design and execution of the DNA Repository/Biobank IT Database of the Department of Medical Genetics at the Mother and Child Institute in Warsaw, dedicated to the Diagnostics and Prevention of Genetically Determined Diseases

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Słowa kluczowe: Biobank, DNA repository, medical database, genetic diagnostics.

Genetic information is encoded in DNA. DNA samples constitute the basis for diagnostic testing. Collections of DNA samples represent unique repositories of genetic information. One of the primary challenges in medicine is to integrate genetic and clinical data while ensuring the long-term preservation of DNA sample collections. Biobanking and DNA repositories are of fundamental importance for medical diagnostics, the development of novel therapeutic approaches as well as new pharmaceuticals. Moreover, such repositories constitute unique collections, that are often characteristic of specific populations. The aim of the project is to leverage resources from the DNA sample repository of the Department of Medical Genetics at the Institute of Mother and Child (DMG IMC) as a foundation for developing a computerized genetic database. The project assumes the creation of an electronic database of all DNA samples stored in the DMG IMC repository, linked to patients' diagnostic and clinical data. At the time of the project's initiation, the DMG IMC repository contained over 70,000 DNA samples derived from patients with genetically determined diseases and their families. The collection is continuously used for both diagnostic and research purposes. Given the unique nature of the collected material, its medical, scientific, and social value, we established appropriate conditions for the functioning of the DNA sample repository within a modern genetic laboratory. Transfer of DNA samples to a fully automated Arkitc platform provided multi-level security and storage, as well as improved organization and more efficient management of the sample collection which directly shortened the time between receiving the DNA sample and making a molecular diagnosis for the patient.

C10.5.P

Development of the wound healing model in human conjunctival air-liquid interface cultures

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Słowa kluczowe: conjunctival epithelium, organoids, air-liquid interface culture, wound healing, ocular surface

Background: The conjunctival epithelium plays a critical role in maintaining ocular surface homeostasis and regeneration following injury. Human conjunctival organoid-derived air-liquid interface (ALI) cultures represent a promising platform for studying epithelial repair mechanisms under physiologically relevant conditions. However, establishing a reproducible and standardized wound induction method in multilayered ALI conjunctival cultures remains technically challenging due to the fragility and limited adhesion of the epithelial sheet. The aim of this study was to optimize mechanical wound induction in conjunctival ALI cultures and establish a pilot model for monitoring epithelial wound closure and regeneration. **Materials and methods:** Human conjunctival organoids were dissociated into single cells and seeded onto Cultrex BME-coated 6.5-mm Transwell inserts at 200,000 cells/insert. Cultures were maintained submerged for 4 days and then exposed to air to promote epithelial stratification. On day 13/14, several mechanical wound induction methods were tested, including scratch-based and punch-based approaches. The optimized biopsy punch method was selected to generate localized and reproducible epithelial wounds. Cultures were collected at 0, 24, 48, 72, and 96 hours post-injury and analyzed by whole-mount immunofluorescence staining for ZO-1 and phalloidin to assess epithelial integrity, tight junction remodeling, actin organization, and wound closure.

Results: Conventional scratching methods (originally developed for 2D cell cultures) resulted in epithelial folding, sheet detachment, and poor wound reproducibility. In contrast, the optimized punch-based approach enabled the generation of localized and reproducible epithelial wounds with preserved surrounding tissue architecture. Progressive wound closure and epithelial remodeling were observed over time, with near-complete recovery detected within less than 96 hours after injury.

Conclusions: These findings demonstrate the feasibility of establishing a reproducible wound healing model in human conjunctival ALI cultures. The development of standardized injury protocols is essential for future studies investigating ocular surface regeneration, inflammatory responses, and therapeutic interventions in conjunctival diseases. **Funding:** EMBO Scientific Exchange Grant no. 12129 (to KJR)

C10.6.P (PRACA WYCOFANA)

C10.7.P

Transcriptomic remodeling of dermal fibroblasts exposed to rosmarinic acid

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Słowa kluczowe: rosmarinic acid, dermal fibroblasts, transcriptome, concentration-dependent effects

Rosmarinic acid (RA) is a natural polyphenol with antioxidant, anti-inflammatory and wound-healing properties, but its genome-wide effects on human dermal fibroblasts remain unclear. Primary human dermal fibroblasts were cultured under standard conditions in DMEM GlutaMAX supplemented with 10% FBS and 1% AAs. Cells were treated with RA at 5, 15 and 45 μ M for 12 and 48 hours. Total RNA was sequenced (Illumina technology), and differentially expressed genes were subjected to pathway enrichment analysis (GO BP, KEGG, FDR<0.1). At 12 hours, only 45 μ M RA induced significant transcriptomic changes, upregulating genes involved in complement, coagulation, fibrinolysis and cell migration, while downregulating genes associated with muscle development and actin cytoskeleton organization. At 48 hours, a non-linear dose-dependent response emerged. The lowest dose increased expression of genes linked to tissue morphogenesis, angiogenesis and wound healing, while decreasing transcripts for translation and ribosome biogenesis. The intermediate dose upregulated genes in p53 signaling, senescence, apoptosis and hypoxia pathways, with downregulation of cell cycle and adhesion-related genes. The highest dose strongly induced genes involved in HIF-1, focal adhesion, morphogenesis and vascular development, but profoundly suppressed genes for translation, oxidative phosphorylation and RNA splicing, indicating metabolic stress. Time-course analysis showed that untreated fibroblasts naturally reduce expression of morphogenesis-related genes over time, whereas 5 μ M RA maintains a pro-regenerative gene expression profile, and 45 μ M RA produces a biphasic pattern – early activation of migration-associated genes followed by a metabolic shutdown at the transcript level. In conclusion, RA exerts non-linear, concentration- and time-dependent effects on gene expression within functional pathways. Low-dose RA modulates genes toward a pro-regenerative state. The intermediate concentration alters expression of genes controlling senescence and apoptosis. High-dose RA, despite early upregulation of migratory genes, carries transcriptomic costs that warrant caution. These findings provide a framework for understanding RA's molecular effects in wound healing and fibrosis.

C10.8.P

High-Throughput Genotyping of 22,500 Blood Donors in Poland to Establish National Rare Blood and Genotyped Platelet Donor Registries

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Słowa kluczowe: population-scale genotyping, rare blood group genotypes, blood donor genotyping, high-throughput genotyping

Background: Recipients with red blood cell (RBC) or platelet alloimmunization require rare donors with defined antigen profiles, yet such donors are seldom identified in routine blood inventories. Large-scale donor genotyping was therefore implemented to uncover these rare phenotypes in the Polish donor population. Aim To establish a national genotype–phenotype matched rare blood and genotyped platelet donor registries within the national blood transfusion service.

Material and Methods: Eligible RBC and platelet donors were enrolled in a national programme targeting genotyping of 22,500 donors by the end of 2026. At data cutoff, 15,058 of 18,000 RBC donors had undergone high-throughput genotyping using multiplex MALDI-TOF panels covering 64 blood group antigens and HPA-1, with additional Rh, Kell, Kidd, Duffy and MNS phenotyping. In 3,513 of 4,500 platelet donors, HLA-A/B typing (solid-phase SSO) and HPA genotyping (MALDI-TOF) were performed. Rare or discrepant findings were verified by serology and alternative molecular methods. Results RBC donors genotyping identified 223 rare blood group genotypes (1.5%), and 96 confirmed rare K+k- donors, including novel KEL*02 null/mod variants (n=4). Additional phenotype-genotype discrepancies included novel Yt(a+b+) case with YT*01 allele drop-out, and multiple further yet unresolved cases (n=177). Platelet donors genotyping identified 18 HLA-A and 29 HLA-B alleles at typical frequencies, including 15% homozygous for HLA-A (13 alleles), 10% for HLA-B (18 alleles), and 2.3% for both loci. HPA genotyping revealed rare homozygotes, including 2.9% HPA-1b.

Conclusions: This programme (2021-2026), supported by the Polish Ministry of Health, improves access to antigen-matched red cell and platelet components and has already enabled provision of compatible blood products for highly alloimmunized patients. It has identified novel gene variants underlying observed phenotypes and provides a framework for further expansion of blood donor genotyping beyond 2026.

C10.9.P

Genetic analysis of gingipain-dependent intracellular survival mechanisms of *Porphyromonas gingivalis* during herpesvirus co-infection

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Słowa kluczowe: *Porphyromonas gingivalis*, intracellular survival, herpesviruses, gingipains, gingival keratinocytes

Periodontal diseases are among the most prevalent chronic infectious disorders worldwide. *Porphyromonas gingivalis*, a major periodontal pathogen, possesses numerous virulence determinants, including cysteine proteases known as gingipains. Previous studies demonstrated that *P. gingivalis* can invade and persist within host cells, classifying it as a facultative intracellular bacterium. Moreover, clinical studies have also shown a frequent association between periodontitis and herpesvirus infections. Notably, *P. gingivalis* attenuates interferon responses and promotes viral replication through the activity of its gingipain Kgp. However, the genetic and molecular mechanisms governing intracellular bacterial survival during viral infection remain unclear. Thus, the aim of our studies is to investigate the genetic contribution of gingipains to intracellular persistence, employing genetically engineered *P. gingivalis* mutants lacking functional gingipain activity. Wild-type and gingipain-deficient strains were used to infect immortalized gingival keratinocytes in the presence or absence of herpesvirus co-infection. Host-cell transcriptional responses were evaluated by analyzing genes involved in antimicrobial defense pathways. Our results demonstrate that intracellular survival of *P. gingivalis* is strongly dependent on gingipain expression. Gingipain-deficient mutants exhibited significantly reduced persistence within host cells and were more efficiently eliminated during herpesvirus co-infection compared with the wild-type strain. Interestingly, this enhanced bacterial clearance occurred independently of interferon signaling. Gene expression analyses revealed that gingipains suppress transcription of host genes implicated in intracellular pathogen restriction through iron sequestration and activation of autophagy-related processes. Loss of gingipain activity abolished this suppressive effect, resulting in enhanced antibacterial host responses. Collectively, using genetically modified *P. gingivalis* strains, we identify gingipains as key virulence factors that modulate host gene expression to promote intracellular survival. These findings provide new insights into host-pathogen interactions, gene regulatory networks involved in bacterial persistence, and the molecular basis of bacterial-viral co-infections potentially contributing to the development of novel therapeutic strategies.



Z1.0

Variants in the 5'UTR region of BRCA1 gene in dogs with mammary gland tumours

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Słowa kluczowe: Mammary glands, tumours, dogs, BRCA1 gene

BRCA1 is one of the most important genes associated with both canine mammary tumours and human breast cancers, acting as a tumour suppressor. Mutations in the 5'UTR region of this gene may affect gene expression and consequently alter protein levels or function. The aim of the study was to analyse the changes in 5'UTR region in BRCA1 gene in 32 tumour and matched healthy tissue samples obtained from dogs affected with mammary tumour and determine whether the changes are somatic or germline. DNA from 32 samples obtained from 12 dogs with mammary cancer (2 males and 10 females) were isolated. One dog had 5 separate tumours on the left and right side of 4th and 5th pair of nipples. PCR 5'UTR region of BRCA1 gene was conducted using primers designed by Enginler et al. (2014) and the products were sequenced. Four of the tumours were carcinoma tubulo-papillare, two carcinoma solidum, two carcinoma complexus, one carcinoma anaplasticum, two osteosarcomas and one chondrosarcoma. The mutation c.1818G>C was found in all samples from which 15 were heterozygotic and 17 homozygotic. The mutations were consistent across all samples coming from each individual dog, thus four dogs were heterozygotic and eight had homozygotic genotypes. There was no connection between malignancy level and homozygosity. Since the same variants were found in tumours and healthy tissues of every single dog, and in all tumors from the dog who had multiple tumours, it can be concluded that the changes were hereditary and thus 5'UTR region of BRCA1 gene might be prognostically important. However, its link with carcinogenesis still needs to be determined. The study was funded by the National Science Centre in Poland, grant no. 2019/35/B/NZ5/00775.

Z2.O

Assessment of chromosomal abnormalities in bull sperm using molecular cytogenetic methods

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Słowa kluczowe: Bos taurus, chromosomal aberrations, sperm-FISH, Robertsonian translocation

Analysis of fluorescent signal copy numbers in sperm cells enables the assessment of meiotic segregation patterns and the detection of chromosomal abnormalities such as aneuploidies and translocations. These aberrations may negatively affect fertility and can spread rapidly in breeding populations through assisted reproductive technologies, emphasizing the importance of early detection. In addition to autosomal abnormalities, irregular segregation of sex chromosomes (X and Y) in spermatozoa may also occur and can lead to the formation of aneuploid gametes, potentially affecting embryo viability and reproductive outcomes. The most common chromosomal rearrangement in cattle is the Robertsonian translocation (1;29). However, despite its importance, there is still limited data on the frequency and patterns of chromosomal abnormalities in bull spermatozoa in Poland. Therefore, the aim of this study was to assess the occurrence and distribution of selected chromosomal abnormalities using the sperm-FISH technique. The study was conducted on semen samples from 100 bulls obtained from the Malopolska Biotechnic Centre bank. The sperm-FISH technique was performed using BAC molecular probes targeting chromosomes – BTA1 (INRA-989G02), BTA29 (INRA-317E09) and BTAX (CH240-220K7), whereas the BTAY probe was generated by chromosome microdissection. The analysis enabled the detection of spermatozoa with fluorescent signal patterns corresponding to chromosomal abnormalities, including potential aneuploidies and translocations involving these chromosomes. Statistical evaluation revealed a significant inter-individual variation in the proportion of abnormal spermatozoa (Chi-square and Fisher test, $p < 0.0001$). Fourteen bulls exhibited abnormal signal patterns involving chromosomes 1 and 29, whereas three additional bulls showed abnormalities involving the sex chromosomes X and Y. A significantly higher percentage of abnormal spermatozoa was observed, which may be associated with reduced fertility. These findings highlight the usefulness of sperm-FISH as an effective cytogenetic tool for identifying chromosomal abnormalities affecting fertility and the genetic quality of breeding populations. Financed by the Chromobull project, grant number: 00101.DDD.6509.00197.2022.06.

Z3.0

Reproductive aging and increased sex chromosome aneuploidy in purebred Arabian stallion sperm: a longitudinal sperm-FISH study

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Słowa kluczowe: aging, aneuploidy, purebred Arabian stallion, reproductive

Arabian horses are among the most highly valued equine breeds worldwide due to their versatility, endurance, and exceptional genetic background. Because of their high breeding value, maintaining reproductive health and fertility is of major importance in modern horse breeding. Age is known to influence the reproductive potential of stallions: sexual maturity is typically reached between 3 and 4 years of age, whereas reproductive performance changes with age. Therefore, the aim of this study was to evaluate the effect of age on the frequency of sex-chromosome aneuploidies in the semen of Arabian stallions. Semen samples were collected longitudinally from 10 purebred Arabian stallions at three time points (2008, 2010, 2014). Fluorescence in situ hybridization (FISH) with probes specific for sex chromosomes was applied to assess sperm aneuploidy rates. In total, 3,000 sperm nuclei per sample were analysed. Stallions were classified into three age groups: young (≤ 5 years), adult (6–15 years), and senior (≥ 16 years). Statistical analyses were performed using quasi-binomial generalized linear models adjusted for repeated measurements within stallions. Significant age-related differences were observed for YY aneuploidy ($F(2,18)=5.44$, $p=0.014$) and for the total number of chromosomal aberrations ($F(2,18)=6.28$, $p=0.009$), while XX aneuploidy showed a marginal effect ($F(2,18)=3.18$, $p=0.066$). Adult stallions exhibited the lowest aberration rates, whereas both young and senior stallions showed increased frequencies of aneuploid spermatozoa, supporting a U-shaped relationship between age and chromosomal instability. The highest risk was observed in senior stallions, with odds ratios exceeding two-fold compared with adult animals. These findings indicate that stallion age significantly influences the occurrence of sex-chromosome aneuploidies in spermatozoa. The increased frequency of chromosomal abnormalities observed in both young and senior stallions may have implications for fertility and breeding management in Arabian horses.

Z4.O

Heteroplasmy in the mitochondrial genome observed in dogs with malignant mammary gland tumours

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Słowa kluczowe: canine mammary tumours; mitochondrial DNA; mtDNA mutations; VNTR heteroplasmy; canine oncology

Mitochondrial DNA (mtDNA) alterations are potentially implicated in tumourigenesis, yet their contribution to canine cancer remains insufficiently defined. Because mitochondria regulate oxidative phosphorylation, metabolism, reactive oxygen species generation and apoptosis, mtDNA variability may affect processes relevant to neoplastic transformation. This study aimed to identify heteroplasmic sites in complete mitochondrial genomes of dogs with malignant mammary tumours and to evaluate their potential association with tumour development. Mitochondrial genomes from the Polish canine population were analysed, including tumour tissue, blood and healthy tissue from 25 dogs. Tumour types were confirmed histopathologically. MtDNA was amplified by long-range PCR, sequenced using an Illumina MiSeq platform, assembled and compared with the reference sequence from GenBank. Variant annotation was performed using UGENE and Canis mitoSNP tool. Overall, 198 heteroplasmic variants in the mitogenome were found, from which only four were located outside the VNTR region (m.8617G/A, m.9470G/A, m.11028T/C, m.13056A/G). There were no heteroplasmic positions in healthy tissue. Interestingly, the heteroplasmy occurred in 11 out of 25 dogs and its level was comparable between tumour and blood. The most frequently heteroplasmic sites were: m.16168A/G, m.16188G/A, m.16288A/G, m.16298A/G. These findings demonstrate increased mtDNA variability in both blood and tumour tissues in canine malignant mammary tumours, yet it is limited to non-coding region of the mitogenome. This may suggest that protein-coding regions are more conserved and less prone to heteroplasmy, yet its relationship with carcinogenesis remains unclear. This research was funded by the National Science Centre in Poland, grant number 2019/35/B/NZ5/00775.

Z5.0

Reproductive aging in stallions assessed by sperm RNA profiling: preliminary insights

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Słowa kluczowe: stallion, sperm, RNA profile, RNA integrity

Sperm quality is known to decline with age, leading to reduced motility, impaired fertilizing capacity, and increased molecular damage within sperm cells. Although age-related changes in semen characteristics have been widely described, the molecular mechanisms underlying these processes remain insufficiently understood, particularly in horses. Therefore, transcriptomic profiling of equine sperm cells may provide valuable insight into age-associated alterations in reproductive biology and help identify molecular markers related to semen quality and fertility in different horse breeds. Due to the low quantity and highly fragmented nature of sperm RNA, transcriptomic analyses of spermatozoa remain technically challenging. In the test samples analyzed in this study, RNA Integrity Number (RIN) values ranged from 1.6-2.6, while RNA purity parameters reached $A_{260}/_{280} = 1,81-2,02$; $A_{260}/_{230} = 0,68-1,29$, with RNA quantity ranging from 425,6-980 ng. Such characteristics may limit the effectiveness of conventional RNA sequencing methods, highlighting the need for approaches suitable for degraded and low-input RNA. In this preliminary study, we evaluated the applicability of a 3' RNA sequencing approach for transcriptomic analysis of equine sperm samples, with particular emphasis on the methodological challenges associated with the low RNA yield and extensive RNA fragmentation characteristic of stallion spermatozoa. The generated test libraries exhibited the expected quality parameters and sequencing characteristics. Next-generation sequencing (NGS) produced an average of 9.3 million raw reads per sample ($SD = 0.66$), of which 73.9% mapped to the equine reference genome and 47.0% were uniquely mapped. However, subsequent analyses revealed that only 25.3% of the reads aligned to annotated gene regions. This relatively low proportion of on-target reads highlights the need for further optimization of RNA-Seq methodologies for equine spermatozoa, particularly with respect to library preparation and transcript enrichment strategies, in order to improve library specificity and overall data quality.

Z6.0

Omentin-1 remodels the transcriptome of GT1-7 GnRH neurons and identifies calcium, MAPK and GnRH secretion pathways as targets of metabolic regulation

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Słowa kluczowe: omentin-1; GnRH neurons; transcriptome; GT1-7 cells; calcium signaling; reproductive genetics

Background: Omentin-1 (OMNT1) is a metabolism-related adipokine potentially involved in reproductive regulation, but its role in GnRH neurons remains unclear. We hypothesized that OMNT1 alters gene expression and signaling pathways controlling GnRH synthesis and secretion. This study aimed to investigate OMNT1-induced transcriptomic changes in hypothalamic GT1-7 cells.

Methods: GT1-7 cells were treated with OMNT1 at 100 ng/mL and subjected to RNA-seq. Differentially expressed genes were annotated using DAVID, KEGG, GeneMANIA, and Reactome. Functional validation included dose- and time-dependent analyses of *Gnrh1* mRNA, GnRH protein and secretion, cAMP levels, and ERK1/2 and PKC phosphorylation, followed by pathway inhibition. RNA-seq and functional data were analyzed using five biological replicates, with significance set at $q < 0.05$ and $p < 0.05$, respectively; functional assays were assessed by one-way ANOVA with Tukey's post hoc test.

Results: Transcriptomic analysis revealed that OMNT1 altered the expression of 863 genes, including 481 upregulated and 382 downregulated genes. Functional enrichment showed that OMNT1 affected gene categories associated with neuronal function and synaptic communication, signal transduction, metabolism and bioenergetics, as well as cell cycle and apoptosis. KEGG pathway analysis identified calcium signaling, MAPK signaling, cAMP signaling, and GnRH secretion as relevant pathways modified by OMNT1. Among the regulated genes were components involved in calcium handling and intracellular signaling, including *Cacna1g*, *Slc8a2*, *Camk1d*, *Calm5*, *Prkcg*, *Plcb2*, *Kiss1r*, and *Gnrh1*. Functionally, OMNT1 reduced *Gnrh1* mRNA expression after 30 min and 24 h, while transiently increasing GnRH protein levels after 15 and 30 min and stimulating GnRH secretion after 30 min. OMNT1 decreased cAMP levels and activated ERK1/2 and PKC signaling, whereas ERK1/2 or PKC inhibition reduced OMNT1-induced GnRH secretion.

Conclusions: These findings indicate that OMNT1 induces transcriptomic remodeling in GnRH neurons and identifies calcium-, MAPK-, cAMP-, and PKC-related pathways as molecular targets linking metabolic signals with reproductive neuroendocrine regulation.

Funding: National Science Centre grant 2024/53/N/NZ9/01916; Foundation for Polish Science START 2026

Z7.0

The impact of aflatoxin B1 on the mitochondrial MT-ATP6 gene in the domestic pig

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Słowa kluczowe: aflatoxin, domestic pig, mutations, mtDNA, MT-ATP6

Aflatoxin B1 (AFB1) present in feed generates cytotoxic oxidative stress, posing a direct threat to mitochondrial integrity in the domestic pig. Investigating changes within the mitochondrial MT-ATP6 gene may help to determine whether AFB1 exposure is associated with genetic alterations potentially affecting oxidative phosphorylation.. The aim of the study was to examine the molecular changes in the MT-ATP6 gene of the domestic pig after AFB1 exposure. Moreover, the hepatoprotective effect of *Silybum marianum* (SM) was analysed. Blood samples from 26 domestic pigs were analyzed and divided into a control group (untreated) and two experimental groups. Animals in the test groups received AFB1 at a concentration 120 µg/kg of body weight or AFB1 (120 µg/kg) in combination with SM at a dose of 90 µg/kg. The genetic material isolated from all individuals was then subjected to PCR amplification and Sanger sequencing. In the studied groups, 16 polymorphic variants were identified at four variable positions (m.9355C>T, m.9369T>C, m.9473T>C and m.9525T>C). The m.9355C>T variant was synonymous, whereas the remaining variants were non-synonymous and resulted in non-conservative amino acid changes. These variants were detected in 3 of 8 individuals from the AFB1-treated group and in 1 of 8 individuals from the control group. The identical presence of genetic variants in both the control and toxin-treated groups might indicate that the animals were born with these changes, which excludes their association with aflatoxin administration. No modulatory effect of SM on the MT-ATP6 variant profile was observed in the studied groups, as the same polymorphisms were present regardless of exposure. The research is being conducted as part of a research project funded by NCN–OPUS-22LAP, project number: UMO-2021/43/I/NZ9/02612B. Keywords: aflatoxin, domestic pig, mutations, mtDNA, MT-ATP6

Z8.O

Tissue-specific microRNA expression following in ovo administration of galactooligosaccharides and sodium butyrate in broiler chickens

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Słowa kluczowe: miRNA, tissue-specific expression, in ovo administration, broiler

Background: Early-life nutritional interventions administered in ovo may induce long-term physiological changes in poultry. In our previous study, we demonstrated that in ovo administration of galactooligosaccharides (GOS) and sodium butyrate (SB) altered the intestinal microbiota of broiler chickens. However, their influence on host microRNA (miRNA) expression remains poorly understood. Therefore, this study evaluated tissue-specific changes in miRNA expression following in ovo administration of GOS and SB. **Materials and methods:** Fertilized Ross 308 eggs were randomly assigned to four experimental groups: non-injected control, saline-injected control, GOS, and SB. Bioactive compounds were administered on day 12 of incubation. Liver, ileum, and cecum samples collected at 42 days of age were subjected to small RNA sequencing using the Illumina platform. Differential miRNA expression was analyzed using DESeq2, with the saline-injected group serving as the reference. **Results:** The effect of the injection procedure differed among tissues, affecting hepatic and cecal miRNA profiles but not the ileum. After accounting for this effect, both GOS and SB were associated with tissue-specific changes in miRNA expression, with a greater number of differentially expressed miRNAs observed following SB administration. Several identified miRNAs have previously been associated with immune regulation, epithelial homeostasis, and metabolic processes.

Conclusions: The results indicate that in ovo administration of GOS and SB may be associated with tissue-specific alterations in miRNA expression profiles in broiler chickens. These findings extend previous observations on microbiota modulation and identify miRNA expression profiling as a promising approach for investigating the molecular responses to early-life nutritional interventions.

Z9.0

Scaling up bovine muscle satellite cell culture towards cultivated meat production: from flasks to bioreactors - preliminary studies

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Słowa kluczowe: bioreactor, 3D cell culture, gene markers expression, muscle satellite cells, cultured meat

With advances in bioengineering tools, recreating the process of myogenesis in vitro has become progressively feasible. By utilizing muscle satellite cells (MuSCs) - muscle precursors - and appropriately modulating the culture environment, it is possible to obtain advanced structures resembling muscle tissue, thereby influencing the expression of myogenic markers. The aim of this study was to examine changes in the expression of key myogenic markers - PAX7, ITGA7, and genes from the MRF family (MYF5, MYOD, MYF6, and MYOG) in CD31-/CD45-/CD29+/CD56+ bovine MuSCs. Cells were cultured across three sequential stages: i) in standard two-dimensional culture in flasks for one week, ii) pre-culture on gelatin microcarriers in a spinner flask for one week, and iii) on gelatin microcarriers in 5L bioreactors (BioStat B Twin, Sartorius) for one week. Relative gene expression was determined by qPCR using the RT HS-PCR MIX SYBR kit (A&A Biotechnology), with RPL27 and OAZ1 used as endogenous controls. The results showed significantly lower relative expression of MYF5 (involved in proliferation) under 2D cell culture conditions, compared to spinner flasks and bioreactors. MYF6 expression (involved in early myogenic differentiation) was significantly lower under 2D cell culture conditions compared to spinner flask culture. Furthermore, significantly the lowest relative expression of PAX7 (critical for MuSCs activation and self-renewal) was observed in cells cultured in spinner flasks. These results indicate the potential of using bioreactors as a culture system to support muscle cell expansion and recreate myogenesis in vitro. Moreover, our recent studies suggest that myogenesis can be modulated by plenty of factors such as: i) fibroblast-derived paracrine signaling, which promotes early myogenic differentiation (especially extracellular vesicles), chemical modulators (like addition of resveratrol) which promote proliferation of MuSCs and re-oxygenation modulation, which support self-renewal of MuSCs. Combining these factors with further development of bioreactor cultivation methodology is crucial for further research.

Z10.O

Effects of dietary *Lepidium meyenii* supplementation on molecular and metabolic traits in pigs

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Słowa kluczowe: RNA-seq, qPCR, DNA methylation, fatty acid profile, oxidative stability

Functional feed additives may affect animal physiology through transcriptional and epigenetic regulation of metabolic pathways. This study investigated the effects of dietary supplementation with *Lepidium meyenii* (Peruvian maca) on gene expression, promoter DNA methylation, fatty acid composition, oxidative stability, and blood lipid profile in pigs. Forty female pigs were allocated to two groups (n = 20/group). The control group received a standard diet, whereas the experimental group was supplemented with 1% maca (10 kg/t feed). After 108 days, animals were slaughtered and tissues collected for analysis. Muscle transcriptome profiling (n = 10/group) revealed no differentially expressed genes (DEGs). In contrast, liver transcriptome analysis identified differences in gene expression and 8 DEGs (CLDN4, INHBE, DCN, SREBF1, PPP1R3C, PPP1R3B, TUBA4A and GCK), including genes associated with glucose metabolism, glycogen synthesis, energy homeostasis, and cytoskeletal organization were included in further analysis. Expression changes in five genes (INHBE, PPP1R3C, PPP1R3B, TUBA4A and GCK) were confirmed by qPCR in a larger cohort (n = 16/group). Promoter methylation analysis showed significant differences at three CpG sites in INHBE, one in PPP1R3C, and eleven in PPP1R3B. Maca supplementation induced only minor changes in fatty acid profiles of muscle and backfat tissues. Blood biochemical parameters were largely unaffected, except for a higher glucose concentration in the experimental group. Lipid oxidation increased during storage in both groups and was not influenced by treatment; however, oxidative induction time was significantly prolonged in backfat from maca-fed pigs. Expression of antioxidant-related genes (SOD2, CAT, GSR, and NFE2L2) in adipose tissue did not differ between groups. In conclusion, dietary *L. meyenii* supplementation induced modest changes in hepatic gene expression and DNA methylation, while exerting limited effects on tissue lipid composition, blood lipid profile, and antioxidant gene expression. This study was funded by the National Science Centre, Poland (grant no. 2020/39/O/NZ9/00816).

211.O

Evaluating the Hepatoprotective Effects of Medicinal Herbs Against Aflatoxin B1 Anatomic and Molecular Toxicity: An In Vivo Pig Model

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Słowa kluczowe: liver, mycotoxin, herbs, domestic pig, multi -omics, transcriptomics, proteomics, metabolomics

Background: Aflatoxin B1 (AFB1) is a pervasive mycotoxin in animal feed that induces severe hepatotoxicity, jeopardizing livestock health and production. The presentation includes the ongoing OPUSLAP-DFG preliminary results and evaluation of the capacity of dietary hepatoprotective herbs—*Andrographis paniculata* (AP), *Silybum marianum* (SM), and *Curcuma longa* (CL)—to attenuate AFB1-induced liver damage in growing piglets.

Methods: The in vivo protocol utilizes post-weaning piglets over 4 weeks across two distinct stages. In Stage 1 (Pilot, n=24), we established the subacute toxicity thresholds using escalating dietary doses of AFB1 (30, 60, and 120 µg/kg) against a control group to avoid acute liver failure. In stage 2 (main study, n=168), we evaluated the interaction between AFB1 and varying doses of AP, SM, and CL extracts across 12 experimental subgroups, including phenotypic and systemic changes via blood profiling and liver histopathology. At the molecular level, liver tissue samples underwent comprehensive transcriptomic, proteomic, and metabolomic profiling, alongside the analysis of mitochondrial DNA variations. Integrative bioinformatics road-mapped the multi-omics dataset to identify disrupted and protected metabolic pathways. Preliminary Results: are presented as six posters to define the precise physiological and systemic thresholds of AFB1 toxicity in weaners and identify the specific molecular pathways through which AP, SM, and CL extracts exert their hepatoprotective effects.

Conclusion: Preliminary results delivered a comprehensive, systemic framework for using targeted herbal supplementation to protect young livestock from severe mycotoxin-induced metabolic disorders, optimizing swine health and feed safety. Acknowledgements This research was funded by the NCN (Narodowe Centrum Nauki)—Project number UMO-2021/43/I/NZ9/02612—and the Deutsche Forschungsgemeinschaft (DFG: German Research Foundation)—Project number 504983453 (WI 1754/18).

Z12.O

Analysis of mutations in the mitochondrial MT-ND1 gene in dogs with mastocytoma

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Słowa kluczowe: mast cell tumours, dogs, mtDNA

Introduction: Mast cell tumours (MCT) are one of the most common skin tumours in dogs. Due to its complex aetiology, varying degrees of malignancy and strong links to breed predisposition and genetic mutations, MCT is a key focus of research aimed at improving diagnostic and prognostic methods. The aim of this study was to analyse changes in the mitochondrial MT-ND1 gene in dogs diagnosed with mast cell tumours.

Materials and methods: The study included a group of 7 dogs from which tumour tissue and peripheral blood were collected. All tumours underwent histopathological assessment. Molecular analysis involved the amplification of the mitochondrial MT-ND1 gene, followed by Sanger sequencing of the PCR products. The results were subjected to bioinformatic analysis using the Unipro UGENE software, which enabled the identification of nucleotide changes. Furthermore, the CanisMito SNP tool was used to analyse changes at the protein level. Results: Four major polymorphisms were detected in blood and tumour samples, at positions: m.2962C>T, m.3028A>C, m.3196T>C, m.3247T>C. All were synonymous, did not alter the amino acid sequence of the MT-ND1 protein or affect its structure or function. These changes indicate polymorphisms of mtDNA of a germinal nature. One potential tumour-specific somatic mutation was identified: m.3194C>T leading to a p.Leu150Phe amino acid substitution in the α -helix region of the MT-ND1 protein. This change may affect the protein structure and potentially modulate the mitochondrial metabolism of cancer cells.

Conclusions: Most variants identified in the MT-ND1 gene were synonymous polymorphisms, likely reflecting natural mtDNA variability. One m.3194C>T variant found in tumour tissue may represent a potential somatic mutation causing a protein substitution. This finding and its potential effect on carcinogenesis process require confirmation on a larger cohort.

Keywords: mast cell tumours, dogs, mtDNA

Z13.O

A candidate piRNA drives DNA methylation changes in equine sarcoid cells

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Słowa kluczowe: DNA methylation, equine sarcoid cells, piRNA, RRBS

Equine sarcoids are the most common skin tumours in horses, associated with bovine papillomavirus (BPV) infection, yet the molecular mechanisms underlying their neoplastic transformation remain unclear. To our knowledge, this is the first study to investigate the functional role of piRNAs in equine sarcoid pathogenesis, opening a new avenue in the epigenetic understanding of this common equine neoplasm. Recent evidence suggests that Piwi-interacting RNAs (piRNAs), key regulators of epigenetic processes, may contribute to cancer-related processes. Based on previous findings, we selected one of the most significantly overexpressed candidate piRNAs in sarcoid tissues for functional analysis. Primary sarcoid cell lines derived from seven equine sarcoids were transfected with the piRNA mimic (Genomed) alongside appropriate controls. Global DNA methylation levels, assessed using the MethylFlash Global DNA Methylation ELISA Kit, revealed a significant increase in transfected cells (0.1%; $p < 0.05$). To investigate site-specific methylation changes, we prepared Reduced Representation Bisulfite Sequencing (RRBS) libraries sequenced on a NovaSeq X Plus. A total of 98,127 differentially methylated sites (DMSs) were identified ($|\text{met. diff}| \geq 25$; $\text{FDR} < 0.1$), including 63,312 sites localised within gene bodies and promoter regions ($\text{TSS} \pm 2 \text{ kbp}$). Functional enrichment analysis revealed hypomethylated DMS-associated genes were overrepresented in tumorigenesis-related pathways, including PI3K-Akt, and calcium signalling, while hypermethylated DMS-associated genes were enriched in MAPK and Rap1 signalling. Notably, growth factor signalling genes, such as FGFR2, VEGFA, and KIT, were identified, suggesting potential involvement in fibroblast activation and neoplastic transformation. The next stage of the study will integrate methylation and transcriptomic profiles of transfected sarcoid cells to identify genes potentially regulated by piRNA-associated methylation changes. Together, these preliminary results indicate that the candidate piRNA plays a significant role in the epigenetic regulation of equine sarcoid development, influencing DNA methylation across pathways governing fibroblast activation and cell proliferation. Financed from: National Science Centre (Poland) grant UMO-2021/43/D/NZ9/02763.

Z14.O

Deciphering the Hepatic MicroRNA Response to *Silybum marianum* Herbal Supplementation in a Porcine Model

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Słowa kluczowe: silymarin; silibinin; pigs (*Sus scrofa*); hepatoprotection; liver dysfunction; oxidative stress; nutraceuticals

Background: *Silybum marianum* (SM) is widely recognized for its hepatoprotective properties, yet its direct influence on the hepatic microRNA (miRNA) regulatory landscape in livestock remains poorly characterized. This study investigated the specific effects of dietary SM supplementation on the porcine liver miRNA expression profile to clarify its underlying physiological mechanisms.

Methods: Commercial crossbred TN70 pigs (Norsvin Landrace × Large White) underwent a 7-day adaptation period before allocation into experimental groups, including a control group (basal diet; n = 4) and an SM group (n = 10). Dietary supplementation was administered from day 42 until the conclusion of the trial on day 70, and hepatic tissues were collected for total RNA extraction. Small RNA libraries (145–160 bp) were prepared and sequenced on an Illumina NovaSeq 6000 platform. High-quality reads were mapped to the *Sus scrofa* reference

genome (Sscrofa11.1) using Bowtie, and differential expression analysis was executed via DESeq2 (padj < 0.10). Downstream functional pathway enrichment was evaluated using ClueGO.

Results: Transcriptomic profiling of the SM group (n = 10) versus the control group (n = 4) identified 15 significantly differentially expressed miRNAs (13 upregulated, 2 downregulated). The most robustly induced molecules were ssc-miR-100 ($\log_2FC = +3.48$), ssc-miR-1271-5p ($\log_2FC = +3.22$), and members of the miR-99 family (ssc-miR-99b and ssc-miR-99a-5p). Downregulation was restricted to ssc-miR-324 and ssc-miR-26b-5p. Functional enrichment analysis linked the upregulated miRNA profile to the suppression of intracellular signal transduction, macromolecule catabolic processes, and the MAPK signaling pathway. Conversely, the downregulated miRNA signature was associated with the activation of macroautophagy and IgSF CAM signaling.

Conclusion: Dietary SM supplementation characteristically modulates the porcine hepatic miRNA climate, primarily driving the attenuation of proliferative signaling cascades and the fine-tuning of selective metabolic and autophagic pathways.

Acknowledgements: This research was funded by Narodowe Centrum Nauki UMO-2021/43/I/NZ9/02612, and Deutsche Forschungsgemeinschaft: 504983453 (WI 1754/18).

Z15.O

Transcriptomic Landscape of Coding and Non-Coding RNAs in Normal and Abnormal Canine Gonads

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Słowa kluczowe: mRNA, lncRNA, miRNA, piRNA, RNAseq, spatial transcriptomics

Cryptorchidism and testicular or ovotesticular disorders of sex development (DSD; XX, SRY-negative) are among the most common congenital abnormalities of the canine urogenital system. Despite their clinical relevance, the molecular mechanisms underlying these conditions remain incompletely understood. This study aimed to comprehensively characterize coding and non-coding transcriptomes in abnormal canine gonads and to identify molecular signatures associated with impaired gonadal development. Comparative analyses were performed on abdominal testes of dogs with cryptorchidism and abdominal testes or ovotestes of XX (SRY-negative) DSD dogs. Scrotal testes and ovaries were used as controls. Transcriptomic profiling of coding (mRNAs) and non-coding RNAs (miRNAs, piRNAs and lncRNAs) was conducted using RNA sequencing (RNA-seq). In addition, spatial transcriptome profiling of protein-coding genes was performed with Visium Spatial Gene Expression technology on abdominal and inguinal testes and their contralateral scrotal testes of dogs with unilateral cryptorchidism. RNA-seq analysis revealed extensive transcriptional remodeling in abnormal gonads. More than 9,000 differentially expressed genes (DEGs) were identified in abdominal testes from cryptorchid dogs and in abdominal testes or ovotestes from dogs with testicular or ovotesticular XX DSD when compared with normal scrotal testes. Furthermore, over 4,100 DEGs were detected between ovotestes and normal ovaries. Substantial alterations in non-coding RNA expression were also observed, including more than 450 differentially expressed miRNAs, 433 piRNAs, and over 2,400 lncRNAs in comparisons of undescended and descended testes. Spatial transcriptomic analyses confirmed these findings, revealing more than 5,600 DEGs between abdominal and control testes and over 7,400 DEGs between inguinal and control testes. In conclusion, these results demonstrate profound transcriptomic dysregulation in abnormal canine gonads and highlight the involvement of both coding and non-coding regulatory networks to gonadal pathology. [This study was funded by the National Science Centre, Poland, grant no. 2023/51/B/NZ9/01165]

Z16.O

Beyond morphological equivalence: heterogeneity of mtDNA, lipid, and transcript profiles in sister blastomeres of 2-cell parthenogenetic porcine embryos.

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Słowa kluczowe: lipids, transcriptome, embryo, mtDNA, single cell, cell fate

The very first stages of mammalian embryonic development are governed by transcripts and proteins of maternal origin. At strictly species-specific time, following a number of mitotic divisions, embryonic genome activation occurs to take control over development. Some models are proposed for segregation of transcripts, proteins and organelles during first cell divisions which may drive the cell fate. We aimed to analyse the differences in the number of mtDNA copies, lipid droplet content and transcriptional profile of single blastomeres during first embryo cleavage. Porcine cumulus oocyte-complexes (COCs) were matured in vitro and activated with ionomycin (5uM) followed by 6DMAP (2mM) treatment and cultured using Primovision time laps system to monitor timing of first cleavage. The 2-cell stage embryos were fixed and stained with Bodipy 493/503 to visualize lipid droplets (confocal microscopy). In parallel embryos were disaggregated and single blastomeres used for transcriptomic (scRNA-seq) and mtDNA (qPCR) analyses. RNA was isolated using Smarter Ultra Low Input RNA Sequencing kit for Illumina (Clontech) followed by library prep using Nextera XT (Illumina). RNA-Seq was performed using Novaseq platform (Illumina). mtDNA was quantified using absolute quantification method in real-time PCR (single cell level). Sister blastomeres originating from individual 2-cell embryos, differed in lipid droplet (LD) content by 16.7% to 561.7%. Only 25% of embryos showed a similar LD distribution in blastomeres. Similarly, mtDNA was asymmetrically distributed in as many as 65% of embryos. Single-cell RNA sequencing revealed, on average, 14,126 aligned transcripts per blastomere. Blastomeres showed differential gene expression in all embryos, with 96 to 489 DEGs identified per embryo. The top enriched pathways include meiosis, proteolysis, phosphorylation, mitochondrial respiratory chain, mTOR signaling and cell-cell junction development. These data show uneven distribution of oocyte storage materials during first cleavage which may influence cell fates during subsequent mitotic divisions. Funding: NSC Poland, SONATA (2014/13/D/NZ2/03901), Statutory funding of the Faculty of Vet. Med. and Animal Sciences PULS.

Z17.O

Hepatic microRNA Profiling Reveals Novel Regulatory Transcripts in Pigs Exposed to Silymarin or Aflatoxin B1

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Słowa kluczowe: Silybum marianum, Herb supplementation, Liver health, Mycotoxin, Nutrient utilization, MicroRNA.

Background: Aflatoxin B1 (AFB1) is a mycotoxin known to damage the liver, increase oxidative stress, and disturb normal metabolic balance in livestock. Silymarin (SM), a lipophilic extract from *Silybum marianum*, is widely recognised for its antioxidant and hepatoprotective effects, yet its miRNA-mediated role during AFB1 exposure remains unclear. In this study, we used small RNA sequencing to profile hepatic miRNAs in pigs and examine the regulatory changes associated with SM treatment.

Methods: The study included 16 TN70 crossbred pigs (Norsvin Landrace × Large White) housed under controlled conditions and given water ad libitum. Small RNA libraries from liver samples were sequenced to identify differentially expressed miRNAs (AFB1, n = 6; SM, n = 10). Putative targets were predicted with RNAhybrid, followed by GO and KEGG enrichment analyses with Bonferroni step-down correction. Results SM and AFB1 groups differed in the expression of 18

miRNAs, with 14 showing higher expression and 4 showing lower expression, and two groups separated in both the heatmap and volcano plot. Clearest increases were seen for ssc-miR-100 ($\log_2FC = +3.03$), ssc-miR-1271-5p, and ssc-miR-221-5p. Analysis of their predicted targets indicated significant suppression ($p_{adj} < 0.001$) across 412 pathways, particularly those involved in intracellular signal transduction, macromolecule catabolic processes, and MAPK signalling ($p_{adj} < 0.0004$). Four miRNAs showed lower expression, namely ssc-miR-802 ($\log_2FC = -0.81$), ssc-miR-140-5p, ssc-miR-26b-5p, and ssc-miR-155-5p. Predicted de-repression of their target genes was associated with 12 enriched pathways ($p < 0.1$), most notably Autophagy - animal ($p_{adj} < 0.05$), with IgSF CAM signalling and Endocytosis.

Conclusion: These findings suggest that silymarin may protect the porcine liver against AFB1-induced injury through coordinated miRNA-mediated regulation, including suppression of toxin-related signalling and catabolic pathways and support of protective processes such as autophagy. **Acknowledgements** This work was supported by National Science Centre, Poland UMO-2021/43/I/NZ9/02612, and Deutsche Forschungsgemeinschaft 504983453/WI 1754/18.

Z18.O

Transcriptomic evidence for tumor suppressor-like activity of eca-miR-200b in equine sarcoid cells

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Słowa kluczowe: equine sarcoid cells, transcriptome profiling, microRNA, eca-miR-200b

Bovine papillomavirus (BPV)-induced equine sarcoids represent the most prevalent cutaneous neoplasm in horses, yet the epigenetic and molecular drivers of their pathogenesis remain largely unresolved. In our previous study, we identified significant downregulation of eca-miR-200b in sarcoid tissues, implicating its involvement in sarcoid pathogenesis. To investigate its biological role, we performed transcriptome profiling of equine sarcoid cells overexpressing eca-miR-200b. Primary sarcoid cell lines (n=7) were transfected with a synthetic mimic (Genomed) using X-tremeGENE™ 9 DNA Transfection Reagent (Roche) and rRNA-depleted libraries were prepared using the QIAseq FastSelect kit (Qiagen) for next-generation sequencing. Differential expression analysis revealed 564 significantly altered genes between mimic-transfected and control cells (padj<0.1), of which 120 were upregulated and 444 downregulated. Functional enrichment analyses, including Over-Representation Analysis (ORA) and Gene Set Enrichment Analysis (GSEA), consistently demonstrated strong suppression of pathways associated with mitotic cell cycle progression, chromosome segregation, DNA replication, and cell division. Concurrently, pathways related to DNA damage response, cellular stress, FoxO signaling, and cellular senescence were enriched, alongside profound alterations in kinetochore, spindle, centromere, and cytoskeleton-associated structures. Notably, the oncogenic transcription factor FOXM1, known to repress miR-200b expression, was significantly downregulated; which is consistent with the established miR-200b/FOXM1 negative feedback loop in cancer. Collectively, these findings indicate that restoration of eca-miR-200b expression exerts strong antiproliferative and tumor suppressor-like effects in equine sarcoid cells. The observed downregulation of eca-miR-200b in sarcoids may therefore contribute to uncontrolled proliferation and maintenance of the transformed phenotype. To the best of our knowledge, this study provides the first comprehensive insight into the role of eca-miR-200b in equine sarcoid biology and highlights its potential importance in sarcoid tumor progression. Financed from: National Science Centre (Poland) grant UMO-2021/43/D/NZ9/02763.

Z19.O

Hepatic microRNA Profiling in Pigs Identify a Novel DE Transcripts Associated with DMSO Administration

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Słowa kluczowe: Toxicogenomics, Transcriptomic analysis, hepatoprotection, miRNA profiling

Background: Dimethyl sulfoxide (DMSO) is widely used as an in vivo solvent, but its neutrality as a vehicle control must be validated. This study evaluated the background effects of DMSO on the porcine hepatic microRNA (miRNA) landscape relative to untreated controls to define its baseline regulatory footprint.

Methods: A total of 7 commercial crossbred TN70 pigs (Norsvin Landrace x Large White) were investigated. Small RNA libraries were constructed and sequenced from the livers of crossbred pigs (n=4 DMSO; n=3 Control). Differentially expressed miRNAs were identified, and an in silico miRNA-mRNA target interaction map was generated using RNAhybrid, strictly filtered for thermodynamic stability and statistical significance ($P < 0.05$). Downstream transcriptomic effects were characterized using GO and KEGG pathway enrichment analyses utilizing Bonferroni step-down correction.

Results: Comparative profiling between the DMSO and Control groups revealed an exceptionally stable homeostatic baseline, with zero upregulated miRNAs and only a single significantly altered transcript identified. •Suppressed Transcriptomic Signatures: Because

no miRNAs were upregulated by DMSO, no downstream gene suppression layer was identified, confirming that the solvent does not impose de novo repressive stress on the hepatic transcriptome. • **Activated Transcriptomic Signatures:** The lone responsive transcript, ssc-miR-503, was significantly downregulated ($\log_2FC = -2.35$). The resulting de-repression of its 3,518 predicted high-confidence target genes revealed highly focused functional clustering ($p_{adj} < 0.005$) toward baseline catabolic and cellular transport machinery. "Cellular macromolecule catabolic process," "protein catabolic process," "Ubiquitin-mediated proteolysis," and the "mTOR signalling pathway" were among the top-enriched phrases; "endocytosis" was the most prevalent. **Conclusion** These data show that DMSO solvent does not alter the liver miRNA expression profile and causes minimal background. Targeted downregulation of ssc-miR-503 implies that DMSO has a mild effect on baseline cellular transport and protein turnover pathways. This confirms the use of DMSO as a biochemically sound vehicle control in pig toxicogenomic modelling.

Acknowledgements: This research was funded by Narodowe Centrum Nauki UMO-2021/43/I/NZ9/02612, and Deutsche Forschungsgemeinschaft: 504983453 (WI 1754/18).

Z20.O

Native chicken breeds, characteristics of genomes and genetic distances.

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Słowa kluczowe: NGS, Greenleg partridge, Rhode Island Red, Rhode Island White, White Leghorn

The National Research Institute of Animal Production implements a unique program dedicated to the conservation of genetic resources of farm animals. The primary objective of this program is to preserve individual populations and protect them from extinction by maintaining genetic balance within each conserved flock, while simultaneously retaining characteristic phenotypic traits of birds. Long-term studies have demonstrated that these populations are well adapted to local environmental conditions, exhibiting high reproductive performance and survival rates under unfavorable conditions. Consequently, native chicken breeds constitute a valuable reservoir of genetic diversity and an important source of biological material for future research and sustainable breeding programs. Despite their importance, comprehensive molecular analyses of these genetic resources have remained limited. The present study was based on Pool-Seq data obtained from four laying hen breeds: Greenleg Partridge, Rhode Island Red, Rhode Island White, and White Leghorn. Variant calling was conducted using a standardized bioinformatics pipeline, including FastQC for quality control, Trimmomatic for read trimming, BWA-MEM for alignment to the reference genome bGalGal1.mat.broiler.GRCg7b (GCA_016699485.1), post-processing of BAM files with Samtools, and variant detection using FreeBayes. Genetic differentiation among breeds was quantified using Bray–Curtis distances calculated across 80,576,624 identified polymorphic sites. Comparative analyses focused on two genomic regions on chromosome GGC14 showing pronounced differentiation between populations. Region R1 (1.36–1.377 Mbp) contains the gene ENSGALG00010022090, whereas region R2 (1.42–1.45 Mbp) harbors the lncRNA gene ENSGALG00010022798. Additionally, two quantitative trait loci (QTL) regions were analyzed. Principal component analysis (PCA) was performed for each selected region. Genetic distances between the Greenleg Partridge breed and the Rhode Island Red, Rhode Island White, and White Leghorn breeds ranged from 0.365 to 0.865, indicating substantial genetic differentiation, particularly within regions R1 and R2 on chromosome GGC14. This research was funded by the National Science Centre of Poland, project no. UMO 2023/51/B/NZ9/00479

Z21.O

Changes in the genetic characteristics of rainbow trout in the Rutki Fish Farm in 2011–2025 and their potential importance in aquaculture and conservation of salmonid fish

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Słowa kluczowe: rainbow trout; *Oncorhynchus mykiss*; microsatellites; genetic variability; genetic drift; domestication

Salmonid stocks maintained under aquaculture conditions are affected by genetic drift and domestication. Domestication is the adaptation of fish to human-controlled conditions through intentional selection for specific traits and unintended selection associated with the hatchery environment. Genetic drift involves random changes in allele frequencies, especially in populations with low effective population size, and may reduce genetic variation. This study estimated genetic variation in three rainbow trout (*Oncorhynchus mykiss*) lines maintained at the Salmonid Fish Breeding Centre in Rutki, Poland, and assessed changes in their genetic characteristics in 2011–2025. The material consisted of 390 fin clips from fish representing the outbred line (OUT), the autumn-spawning line (A), and the spring-spawning line (S). Genetic variation was assessed using polymorphism of 12 microsatellite loci amplified in three multiplex PCR reactions, with locus OMM1034 amplified separately; genotypes were determined by automated capillary electrophoresis. The investigated stocks showed moderate genetic variation. The highest values of the number of alleles (A_n) and alleles per locus (A_n/locus) occurred in the OUT line, lower values in the S line, and the lowest in the A line. Observed and expected heterozygosity (H_o and H_e) showed no clear decreasing trend, while low, statistically non-significant F_{IS} values indicated to very limited inbreeding. F_{ST} and NMDS analyses confirmed clear genetic differentiation among the S, A, and OUT lines, although genetic distances slightly decreased in 2011–2025. The highest rate of genetic change was estimated in the A line, followed by the S and OUT lines. The results suggest parallel effects of genetic drift and domestication, with domestication probably playing the more important role, although this requires verification. Because these processes may reduce genetic variation important for adaptation and the quality of stocking material, systematic genetic monitoring, high effective population size, rotational use of broodstock, and introduction of unrelated material are important for salmonid conservation and aquaculture.

Z22.O

Associations Between Rumen Microbiome, Production Traits, and Host Genomic Variation in Holstein Cows

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Słowa kluczowe: rumen, microbiome, selection, heritability

Rumen microbiome composition is partly influenced by host genetics, making it a potential target for selection in dairy cattle breeding. The objective of this study was to identify rumen microbial taxa associated with production and health-related traits and to quantify the contribution of host genetic variation to their abundance. 192 high-producing Holstein cows were studied. Rumen microbiome profiles were characterised using 16S rRNA gene sequencing (V3–V4 region). Genomic analyses were performed using 39,054 SNP markers. Associations between microbial abundances and phenotypic traits were evaluated using a MaAsLin2 model. A microbiome-GWAS was conducted using a mixed linear model (MLMA). SNP-based heritability was estimated using GREML approach. At the phylum level, 25 significant associations involving 10 taxa were identified for four traits: milk fat percentage, milk fat-to-protein ratio, rumen pH, and milk urea concentration. At the genus level, associations were detected for seven traits, including milk yield, milk protein percentage, milk fat percentage, casein content, somatic cell count, milk urea concentration, and rumen pH. The microbiome-GWAS for the trait-associated bacteria identified three genome-wide significant SNPs associated with Planctomycetota and Verrucomicrobiota, as well as nine suggestive signals for Chloroflexi, Cyanobacteria, Elusimicrobiota, and Patescibacteria abundances. At the genus level, 10 significant and 18 suggestive SNPs were detected. These SNPs were located near candidate genes involved in immune response and ruminal acidity, potentially constituting a system for rumen protection and maintenance of ruminal homeostasis. Heritability estimates indicated that a subset of microbial taxa is under moderate-to-high genetic control, with the highest values observed for Planctomycetota, Cyanobacteria, and Patescibacteria. These results demonstrate that selected components of the rumen microbiome are associated with both production traits and host genomic variation, providing a foundation for further development of microbiome-informed selection strategies in dairy cattle. Supported by the Implementation Doctorate program of the Polish Ministry of Education and Science (MEiN) (project DWD/6/0206/2022) and by Kombinat Rolny Kietrz Ltd.

Z23.O

Parentage testing, genetic diversity and population structure of Huacaya Alpacas

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Słowa kluczowe: Huacaya alpacas, microsatellite markers, genetic structure

Alpacas have been bred for centuries for their high-quality, hypoallergenic fiber, which is highly valued in the textile industry. This study aimed to evaluate the usefulness of three microsatellite marker panels for parentage testing in Huacaya alpacas and to investigate the genetic structure of the population. Panel I consisted of 16 markers, Panel II of 17 markers, and Panel III of 13 markers. In Panel I, two markers (SRY and YWLL02) were excluded from further analyses because they were monomorphic. A total of 239 randomly selected alpaca DNA samples were analyzed. DNA amplification was performed using multiplex PCR, and fragment analysis was carried out by capillary electrophoresis on a 3500xl Genetic Analyzer. Genotyping was conducted using GeneMapper 5.0, and the resulting DNA profiles were subjected to bioinformatic analyses using CERVUS, GenAEx, and STRUCTURE software. The average polymorphic information content (PIC) across the 44 markers was 0.727. However, PIC values below 0.5 were observed for markers P194 and CMS3, indicating low levels of polymorphism. When all markers were analyzed together, the combined exclusion probability for one candidate parent and for a candidate parent pair reached 99.99% in both cases. The highest values of the effective number of alleles (N_e), Shannon's information index (I), observed heterozygosity (H_o), and expected heterozygosity (H_e) were recorded for marker YWLL08, whereas the lowest values were observed for marker YWLL46. The results demonstrate that each microsatellite marker panel can be used independently for individual identification and parentage verification. Furthermore, analyses of the population structure of Huacaya alpacas maintained in Poland, based on PCoA, heatmap visualization, and neighbor-joining (NJ) tree analysis, indicated a genetically homogeneous population with no clear internal genetic differentiation. In contrast, Bayesian clustering analysis suggested the presence of two potential genetic clusters ($K = 2$) within the studied population, indicating that Bayesian approaches may be more sensitive in detecting subtle genetic structure.

Z1.P

Comparative transcriptomic profiling of abdominal and scrotal testes in dogs using RNA sequencing

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Słowa kluczowe: RNAseq, disorder of sex development, cryptorchidism, gene expression

Abdominal testes are encountered in two distinct conditions in dogs: cryptorchidism in XY males, resulting from failure of testicular descent, and XX disorders of sex development (XX DSD), in which testicular tissue develops despite a 78,XX karyotype (SRY-negative) due to abnormal bipotential gonad differentiation. Whereas retained testes in cryptorchid dogs are most often found in the inguinal region and only occasionally in the abdomen, testes in dogs with XX DSD are usually located within the abdominal cavity. Ectopic testicular location is associated with impaired spermatogenesis, altered testicular function, and an increased risk of neoplastic transformation. However, despite their different developmental origins, the molecular mechanisms underlying testicular dysfunction in abdominal testes remain poorly understood. In the present study, RNA sequencing (RNA-seq) was used to compare the transcriptomes of abdominal testes collected from XY dogs with cryptorchidism (n = 5) and from individuals with testicular XX DSD (n = 5) with those of control scrotal testes from healthy XY dogs (n = 8). Differentially expressed genes (DEGs) were identified using the criteria $FDR < 0.05$ and $|\log_2FC| > 1.5$. Principal component analysis (PCA) demonstrated a clear separation of samples into two clusters: (1) abdominal testes from XY dogs with cryptorchidism clustered together with gonads of XX DSDs and (2) control scrotal testes from XY dogs. Comparisons of abdominal testes from XY dogs with cryptorchidism and dogs with testicular XX DSD against control scrotal testes revealed a similar number of DEGs (9,686 and 9,252, respectively), whereas direct comparison between the two groups of abdominal testes identified only 1,242 DEGs. These findings indicate that abdominal testicular retention, regardless of whether it occurs in XY cryptorchid dogs or in dogs with XX DSD, is associated with extensive transcriptomic alterations and massive dysregulation of gene expression. [This study was funded by the National Science Centre, Poland, grant no. 2023/51/B/NZ9/01165.]

Screening genetic backgrounds for Blue Eyed White (BEW) alpaca using WGS

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Słowa kluczowe: BEW, alpaca, blue eye, deafness, coat color, pigmentation

Blue-eyed white (BEW) alpacas are an undesired phenotype in Polish breeding because they combine a solid white fleece, blue irises and a high risk of congenital deafness, reported in some populations in 50–80% of BEW animals. The phenotype is usually interpreted as the outcome of white-spotting and grey-associated backgrounds, but BEW individuals may appear unexpectedly, complicating mating decisions. In the BEW phenotype, the known grey-associated KIT variant c.376G>A is found as a heterozygote. The homozygous variant of this mutation is presumed embryonically lethal. Additionally, it occurs as a cryptic mutation in non-grey animals. We aimed to characterise the genetic background of BEW alpacas and identify candidate variants that could support safer crossbreeding decisions. The wider dataset included 10 BEW alpacas and approximately 400 animals of different coat colours. All BEW animals carried KIT c.376G>A in heterozygous form. MC1R and ASIP haplotypes showed that BEW animals were not genetically uniform in base colour, including light fawn, black, brown, very light fawn/white, and rainbow genotypes. Whole-genome sequencing was performed using short-read Illumina sequencing for 4 white and 4 BEW alpacas. The analysis followed standard WGS best practices for SNP discovery, including quality control, reference alignment, duplicate marking, base-quality recalibration, variant calling and annotation, followed by GWAS-style case-control testing under various genetic models. Variant-level association analysis highlighted two main candidate regions. The strongest BEW-enriched signal was detected in SLC24A5 on NC_132992.1, where the alternative allele count was 8/8 in BEW cases and 1/8 in white controls (allelic model, $p = 0.000699$). A second strong cluster occurred in the ASIP region on NC_133005.1, but with the opposite direction, as alternative alleles were enriched in white controls. These results indicate that BEW cannot be explained by KIT alone and may involve additional pigmentation loci, although findings remain exploratory due to the small WGS sample size.

Gold nanoparticles and genomic stability in geese

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Słowa kluczowe: genomic instability, SCGE assay, micronucleus test, gold nanoparticles, Anser anser

Maintaining the structural integrity of DNA and the efficiency of enzymatic repair pathways constitute the bedrock of cellular homeostasis and genomic stability. In recent years, the dynamic development of nanotechnology has led to the widespread application of gold nanoparticles (AuNPs) in targeted drug delivery systems, bioimaging, and diagnostics. However, their high surface-to-volume ratio and ability to cross biological barriers raise critical concerns regarding their long-term biocompatibility and potential to induce oxidative stress, clastogenicity, or aneugenic events. While nanomaterial toxicity is extensively studied in mammalian models, there remains a critical knowledge gap regarding their impact on the avian genome, particularly in economically viable poultry species. Therefore, the aim of this study was to assess the genotoxic profile of AuNPs stabilized in a polymer matrix regarding the genomic stability of Zatorska geese (*Anser anser*). The experiment was conducted in vitro on primary peripheral blood cells isolated from 10 *Anser anser* individuals. Two AuNP solutions (10 ppm) suspended in a 2% sodium alginate matrix were synthesized using either xylose or maltose as reducing agents. Cells were subjected to a 24-hour exposure to the tested nanostructures. Biomarkers of DNA damage were analyzed using the micronucleus test (MN assay) and the alkaline comet assay (SCGE). Exposure to AuNPs significantly induced genomic instability in goose blood cells compared to the control group. Both an increased percentage of DNA in the comet tail and an elevated micronucleus index were observed. Furthermore, the intensity of the genotoxic effect depended on the type of sugar reducer used during synthesis. Gold nanoparticles induce genotoxic stress in goose blood cells during a 24-hour exposure, leading to DNA damage. The type of organic reducer modifies the mutagenic potential of AuNPs within the alginate matrix.

Z4.P

Tissue-dependent cellular chimerism in dogs detected by STR profiling and array comparative genomic hybridization (aCGH)

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Słowa kluczowe: cellular chimerism, dog, STR, aCGH, tissue-specific mosaicism

Cellular chimerism in dogs is a rarely reported phenomenon that may remain undetected in routine genetic testing and parentage verification, which are usually based on the analysis of a single biological sample. The aim of this study was the molecular characterization of two cases of canine chimerism identified during routine parentage testing using STR analysis and array comparative genomic hybridization (aCGH), with particular focus on tissue-specific differences. The study included two dogs representing the Great Dane and Chow Chow breeds. DNA isolated from peripheral blood, buccal epithelium, and hair follicles was analyzed. Genetic profiling was performed at 21 STR loci (ISAG) using 3500xl capillary sequencer (AB). aCGH analysis was conducted using SurePrint G3 Canine CGH Microarrays (Agilent) based on the CanFam3.1 reference genome assembly. In both cases, the presence of more than two alleles at multiple STR loci indicated the coexistence of different cell lines. In the Great Dane, additional alleles were detected in 6 loci in blood, 5 loci in buccal epithelium, and 3 loci in hair follicles. All alleles were consistent with parentage genotypes. In the Chow Chow, a more complex genetic profile was observed, including up to four alleles at a single locus and additional alleles in 17 loci in hair follicles. aCGH analysis confirmed tissue-dependent chromosomal imbalances. The highest number of aberrations was detected in buccal epithelium, whereas the lowest number was identified in hair follicles. In the Great Dane, differences in the X chromosome profile between tissues suggested the coexistence of XX and XY cell lines. The results indicate that cellular chimerism in dogs may occur more frequently than previously assumed and may show a tissue-dependent pattern. The combination of STR analysis and aCGH constitutes an effective diagnostic approach for chimerism detection with important implications for genetic diagnostics and breeding practice.

Z5.P

Gene encoding myelin and lymphocyte protein (MAL) is associated with antigenic variability of the equine blood system P

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Słowa kluczowe: horse, blood group, erythrocyte antigens, genetic variants

Although serological blood typing is no longer applied for routine parentage verification in horses, understanding the genetic basis of erythrocyte antigen diversity remains important for preventing neonatal isoerythrolysis and ensuring compatibility in blood transfusions. Despite decades of serological studies of equine blood groups, the molecular basis of erythrocyte antigen variability remains largely unknown. Recently, through comparative analysis of blood transcriptomes from 100 horses with serologically defined erythrocyte antigens, we demonstrated that blood group system K is associated with a missense variant in the glycophorin A (GYPA) gene. To date, this remains the only equine blood group system characterized at the molecular level. In the present study, we applied a similar transcriptome-based (TWAS) approach to identify the genetic basis of serological variation in the equine blood group system P. Comparison of blood transcriptomes from horses carrying different P-system antigens revealed significantly associated variants on ECA15 ($p < 3.9 \times 10^{-7}$). The top SNPs were detected within the MAL (myelin and lymphocyte protein) gene. Since this gene is considered the molecular basis of a recently recognized human MAL blood group system, it emerged as a strong functional candidate for further investigation. Sanger sequencing of the complete coding sequence of the equine MAL gene identified eight missense variants (c.55G>C, p.Val19Leu; c.220A>T, p.Arg74Trp; c.335A>C, p.Asn112Thr; c.355C>G, p.His119Asp; c.432C>G, p.Ile144Met; c.439G>A, p.Val147Met; c.443T>G, p.Ile148Ser; c.444T>G, p.Ile148Met). Preliminary comparison of the resulting amino acid substitutions with serologically defined antigens suggests that variants affecting residues 112, 119, and 148 may underlie the observed variability of the equine P blood group system. Ongoing analyses in a larger cohort representing multiple serotypes are aimed at validating these associations. Our study confirmed that blood transcriptome based approaches, despite certain limitations, can be effective in uncovering the molecular background of equine erythrocyte antigen variability. The work was supported by the National Science Centre, Poland, under research grant 2022/47/B/NZ9/02331.

Z6.P

Analysis of transcript levels of MET, PRDM14, INHA, and CLPTM1L genes in dogs with abdominal and inguinal cryptorchidism

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Słowa kluczowe: RNAseq, cryptorchidism, gene expression, cancer-related genes

Cryptorchidism is the most common congenital disorder of the male reproductive system in dogs and is classified as abdominal or inguinal according to the location of the retained testis. It may lead to sterility or subfertility and represents a significant risk factor for testicular carcinogenesis. Despite its clinical importance, the molecular basis of cryptorchidism remains poorly understood, particularly with respect to the mechanisms responsible for the increased susceptibility of undescended testes to malignant transformation. The aim of this study was to characterize transcriptomic differences between descended and undescended testes in dogs with abdominal and inguinal cryptorchidism using RNA sequencing (RNA-seq) and to validate the expression of selected cancer-related candidate genes by RT-qPCR. RNA-seq was performed on gonadal samples from dogs with abdominal (5 undescended testes and 8 control testes) and inguinal (7 undescended testes, 8 contralateral descended testes, and 7 control testes) cryptorchidism. Validation was carried out in an independent cohort of 30 dogs with unilateral cryptorchidism, comprising 15 abdominal and 15 inguinal cases by qPCR for selected genes: MET, PRDM14, INHA, and CLPTM1L. RNA-seq analysis revealed significant downregulation of PRDM14, MET, and CLPTM1L and upregulation of INHA gene in both types of undescended testes. The qPCR results were in agreement with the RNA-seq findings in dogs with inguinal cryptorchidism, demonstrating significant downregulation of MET ($p = 0.000284$), PRDM14 ($p = 0.000003$) and CLPTM1L ($p = 0.000008$) in undescended testes compared to descended testes. In contrast, INHA expression was significantly elevated in undescended gonads ($p = 0.000015$). These results indicate that the studied genes may be involved in molecular mechanisms underlying the increased susceptibility of cryptorchid testes to malignant transformation. Further RT-qPCR analyses of abdominal cryptorchid samples are ongoing to validate and expand upon these findings. This study was financed by the National Science Centre in Poland, grants: 2018/29/B/NZ9/01065 and 2023/51/B/NZ9/01165.

Z7.P

Differential expression of candidate genes in periumbilical skeletal muscle of dogs with umbilical hernia

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Słowa kluczowe: canine umbilical hernia; gene expression; skeletal muscle; RT-qPCR; transcriptional dysregulation; candidate genes

Canine umbilical hernia (UH) is a frequent congenital defect characterised by incomplete closure of the abdominal wall; however, its molecular etiology remains insufficiently elucidated. Evidence from comparative studies suggests that dysregulation of genes involved in myogenesis, musculoskeletal development and tissue homeostasis may contribute to hernia pathogenesis. The objective of this study was to investigate the transcriptional profiles of selected candidate genes in periumbilical muscle tissue of dogs affected by UH and to identify molecular mechanisms potentially associated with abdominal wall insufficiency. Periumbilical muscle samples were collected from 37 dogs diagnosed with UH and eight unaffected controls. Relative transcript abundance of SIM1, PITX1, METTL21C, MYH2 and MYL2 was quantified by reverse transcription quantitative polymerase chain reaction (RT-qPCR). Gene expression data were normalised using GAPDH and ACTB as endogenous reference genes and statistically evaluated using the Mann–Whitney U test. All investigated genes exhibited significantly decreased transcript abundance in UH-affected dogs compared with controls. The most pronounced downregulation was observed for MYH2 and MYL2 ($p < 0.001$), whereas METTL21C demonstrated a less marked, although statistically significant, reduction ($p < 0.05$). SIM1 and PITX1 were likewise significantly downregulated. These findings indicate substantial transcriptional perturbations affecting genes implicated in myofibre organisation, contractile apparatus function, myogenic differentiation and maintenance of musculoskeletal integrity. The observed transcriptional dysregulation suggests impaired molecular pathways governing skeletal muscle development and structural maintenance in dogs affected by UH. Reduced expression of these genes may contribute to compromised abdominal wall integrity. This study was financed by statutory funding 506.534.05.00 from the Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences, Poland

Z8.P

Identification of the ECA3(q13;q21) inversion associated with the crypto-tobiano coat pattern in Hucul horses using FISH and PCR techniques

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Słowa kluczowe: horse, Hucul horse, FISH, crypto-tobiano, inversion

Hucul horses are one of the oldest primitive horse breeds in Poland and are included in a genetic resources conservation program. According to the breed standard, bay, mouse-gray, piebald, dun, and black coat colors are accepted, whereas the presence of white markings on the head and limbs is considered a disqualifying trait for breeding. A particular challenge is posed by horses exhibiting the minimal tobiano pattern (crypto-tobiano), in which the characteristic coat features may resemble ordinary white markings. This can lead to incorrect phenotypic assessment and the exclusion of genetically valuable animals from breeding programs. The tobiano coat pattern is associated with the dominant TO allele and a paracentric inversion, ECA3(q13;q21), located on equine chromosome 3. The aim of this study was to identify this chromosomal rearrangement in Hucul horses displaying the minimal tobiano pattern. The biological material consisted of whole-blood samples collected from horses originating from the Hucul Horse Stud "Gładyszów" in Regietów. Cytogenetic analysis was performed using fluorescence in situ hybridization (FISH) with BAC probes specific for the CLOCK and PDGFRA genes. In addition, PCR analysis targeting the detection of the ECA3(q13;q21) inversion was carried out, and the amplification products were separated by electrophoresis. FISH analysis revealed the presence of the ECA3(q13;q21) inversion in all examined individuals. These results were confirmed by PCR and electrophoretic analysis of the amplification products, indicating the occurrence of the heterozygous genotype associated with the tobiano coat pattern. The obtained results confirm the usefulness of FISH and PCR techniques for identifying the genetic basis of the crypto-tobiano coat pattern and indicate their potential value in diagnostics supporting breeding programs and the conservation of genetic resources in the Hucul horse breed.

Plastic-Induced DNA Damage in Porcine Cells Assessed by the Comet Assay

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Słowa kluczowe: Nanoplastics, Bisphenol A, DNA damage, Genotoxicity, Pig Cell, Comet assay

Bisphenol A (BPA) and nanoplastics have attracted considerable attention due to their potentially harmful effects on cellular function. However, information regarding their impact on animal cells remains limited. Given the increasing presence of nanoplastics in agricultural ecosystems, investigating their effects on porcine cells is important for understanding potential risks to animal health and livestock production. The aim of this study was to evaluate the effects of BPA and nanoplastics on in vitro-cultured porcine fibroblasts, mesenchymal stem cells (MSCs), and adipocytes, with particular emphasis on their genotoxic potential. DNA damage was assessed using the alkaline comet assay (OxiSelect™ Comet Assay Kit, Cell Biolabs, USA). Cells were cultured under standard conditions and exposed to BPA (10, 25, and 50 µg/mL) or nanoplastics (50, 100, and 200 µg/mL). Following treatment, cells were embedded in agarose, lysed, subjected to alkaline DNA unwinding and electrophoresis, stained with Vista Green DNA Dye, and analyzed by fluorescence microscopy. DNA damage parameters, including tail length and the percentage of DNA in the tail, were quantified using OpenComet software (ImageJ). Exposure to nanoplastics induced a concentration-dependent increase in DNA damage in all examined cell types. Among the analyzed cells, MSCs exhibited the highest susceptibility, whereas fibroblasts showed a comparable response at the highest concentration tested (200 µg/mL). In contrast, adipocytes exhibited the lowest level of DNA damage. Similarly, BPA exposure resulted in the greatest DNA damage in fibroblasts, followed by MSCs, while adipocytes remained the least affected. Overall, adipocytes appeared less sensitive to both BPA- and nanoplastic-induced genotoxicity, which may be associated with their lower proliferative activity and distinct metabolic characteristics compared with fibroblasts and MSCs. These findings demonstrate that both BPA and nanoplastics induce DNA damage in porcine cells, with the magnitude of the genotoxic response depending on cell type. This study was financed by the statutory funds of the Department of Genetics and Animal Breeding (No. 506.534.09.00).

Z10.P

In vitro evaluation of the genotoxic effects of acrylamide in Olkuska sheep lymphocytes

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Acrylamide is an organic chemical compound with proven neurotoxic, carcinogenic, and mutagenic properties. It poses a significant environmental and feed-borne hazard (present in industrially processed pellets subjected to aggressive thermal treatment) capable of inducing neurological symptoms in animals. The aim of this experiment was to evaluate the cytogenetic effects induced by acrylamide in Olkuska sheep cells using the sister chromatid exchange (SCE) assay. Material and Methods: The experimental material consisted of whole peripheral blood collected from five female sheep. In vitro lymphocyte cultures (RPMI-1640 medium, penicillin-streptomycin, pokeweed mitogen, fetal bovine serum) were incubated for 72 hours. At the 24th hour, 5-bromo-2'-deoxyuridine (20 µg/ml) was added for differential staining of sister chromatids. Acrylamide exposure was initiated at the 28th hour of culture, utilizing concentrations of 50 µM, 250 µM, and 1000 µM, alongside a negative control. Microscopic slides were stained using the FPG technique. For each of the 4 experimental variants across 5 subjects, 20 metaphases were analyzed (totaling 400 structures). Data verified by Shapiro-Wilk and Levene's tests were analyzed using one-way analysis of variance (ANOVA) and Tukey/Dunnnett post-hoc tests ($\alpha = 0.05$). Cytogenetic analysis confirmed a normal karyotype ($2n=54, XX$) for all examined females. In the control groups, the spontaneous frequency of SCE was 5.23 ± 1.85 per metaphase. Acrylamide induced a dose-dependent increase in SCE frequency. The 50 µM concentration did not significantly increase the number of SCEs compared to the control ($p > 0.05$). A statistically significant increase in DNA damage was observed at 250 µM ($p < 0.05$) and 1000 µM ($p < 0.01$). Furthermore, the highest dose (1000 µM) exhibited strong cytotoxicity, inhibiting the cell proliferation rate index (PRI). These findings demonstrate the genotoxic and cytotoxic potential of acrylamide in ovine lymphocytes in vitro.

Keywords: Acrylamide, Sister chromatid exchange (SCE), Genotoxicity, Cytotoxicity, Peripheral blood lymphocytes

Z11.P

Widespread dysregulation of long non-coding RNAs and PIWI-interacting RNAs in undescended testes of dogs with unilateral cryptorchidism

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Słowa kluczowe: RNA-seq, piRNA, lncRNA, cryptorchidism

Cryptorchidism is among the most common congenital reproductive disorders in male dogs and is associated with impaired spermatogenesis, reduced fertility, and an increased risk of testicular tumors. Non-coding RNAs are one of the regulators of testicular development and germ cell maintenance; however, their involvement in canine cryptorchidism remains poorly understood. This study aimed to investigate the expression patterns of long non-coding RNAs (lncRNAs) and PIWI-interacting RNAs (piRNAs) in undescended testes of dogs with unilateral cryptorchidism. Testicular samples were collected from dogs with unilateral cryptorchidism, including undescended (UD) and contralateral descended (D) testes, as well as scrotal testes from healthy controls (C). LncRNA expression was analyzed using RNA sequencing in UD (n = 7), D (n = 8), and C (n = 7) samples. Small RNA sequencing was performed for piRNA profiling in 9 UD, 9 D, and 8 C testes. Because of the limited annotation of canine piRNAs, sequencing reads were mapped to a reference set of known human piRNAs. Differential expression analyses were performed for UD vs. D and UD vs. C comparisons using thresholds of $FDR < 0.05$ and $|\log FC| > 1$. In the UD vs. D comparison, 2441 lncRNAs were differentially expressed, including 2022 down-regulated and 419 up-regulated transcripts. Similarly, 2244 lncRNAs showed altered expression in UD vs. C, of which 1822 were down-regulated and 422 up-regulated. piRNA profiling revealed 433 differentially expressed molecules in UD vs. D (273 down-regulated and 160 up-regulated) and 408 in UD vs. C (281 down-regulated and 127 up-regulated). These findings demonstrate profound disruption of both lncRNA and piRNA expression in undescended canine testes. Together with previous observations of widespread miRNA dysregulation, the results highlight a substantial contribution of non-coding RNAs to the molecular pathology of canine cryptorchidism. Study financed by the National Science Centre, Poland (grants no. 2018/29/B/NZ9/01065 and 2023/51/B/NZ9/01165).

Z12.P

The impact of the T674I polymorphism in the TLR4 gene on oxidative activity and NETosis in bovine neutrophils following LPS stimulation

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Słowa kluczowe: bovine TLR4 polymorphism, PMNs, oxidadative burst, NETosis, gene expression,

The TLR4 receptor plays a pivotal role in recognizing lipopolysaccharide (LPS) from Gram-negative bacteria and activating the innate immune response. The T674I variant is located within the TIR (Toll/Interleukin-1 Receptor) domain of this protein, which is responsible for recruiting adaptor proteins and intracellular signal transduction. This study aimed to evaluate the impact of the T674I variant in the TLR4 gene on the intensity of reactive oxygen species (ROS) production, the capacity for dsDNA release (indicating NETosis), and the expression of key genes in the TLR pathway. Polymorphonuclear neutrophils (PMNs) were isolated from whole blood of Polish Holstein-Friesian cows with CC (n=15) and CT (n=10) genotypes. PMNs were stimulated with LPS (12.5–50 µg/mL). Oxidative activity was assessed using the nitroblue tetrazolium (NBT) reduction assay, while NETs release (dsDNA) was quantified fluorometrically using the PicoGreen dye. Gene expression (TLR4, TLR2, NCF1, PADI4, IRF3, NFkB p65) was determined via RT-qPCR. Following LPS stimulation, neutrophils with the CC genotype released more ROS than those with the CT genotype, though this difference was not statistically significant (p=0.553). Conversely, a significantly lower capacity for extracellular dsDNA release was observed in CT individuals compared to CC individuals (p < 0.01). Transcriptional analysis revealed that CT neutrophils were characterized by higher IRF3 expression and an upward trend for TLR4 and TLR2. Despite the differences observed in the functional assay for NETosis, the expression of PADI4 and the NFkB p65 factor showed no significant genotypic differences under LPS stimulation. These results suggest that the T674I polymorphism shifts the cellular response away from LPS-induced NETs release toward the activation of an alternative, TRIF-dependent signaling pathway (evidenced by increased IRF3 gene expression), leading to a milder, interferon-mediated immune response. In CC individuals, the TIR domain more effectively promotes the pathway leading to NETosis, which correlates with a higher transcriptional potential of the oxidative machinery (NCF1).

Z13.P

Assessment of changes in the MT-ND4 gene affected by aflatoxin B1 and plant extract in the liver of pigs (*Sus scrofa*)

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Słowa kluczowe: AFB1, swine, hepatoprotective, silibinin, andrographolide, curcumin

AFB1 is a toxic, carcinogenic metabolite produced by *Aspergillus* fungi, which enters the body via the digestive tract, the respiratory tract or the skin. It causes genotoxic effects and weakens the immune system. Pigs are particularly susceptible to it, resulting in organ damage and reproductive problems. Plants such as *Andrographis paniculata* (AP), *Silybum marianum* (SM) and *Curcuma longa* (CL) exhibit hepatoprotective properties, protecting the body against the effects of the toxin. The aim of the study was to determine the relationship between the occurrence of mutations and polymorphisms in the MT-ND4 gene and the impact of AFB1 on the pig liver, as well as the hepatoprotective effects of selected herbs. 50 piglets were divided into five groups of 10. Following acclimatisation, from day 56 to day 70 of life, AFB1 (120 µg/kg body weight) was administered orally according to the following regimens: Group 1: AP (30 µg/kg) + AFB1, Group 2: SM (90 µg/kg) + AFB1, Group 3: CL (90 µg/kg) + AFB1, Group 4: Control, Group 5: AFB1 (120 µg/kg). Four synonymous polymorphisms (m.12162C>T, m.12219C>T, m.12291G>A, m.12390G>A) were detected in the MT-ND4 gene, which did not affect the protein structure. These variants were present in 30% of individuals in the group 5, and accordingly 40% (group 1), 50% (group 3), whereas in the group 4 and 2, they occurred in only 10% of individuals. One case of heteroplasmy (m.12390G/A) was reported in the AP+AFB1 group. Polymorphisms in the MT-ND4 gene sequence were observed in all study groups, and differences in their prevalence were noted between groups; however, further research should be conducted to determine the hepatoprotective effects of these herbs on the liver. This research is financed and supported by the scientific project NCN-OPUSLAP (UMO-2021/43/I/NZ9/02612) entitled: Multilevel molecular analysis of the hepatoprotective effect of medicinal herbs extracts in prevention of liver dysfunction caused by aflatoxin B1 in pig as an animal model (in-vivo), and hepatocyte cell culture analysis in human and pig (in-vitro).

Z14.P

Complete mitochondrial genome of *Apis mellifera carnica*, line „Dobra”, and its phylogenetic analysis.

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Słowa kluczowe: *Apis mellifera carnica*; Dobra line; *Apis mellifera* complete mtDNA; phylogenetic analysis of Dobra line; honeybee genetic resources

The honeybee mitochondrial genome is an important marker in studies of ancestry, genetic variation, and phylogenetic relationships. The aim of this study was to obtain complete mitogenome sequences of the *Apis mellifera carnica* “Dobra” line and assess their similarity and phylogenetic relationship to reference sequences of selected honeybee subspecies. The “Dobra” line is a native, locally adapted line of the Carniolan honeybee associated with the Beskid Wyspowy Mountains. Its value results from its breeding importance, adaptation to local environmental conditions, winter performance of colonies, and effective use of available forage, especially honeydew. For this reason, molecular characterization of this line is relevant for the protection and monitoring of honeybee genetic resources. The research material consisted of adult worker bees (n=8) of the “Dobra” line deposited at the IZ PIB. DNA was isolated from legs using the Sherlock kit (A&A Biotechnology), and DNA quantity and quality were assessed with NanoDrop (Thermo Fisher Scientific). Nanopore sequencing was performed by GenXone on the PromethION 2 Solo platform using a FLO-PRO114M flow cell and SQK-NBD114-96 kit; basecalling was carried out with the sup v5.2.0 model. Seven unique mitochondrial sequences, approximately 16,500 bp long, were obtained and analyzed in Geneious Prime. The analysis included sequence alignment and assessment of phylogenetic relationships with reference sequences of *A. m. carnica*, *A. m. caucasica*, *A. m. ligustica*, and *A. m. mellifera*. The percentage of identical sites among the “Dobra” samples was 99.5%, indicating low mitochondrial variation and high homogeneity. When the *A. m. carnica* reference sequence was included, this value was 98.6%. Phylogenetic analysis confirmed the close relationship of the “Dobra” samples to *A. m. carnica* NC_061380.1 and showed their grouping in a compact cluster. The sequences were deposited in NCBI GenBank under accession numbers PZ267027.1–PZ267033.1, supporting their future use in comparative, population, and phylogenetic studies.

Z15.P

Molecular characterization of CD163 and CD172a-enriched bronchoalveolar lavage cell populations in domestic pigs – preliminary results

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Słowa kluczowe: porcine pulmonary macrophages; bronchoalveolar lavage; immunomagnetic enrichment; CD163/CD172a; qPCR

Porcine pulmonary macrophages are specialized innate immune cells that help maintain respiratory homeostasis, recognize infectious agents, and initiate and modulate the local inflammatory response. Because these cells are directly involved in lung immune responses, isolating macrophages from bronchoalveolar lavage (BAL) fluid and culturing them in vitro are important research tools for studying host response mechanisms to respiratory diseases. However, obtaining a homogeneous macrophage population is challenging. Therefore, the aim of this experiment was to determine the enrichment of cells of the monocyte-macrophage lineage by analyzing the expression profiles of marker genes using quantitative PCR in a population of cells obtained from domestic pig BAL after immunomagnetic enrichment for cells expressing the CD163 and CD172a antigens. This experiment was conducted using biological material obtained post-slaughter from a 990-pig line. Cells isolated from BAL were subjected to immunomagnetic enrichment using anti-CD172a or anti-CD163 antibodies. Molecular characterization of the obtained cell fractions was performed by qPCR, analyzing the expression levels of the marker genes: MARCO, CD68, CD86, EPCAM, VIM, CD163, PTPRC/CD45, and CSF1R. Preliminary results indicate that CD163-based selection most effectively enriched the BAL-derived cell population for cells with a macrophage-like molecular profile, reflected by increased expression of CD163, MARCO, and CD68 compared with the corresponding depleted fraction. CD172a enrichment also retained cells expressing macrophage-associated markers, consistent with the broad expression of CD172a on porcine myeloid cells, but the macrophage-associated profile was less pronounced than after CD163 selection. Reduced expression of the analyzed macrophage markers in the depleted fractions supports the partial enrichment efficiency. However, detectable EPCAM expression, together with high PTPRC/CD45 expression, particularly after CD172a-based selection, suggests that the isolated BAL cell population remained heterogeneous and may include epithelial or other leukocyte populations. These preliminary findings indicate that immunomagnetic enrichment improves macrophage representation but does not yield a fully homogeneous culture.

Z16.P

Replication timing and DNA methylation analyses indicate epigenetic spreading from X-derived chromatin to autosomal regions in marker chromosomes of a Hucul carrying a reciprocal X;1 translocation

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Słowa kluczowe: XIST, DNA methylation, X chromosome abnormalities, disorders of sex development (DSD)

Chromosomal rearrangements involving the X chromosome may profoundly affect genome organization and gene regulation through alterations in replication timing and epigenetic modifications. In females, dosage compensation mechanisms associated with X-chromosome inactivation can influence not only X-linked regions but also adjacent chromosomal segments involved in structural rearrangements. However, little is known about the epigenetic consequences of X-autosome rearrangements in horses. Therefore, the aim of this study was to investigate replication timing and DNA methylation patterns in X-derived marker chromosomes identified in a Hucul mare with karyotype 64,XX,t(X;1)(Xp;1p)(Xq;1q). Replication timing and DNA methylation were investigated in fibroblast cultures derived from a 4-year-old Hucul mare carrying a reciprocal translocation between chromosome X and chromosome 1 [64,XX,t(X;1)(Xp;1p)(Xq;1q)]. Early and late replication patterns were assessed using BrdU incorporation, whereas DNA methylation was evaluated by immunofluorescent detection of 5-methylcytosine on metaphase chromosomes. Combined analyses of replication timing and DNA methylation revealed that the derivative chromosomes retained characteristic epigenetic features of the X chromosome. Autosomal regions originating from chromosome 1 displayed altered replication timing and increased DNA methylation compared with the normal chromosome 1, suggesting that X-derived chromatin may influence the epigenetic organization of adjacent autosomal regions. These findings provide evidence for partial spreading of X-associated chromatin features across the derivative chromosomes.

Z17.P

CRISPR/Cas9-Mediated Deletion of the SREBF1a Isoform Affects Adipogenic Differentiation of Porcine Mesenchymal Stem Cells

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Słowa kluczowe: Adipogenesis, CRISPR/Cas9, Mesenchymal stem cells (MSCs), Genome editing, Pig

Adipogenesis is a tightly regulated process governed by a cascade of transcription factors, including members of the sterol regulatory element-binding transcription factor (SREBF) family. The SREBF1 gene produces two isoforms, SREBF1a and SREBF1c, through alternative transcription start sites. While the role of SREBF1c in adipogenesis has been extensively characterized, the function of SREBF1a remains poorly understood. The aim of this study was to generate genetically modified porcine mesenchymal stem cells (MSCs) carrying a CRISPR/Cas9-mediated deletions within the promoter region specific to the SREBF1a isoform and to evaluate the potential role of SREBF1a in adipogenesis. MSCs were isolated from porcine adipose tissue. Guide RNA (gRNA) sequences targeting the SREBF1a-specific promoter region were designed and cloned into the pX330 vector. Recombinant plasmids were introduced into MSCs by nucleofection, and transfection efficiency was assessed using a GFP-expressing control plasmid. Genomic modifications were verified by PCR and Sanger sequencing. The impact of SREBF1a promoter deletions on adipogenic differentiation was evaluated by quantifying intracellular lipid accumulation using BODIPY staining. Efficient genome editing and successful isolation of modified MSC clones were achieved. Transfection efficiency reached approximately 40%. Clones carrying deletions within the SREBF1a promoter region were identified, including a 549 bp deletion in both homozygous and heterozygous states, as well as a 130 bp heterozygous deletion. Among 49 analyzed clones, seven carried targeted modifications, corresponding to a genome-editing efficiency of 14.3%. The modified MSCs maintained normal proliferative capacity but exhibited variable adipogenic differentiation potential. In conclusion, CRISPR/Cas9-mediated genome editing enabled the efficient generation of porcine MSCs carrying targeted deletions within the SREBF1a promoter region. These cells provide a valuable model for investigating the role of SREBF1a in regulating genes involved in adipogenesis. This study was financed by the the Polish National Science Center (NCN) Grant No. 2018/29/B/NZ2/00956 and by the statutory fund of the Department of Genetics and Animal Breeding (No. 506.534.09.00).

Z18.P

Analysing the role of lncRNA MALAT1 in porcine adipogenesis using a cell culture experiment

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Słowa kluczowe: lncRNA, preadipocyte, pig, adipogenesis

In pig production, the fat content of carcasses has markedly declined due to long-term selective breeding aimed at improving lean meat yield and growth performance. However, this trend is unfavourable because reduced intramuscular fat (IMF) negatively affects meat flavour and quality. In this study, we examined the overexpression and silencing of the lncRNA MALAT1, which was found to be differentially expressed in porcine subcutaneous adipose tissue between fatty and lean pigs. Although MALAT1 has been studied in several species in relation to fat accumulation and obesity, previous research has reported inconsistent and often contradictory results. Firstly, primary preadipocyte lines were established from abdominal subcutaneous fat and cryobanked in medium containing a cryoprotectant. For overexpression, we used a ready-to-use, custom-engineered Lentivirus vector prepared by CreativeBiogene containing a construct with genes for \ MALAT1 (ENSSSCT0000080860), GFP, and an antibiotic resistance gene for selection. Transduction has been performed in growth medium supplemented with 8 µg/mL Polybrene, with the MOI adjusted experimentally. We applied two controls: non-transduction and an empty plasmid (no MALAT1 sequence). Silencing has been achieved using siRNA (siMALAT1) delivered by lipofection or electroporation. Both overexpression and silencing effects were tested during the proliferation stage and adipogenic differentiation. A strong transfection effect was observed. Overexpression experiment showed that during proliferation, MALAT1 expression was 4-fold higher compared to the empty plasmid control, and during differentiation, MALAT1 expression towered by only 40% after 3 days of differentiation (compared to the empty plasmid control). After 7 days of differentiation, overexpression has not been observed. A silencing experiment using lipofection showed 7-fold MALAT1 knockdown at the proliferation stage and 45% knockdown after 7 days of differentiation. The application of electroporation yielded better results, with MALAT1 knockdown remaining at 70% after 11 days of differentiation. Unfortunately, overexpression seems to be abolished at the differentiation stage, but testing proliferation looks promising. Further observations will focus on transcriptome changes.

Z19.P

MicroRNA Expression Profiling of Dietary *Andrographis paniculata* Extracts Exerts Hepatoprotective Effects in Pigs

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Słowa kluczowe: *Andrographis paniculata*, Hepatoprotection, microRNA, Transcriptomics, Swine, Metabolic signalling

Background: *Andrographis paniculata* (AP) is recognized for its potent hepatoprotective properties, yet its exact molecular mechanisms in swine models remain largely unexplored. This study characterized the hepatic miRNA expression profiles of pigs supplemented with dietary AP extracts to decipher the underlying networks driving their beneficial effects.

Methods: A total of 13 commercial crossbred TN70 pigs (Norsvin Landrace x Large White) were investigated. Small RNA libraries from porcine livers (n = 3 control; n = 10 AP) were sequenced to identify differentially expressed (DE) miRNAs. Downstream transcriptomic impacts were predicted using RNAhybrid to construct a high-confidence target interaction map. Modulated networks were characterized via GO and KEGG pathway enrichment analyses.

Results: Transcriptomic profiling identified 12 significantly DE miRNAs in the AP vs. control comparison, demonstrating a predominantly up-regulatory response (11 upregulated, 1 downregulated). Most robustly induced transcripts included ssc-miR-100 ($\log_2FC > 3.60$), ssc-miR-1271-5p ($\log_2FC = +3.18$), ssc-miR-99a-5p ($\log_2FC = +2.79$), and ssc-miR-221-5p ($\log_2FC = +2.57$). Conversely, downregulation was restricted solely to ssc-miR-10386 ($\log_2FC = -1.34$). Eleven upregulated miRNAs targeted a universe of 6,100 unique genes. Functional enrichment analysis revealed that these potentially silenced targets clustered significantly ($p_{adj} < 0.003$) in pathways governing macromolecule catabolism, negative regulation of the cell cycle, endocytosis, autophagy, and metabolic hormone cascades. Conversely, the de-repression of the 3,536 predicted target genes of downregulated ssc-miR-10386 significantly ($p_{adj} < 0.02$) enriched pathways driving cellular communication and adaptive responsiveness, including the MAPK, Rap1, and NF-kappa B signaling pathways.

Conclusion: Dietary AP extract significantly modulates the porcine hepatic miRNA landscape. AP administration exerts a dual regulatory mechanism: it imposes a strong repressive control over metabolic turnover and autophagy via miRNA upregulation while simultaneously de-repressing vital signalling hubs (MAPK, NF-kappa B) via target miRNA downregulation.

Acknowledgements: This research was funded by Narodowe Centrum Nauki: UMO-2021/43/I/NZ9/02612, and Deutsche Forschungsgemeinschaft: 504983453 (WI 1754/18).

Z20.P

Selective hippocampal transcriptional adaptation to chronic cannabidiol exposure in mice

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Słowa kluczowe: CBD, behavior, hippocampus, transcriptome

Cannabidiol (CBD) is a non-psychoactive phytocannabinoid primarily isolated from *Cannabis sativa*. Growing evidence indicates that CBD exerts a wide range of pharmacological effects, including anti-inflammatory, immunomodulatory, antioxidant, anxiolytic, antipsychotic, and anticonvulsant activities. CBD has been extensively explored in both preclinical and clinical studies, particularly in neurological and psychiatric conditions. The present study was designed to assess the effects of varying doses of CBD on hippocampal transcriptomic profiles, corticosterone levels, and behavioral responses in mice. The study was performed using C57BL/6J mice ($n = 48$), which were randomly assigned to experimental groups receiving cannabidiol (CBD) at doses of 0.2, 10, or 20 mg/kg bw. CBD/vehicle was delivered via daily intraperitoneal injections for either 2 or 28 days. Behavioral testing included the Y-maze, light-dark box, Open Field, and Elevated Plus Maze Performance assays to assess spatial memory, anxiety-related behavior, and locomotor activity. Following euthanasia, hippocampal tissues were harvested for transcriptomic analysis using RNA-Seq. Corticosterone levels were determined in the blood plasma samples using an ELISA. Long-term treatment with 10 mg/kg CBD, resulted in the most significant hippocampal transcriptional alterations involving coordinated regulation of genes engaged in mitochondrial oxidative phosphorylation, with a notable representation of respiratory chain complex I subunits, as well as pathways related to purine and nucleotide metabolism. Shared mitochondrial respiratory genes were responsible for the enrichment of KEGG pathways linked to neurodegeneration and retrograde endocannabinoid signaling. Long-term CBD administration significantly reduced

corticosterone levels. Behavioral assessments were inconclusive regarding anxiety-like behavior; however, no negative effects of CBD on spatial and short-term memory were observed. These findings indicate that prolonged administration of a moderate CBD dose induces coordinated transcriptional adaptations in hippocampal pathways related to bioenergetics and nucleotide metabolism, accompanied by modulation of endocrine stress markers without observable behavioral impairment. Study funded by research project no. 2020/39/O/NZ9/00821 financed by the National Science Center, Poland

Z21.P

Whole-genome sequencing of *GTF2I*, *GTF2IRD1*, *CD38* and *BST1* reveals candidate variants for social behaviour in Valais Blacknose sheep

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Słowa kluczowe: sheep; whole-genome sequencing; social behaviour; oxytocin pathway, genetic variants

Background: Haploinsufficiency of *GTF2I* and *GTF2IRD1*, transcription factor genes deleted in Williams-Beuren syndrome (WBS), has been associated with hypersociality in humans and rodent models. Loss-of-function variants in *CD38* and *BST1*, ectoenzymes regulating oxytocin secretion via the NAD⁺/cyclic ADP-ribose pathway, have been linked to impaired social behaviour and increased autism risk. Whether naturally occurring variation in these genes is associated with inter-individual differences in social behaviour in domestic animals remains unexplored.

Methods: In this pilot study, we characterised the landscape of genetic variants in *GTF2I*, *GTF2IRD1*, *CD38*, and *BST1* in 14 Valais Blacknose sheep (*Ovis aries*) classified as human-oriented (n=7) or flock-oriented (n=7) using standardised behavioural tests. Whole-genome sequencing was performed on Illumina NovaSeqX (PE 2x150 nt), reads were aligned to GCA_016772045.2 using BWA-MEM, variants called with GATK HaplotypeCaller, and annotated with Ensembl VEP.

Results: A total of 4,075 unique variants were identified across the four loci. Several group-differentiated patterns were observed, though their interpretation is limited by the small sample size. In *CD38*, 1,365 variants were exclusive to human-oriented individuals vs. 77 in flock-oriented animals, including multiple MODERATE-impact missense variants. In *BST1*, three human-oriented-private missense variants were detected. In *GTF2IRD1*, missense variants showed higher carrier frequency in human-oriented individuals. Additionally, a HIGH-impact splice-donor variant in *BST1* and a predicted loss-of-function allele in *GTF2I* were each detected in single flock-oriented individuals, consistent with a potential role of reduced gene function in lower human-directed sociability.

Conclusions: These findings provide the first variant catalogue for these loci in any sheep population and identify candidate polymorphisms warranting validation in larger cohorts. This work was supported by the National Science Centre, Poland, grant no. 2023/07/X/NZ2/01697.

Z22.P

Effect of ACSL1 gene polymorphism on milk fat content and fatty acid profile in Holstein–Friesian cows – preliminary study

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Słowa kluczowe: ACSL1 gene, polymorphism, fatty acids, milk, dairy cattle

Background: Milk fat content and fatty acid profile are key traits in dairy cattle, influencing the nutritional and technological value of dairy products. The ACSL1 gene encodes acyl-CoA synthetase long-chain family member 1, an enzyme involved in long-chain fatty acids activation and lipid metabolism. Despite its well-established biological function, evidence regarding the effects of ACSL1 polymorphisms on milk fat traits remains limited. The aim of this study was to analyse sequence variation within a selected fragment of the ACSL1 gene and evaluate its association with milk fat content and fatty acid profile in Polish Holstein–Friesian cows. **Materials and Methods:** The study included 27 Polish Holstein–Friesian cows. A selected fragment of the ACSL1 gene, encompassing exon 2 and adjacent intronic regions, was amplified by PCR and analysed by Sanger sequencing. Associations between genotype and milk fat traits were evaluated using general linear models (GLM), taking parity and lactation stage into account. **Results:** A single nucleotide polymorphism (g.15222922T>G) was identified in intron 2 of the ACSL1 gene. Only TT and TG genotypes were detected in the analysed population. The identified polymorphism was associated with milk fat content ($P = 0.034$), as well as with long-chain fatty acids (LCFA), stearic acid (C18:0), oleic acid (C18:1), polyunsaturated fatty acids (PUFA), and trans fatty acids. Compared with the TG animals, cows with the TT genotype had higher milk fat content (4.74% vs. 4.01%) and higher concentrations of LCFA, C18:0, C18:1 and PUFA. Lactation stage was also significantly associated with LCFA, C18:0, C18:1, PUFA, and trans fatty acids.

Conclusions: The identified ACSL1 polymorphism was associated with milk fat content and selected fatty acid fractions, indicating its potential importance in the genetic regulation of milk fat traits. Further studies involving larger populations are required to validate these findings.

RNA-Seq Reveals Hepatoprotective Mechanisms of *Andrographis paniculata* in Aflatoxin B1-Challenged Porcine Hepatocytes

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Słowa kluczowe: RNA-seq; *Andrographis paniculata*; aflatoxin B1; porcine hepatocytes; hepatoprotection; transcriptomics; oxidative stress; mitochondrial remodeling; PPAR signaling; DESeq2.

Background: Aflatoxin B1 (AFB1) is a potent hepatotoxic mycotoxin that induces oxidative stress, mitochondrial dysfunction, metabolic disturbances, and inflammatory responses in hepatocytes. *Andrographis paniculata* (AP), a medicinal plant rich in andrographolide and related diterpenoids, has demonstrated antioxidant and hepatoprotective properties; however, the molecular mechanisms underlying its protective effects remain incompletely understood. **Methods:** Primary porcine hepatocytes were treated with AP under AFB1-induced stress, and transcriptomic alterations were investigated using RNA sequencing. Differential gene expression was analyzed using the DESeq2 pipeline with an adjusted p-value < 0.05 and |log₂ fold change| > 1. Functional characterization of differentially expressed genes (DEGs) was performed through Gene Ontology (GO), KEGG, and Reactome pathway enrichment analyses.

Principal component analysis (PCA), dispersion estimates, sample distance heatmaps, MA plots, and volcano plots were used to assess data quality and treatment-associated transcriptomic changes.

Results: RNA-seq identified 438 significantly differentially expressed genes, including 309 genes with biologically relevant expression changes (208 upregulated and 101 downregulated). Prominent upregulated genes included HSPA6, OSBPL2, DGAT1, APOA1, RBP4, CD74, C1QA, and IGHM, whereas COX1, ND6, GCLC, EIF5B, PCK1, and NDUFC1 were among the most significantly downregulated genes. PCA demonstrated clear separation between experimental groups, confirming a robust transcriptomic response. Functional enrichment analyses revealed significant modulation of oxidative phosphorylation, mitochondrial electron transport, ribosomal organization, protein processing in the endoplasmic reticulum, PPAR signaling, lipid metabolism, antioxidant defense, RNA metabolism, and HMOX1-associated cytoprotective pathways.

Conclusion: AP treatment induced extensive transcriptomic reprogramming in AFB1-challenged porcine hepatocytes, highlighting coordinated regulation of mitochondrial function, oxidative stress adaptation, lipid metabolism, immune signaling, and protein homeostasis. These findings provide transcriptome-wide evidence supporting the multifactorial hepatoprotective mechanisms of AP and identify potential molecular targets for future validation using qRT-PCR, proteomics, and functional assays.

Acknowledgements: This research is financed and supported by the scientific project NCN-OPUSLAP (UMO-2021/43/I/NZ9/02612).

MicroRNA Reprogramming by *Andrographis paniculata* and *Silybum marianum*: Molecular Mechanisms of Hepatoprotection Against Aflatoxin B1 in Pigs

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Słowa kluczowe: RNA-seq; *Andrographis paniculata*; *Silybum marianum*; aflatoxin B1; porcine hepatocytes; hepatoprotection; transcriptomics; oxidative stress; DESeq2

Background: Aflatoxin B1 is a potent hepatotoxin that severely disrupts liver function by interfering with RNA regulation. While medicinal herbs like *Andrographis paniculata* (AP; active component andrographolide) and *Silybum marianum* (silymarin) are recognized for their hepatoprotective properties, the precise underlying molecular mechanisms—specifically regarding microRNA (miRNA) modulation—remain poorly defined. **Materials and Methods:** This study investigated miRNA-mediated transcriptomic changes in porcine livers following AFB1 exposure and herbal supplementation. A feeding experiment was conducted using 130 young growing pigs. Three cohorts received standardized diets supplemented with *Andrographis*, *Silymarin*, or *Curcuma longa* (curcumin), while a control group of 24 pigs was exposed solely to AFB1. High-quality small RNA libraries were sequenced and analysed

using differential expression and weighted gene co-expression network analysis (WGCNA). Downstream mRNA targets will be predicted computationally (e.g., TargetScan, miRanda, RNAhybrid) based on 3' UTR binding stability.

Results: Preliminary data indicate that both *Andrographis* and *Silymarin* supplementation significantly modulated miRNA expression profiles associated with hepatoprotection. These changes were reflected in differentially expressed genes (DEGs) involved in critical immune response and detoxification pathways. Notably, common miRNA signatures were identified across all three herbal treatments, suggesting shared regulatory mechanisms. Ongoing WGCNA and KEGG pathway enrichment analyses aim to further elucidate the specific molecular pathways underlying these protective effects.

Conclusion: These findings demonstrate that *Andrographis paniculata* and *Silybum marianum* exert substantial hepatoprotective effects against AFB1-induced toxicity by driving targeted miRNA-mediated transcriptomic reprogramming. The identification of shared miRNA signatures across successful treatments highlights potential universal molecular targets for herbal-mediated liver protection in swine, serving as a foundational baseline for future integrative RNA-Seq validation.

ACKNOWLEDGEMENTS This research is financed and supported by the scientific project NCN-OPUSLAP (UMO-2021/43/I/NZ9/02612) entitled Multilevel molecular analysis of the hepatoprotective effect of medicinal herbs extracts in prevention of liver dysfunction caused by aflatoxin B1 in pig as an animal model (in-vivo), and hepatocyte cell culture analysis in human and pig.

Z25.P

Genetic parameters estimation of traits assessed during the stallion qualification for performance tests.

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Słowa kluczowe: stallion; performance test qualifications; heritability; genetic correlations

When assessing breeding value and genetic progress, it is important to include factors that may influence the selected traits and to use precisely estimated genetic parameters. Therefore, the study aimed to estimate the heritability and genetic correlations between performance traits. Material and method: The data included results for 550 stallions that entered the qualifications for performance tests between 2017 and 2025. The following traits were registered: walk, trot, canter, jumping, and overall result. Additionally, a trait called "success" was created (passed or failed the qualification). Because of sport specialization, not all horses received notes on all traits. The overall result was available for 447 individuals. The single-trait animal model with the AI-REML method was implemented in the DMU package. The final model included the fixed effects of the year and horse test group, linear regression on the age, and a random direct additive effect of the animal. Results: The heritability was estimated at a moderate level, between 0.39 and 0.58, with SE between 0.01 and 0.13. The highest value was obtained for walk, and the lowest for success. After the liability transformation, the heritability for "success" reached the highest level among all traits, with a value of 0.61 and the SE of 0.02. The genetic correlations were moderate to high. For most traits, positive correlations were observed, ranging from 0.48 to 0.91. Except for the trait "success," which was negatively correlated with all other traits (-0.76 to -0.58). Conclusions: The results suggest that the traits evaluated during the qualification of stallions for the performance test are heritable and can be used for the genetic evaluations. The study should be continued; however, the standard errors for heritability estimations are already very promising. The trait "success" should be treated with caution, as its genetic correlations with other traits unexpectedly differ from those of the rest.

Z26.P

Isolation and silvicultural practices as drivers of genetic variation in relict and pest deadwood-dwelling beetles

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Słowa kluczowe: saproxylic beetles, ddRAD-seq, biodiversity, NGS

Saproxylic beetles play a key role in decomposition, nutrient cycling, and maintaining forest biodiversity. Because they are highly sensitive to habitat change, they serve as valuable bioindicators of human impacts on forest ecosystems. As European forests face increasing management intensity, fragmentation, and structural simplification, understanding how these pressures affect the genetic structure and long-term viability of wood-dwelling species is essential. Using double-digest RAD sequencing (ddRAD-seq), we generated genome-wide SNP datasets for multiple saproxylic beetle species, including rare old-growth specialists, widespread generalists, and common pest taxa. Sampling included both primeval forests, where natural processes remain largely intact, and managed stands with reduced deadwood availability and lower habitat connectivity. We addressed two main questions: (i) how strongly does forest isolation shape population structure, and (ii) to what extent does forest management influence genetic diversity? Many species showed clear genetic divergence among forests. Rare old-growth specialists had significantly higher genetic diversity in primeval forests, reflecting their vulnerability to habitat alteration. In contrast, widespread species were more genetically stable across the landscape, while some pest beetles showed increased diversity in managed forests, highlighting taxon-specific management priorities. These findings reveal key evolutionary and ecological mechanisms shaping insect diversity in human-altered forests and support targeted management strategies such as restoring connectivity, retaining deadwood, and protecting remaining primeval forests to sustain biodiversity and ecosystem functions. This work was supported by NCN, grant no. 2021/43/B/NZ9/00991

Whole genome analysis reveals complex origins of Polish Konik horses

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Słowa kluczowe: Polish Konik, whole genome sequencing (WGS), genetic diversity, Tarpan, population structure, conservation genetics

Polish Konik is a native horse breed from Poland, often regarded as a living descendant of the extinct Tarpan. Although the origins of the breed have never been known, the assumption has become crucial to the breed's conservation status and cultural importance. Our study uses whole genome sequencing to investigate the genetic origins and diversity of the Polish Konik horse. We sequenced 12 Polish Konik individuals (grouped in four pooled samples, representing different sire and dam lines) and compared them with 148 published genomes from modern breeds and ancient horses (including the Tarpan sample). Principal component analysis (PCA) and ADMIXTURE analysis showed that Polish Koniks form a tight cluster, distinct from most modern breeds and sharing ancestry components with primitive and ancient horses. The Neighbor-Joining tree indicated more complex origins, grouping Polish Konik horses with Dülmener and certain ancient samples. Importantly, we found no evidence that the Polish Konik is a direct descendant of the extinct Tarpan. However, the low coverage of the Tarpan genome and the pooled sampling design mean this conclusion must be interpreted with caution. The findings of our research reveal a complex origin for the Polish Konik horse breed.

The evolution of duplications in the mitogenomes of birds

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Słowa kluczowe: Aves, birds, duplications, mitochondrial genomes, rearrangements

Historically, the duplication of the control region and adjacent genes in avian mitochondrial genomes has been considered a rare event that occurs independently across different lineages. However, the apparent scarcity of these duplications is likely an artifact of poorly designed PCR primers, inadequate sequencing methods, or the reliance on un-duplicated reference sequences during genome assembly. To address this, we applied a targeted molecular strategy to identify duplicate mitogenome fragments, revealing repeated regions in 261 taxa. Incorporating additional avian mitogenomes from GenBank expanded this total to 547 taxa, representing 33 of the 43 avian orders. We then mapped the presence and absence of these duplications onto a phylogenetic tree of avian orders to infer ancestral states. Contrary to prevailing assumptions, our results demonstrate that mitochondrial duplications are a widespread phenomenon in birds. Their discovery across a vast number of Neoaves orders suggests that mitochondrial duplication is an ancestral state common to all birds, rather than a feature exclusive to early-diverging lineages. From an evolutionary perspective, a single ancestral duplication followed by independent losses—which our data indicates occurred in at least six orders—is a highly more parsimonious scenario than the traditional hypothesis of countless independent duplication events. This work was supported financially by the National Science Centre Poland (Narodowe Centrum Nauki, Polska) under the number UMO-2021/43/B/NZ8/02136.

Z29.P

Phylogenetic relationships between *Amazona* parrots based on mitochondrial genomes

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Słowa kluczowe: *Amazona*, evolution, mitochondrial genomes, parrots, phylogeny

Parrots of the genus *Amazona*, commonly known as amazons, are a vibrant Neotropical group renowned for their exceptional vocal mimicry and complex social behaviors. Their geographic range extends from northern Mexico through Central America and the Caribbean to South America. The taxonomy of *Amazona* remains fluid and incomplete; widespread disagreement persists regarding species and subspecies boundaries, leaving the phylogenetic positions of many taxa uncertain. Previous phylogenetic studies have relied on limited mitochondrial or nuclear markers, and insufficient taxon sampling has left many relationships unresolved. Because a comprehensive, mitogenome-scale analysis has yet to be performed for this group, the systematic status of many taxa requires verification. To address these gaps, we sequenced the complete mitochondrial genomes for representatives of most recognized *Amazona* taxa to resolve these long-standing phylogenetic and systematic uncertainties. Phylogenetic analyses revealed the presence of two main clades. The first clade comprises two subclades containing species that inhabit the Greater Antilles and Central America. The second includes South American species intermixed with lineages from the Lesser Antilles and Central America. Phylogenetic relationships and geographic distributions indicate that the Greater Antilles were colonized once from Central America, whereas the Lesser Antilles were colonized twice from South America. Additionally, at least four migration events occurred from South to Central America. Phylogenetic analyses, alongside genetic distances, resolved the taxonomic positions of numerous taxa. Specifically, nine taxa currently described as subspecies should be elevated to species status, while one species should be reclassified as a subspecies. Finally, the analyses revealed three instances of mitochondrial DNA introgression between *Amazona ochrocephala* and *A. aestiva*, as well as between different subspecies of *A. ochrocephala*. This work was supported financially by the National Science Centre Poland (Narodowe Centrum Nauki, Polska) under the number UMO-2021/43/B/NZ8/02136.

Z30.P

Reconstructing the Phylogeny of the European Snow Vole (*Chionomys nivalis*) Using Ancient DNA

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Słowa kluczowe: ancient DNA, *Chionomys nivalis*, phylogeny

Repeated climatic changes during the Pleistocene strongly influenced the distribution and genetic diversity of many cold-adapted species. The European snow vole (*Chionomys nivalis*), a glacial relict currently inhabiting fragmented mountain regions of Europe and Western Asia, is a valuable model for studying how past climate changes shaped the evolution of species. To reconstruct the phylogeny and population history of *Chionomys*, we generated the largest mitochondrial genomic dataset available for this genus, including 145 complete mitochondrial genomes from both modern and fossil specimens. The dataset consisted of 75 ancient mitogenomes obtained from 18 palaeontological sites in 10 countries and 70 modern mitogenomes representing 18 countries. We also analysed nuclear genomic data from 37 individuals to investigate population structure. Radiocarbon dating of fossil remains provided a temporal framework for evolutionary analyses. Phylogenetic analyses revealed six major mitochondrial lineages within *C. nivalis*. The oldest lineage was found in Iran and Turkmenistan, suggesting that the Middle East played an important role in the early evolution of the species. Additional distinct lineages occurred in Turkey, Syria, Israel, Georgia, and Iran, indicating long-term survival of populations in this region. European populations formed several regional clades associated with the Balkans, Alps, Apennines, Iberian Peninsula, and Central Europe, consistent with survival in multiple glacial refugia and subsequent postglacial expansion. Nuclear genomic analyses supported the mitochondrial results and confirmed a clear division between Middle Eastern and European populations. Middle Eastern populations showed higher genetic diversity and stronger population structure than European populations, suggesting that this region was an important centre of diversification during the Quaternary. Our results provide the most comprehensive reconstruction of the phylogeny of *C. nivalis* to date and demonstrate the important role of glacial refugia and climatic changes in shaping the genetic diversity of this mountain species.

Phylogenetic analyses of saiga antelope (*Saiga tatarica*) including ancient DNA

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Słowa kluczowe: Ancient DNA, evolution, mitochondrial genomes, phylogeny, Saiga

Saiga (*Saiga tatarica*) is a small steppe antelope and a critically endangered Ice Age relic currently restricted to a few fragmented regions in Central Asia. Consequently, studying this species is a high conservation and scientific priority. To elucidate the evolutionary history and unresolved fossil taxonomy of this species, we analyzed 112 mitogenomes and 278 control region sequences across its historical and contemporary Holarctic range. The oldest haplotype diverged over 150 ka in Northeast Asia, aligning with early fossil records, followed by extinct lineages in Ukraine and Romania. Five major groups diverged during the cold MIS4 period (75–55 ka) and expanded across Eurasia and North America, indicating that modern saigas are a heterogeneous group originating from at least five ancient lineages. Among these, the Mongolian population (*S. t. mongolica*) split approximately 15 ka during late MIS2, confirming its subspecies status, while a Siberian/Northeast Asian lineage (~33 ka) colonized Canada around 27 ka. Phylogeographic analyses revealed widespread sequence intermixing and a lack of population structure, demonstrating that Pleistocene saigas functioned as a vast metapopulation with fluid gene flow across the continuous mammoth steppe. However, effective population size (N_e) fluctuated significantly due to climate-driven habitat shifts. The population was large and stable during the warm MIS3 stage but crashed at the onset of the cold MIS2 period. Although N_e recovered as temperatures rose, prominent declines occurred after the Last Glacial Maximum (LGM) and during the warm MIS1 period (9–6 ka). These demographic shifts reflect the contraction of the steppe-tundra ecosystem, which was disrupted by ice-sheet expansion and heavy snowfall during cold intervals, and by afforestation, increased humidity, and glacial meltwater barriers during warm phases. This work was supported by the National Science Centre Poland (Narodowe Centrum Nauki, Polska) under Grant no. 2020/37/B/NZ8/02595.

Z32.P

Associations Between TLR1 and TLR2 Gene Polymorphisms and Udder Health Indicators in Holstein–Friesian Cattle

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Keywords: cattle, mastitis, polymorphism, Toll-like receptors (TLR)

Mastitis is a complex disorder affecting the mammary gland. It is characterized by inflammation of the milk ducts and glandular tissue of the udder, leading to impaired physiological function. From an economic perspective, mastitis is considered one of the most important diseases in dairy cattle. Despite significant advances in treatment methods, udder infections remain a common problem. Intensive breeding programs focused primarily on increasing milk yield and milk component content may contribute to reduced resistance to mastitis. The aim of this study was to investigate associations between polymorphisms of the selected genes TLR1 and TLR2, whose products are involved in the innate immune response, and resistance to mastitis in Black-and-White Holstein–Friesian dairy cows. The study included a herd of 933 cows. Peripheral blood samples were collected from all animals, and genomic DNA was subsequently isolated. Genotypes were determined using the PCR–RFLP method. Significant genetic interactions were identified for TLR1 and TLR2 polymorphisms. Certain TLR1×TLR2 interactions were associated with increased susceptibility to mastitis, whereas other TLR1×TLR2 and TLR2×TLR2 interactions were associated with a more favorable course of the disease. These findings suggest a potential role of the studied genes in shaping resistance to mastitis; however, further validation in larger and independent dairy cattle populations is required.

Z33.P

Analysis of Genetic Structure and Biodiversity of Selected Horse Breeds in Poland Based on 17 Microsatellite Loci

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Słowa kluczowe: horse; parentage testing; genetic structure; genetic biodiversity

Introduction: Assessing genetic diversity and parentage verification are crucial for conserving native equine resources. Microsatellite markers (STRs) are the international standard for individual identification and pedigree control.

Aim: This study characterised the polymorphism and genetic structure of four horse breeds in Poland, including primitive breeds and regional types, using a recommended STR panel.

Material and Methods: Peripheral blood and hair follicle samples were collected from 801 horses: Hucul horse (hc), Polish Konik (kn), Malopolski horse (m) and Polish Coldblood (z), including two regional types: Sokolski (z/sok.) and Sztumski (z/sztum.). Profiling of 17 STR loci was performed via multiplex PCR using the Equine Genotypes Panel 1.1 Kit.

Results: The overall mean number of alleles (N_a) was 8.647 and the effective number of alleles 4.952. The highest N_a was recorded in the Polish Coldblood (8.882) and the lowest in the Malopolski breed (8.353). The mean values for the inbreeding coefficient within populations ($F_{IS} = 0.071$) and the total population ($F_{IT} = 0.081$) were low. A low level of inter-population differentiation was confirmed by the mean fixation index ($F_{ST} = 0.011$). Mean observed heterozygosity (H_o) ranged from 0.6726 (kn) to 0.7323 (z/sok). The polymorphism information content (PIC) ranged from 0.6597 (kn) to 0.6952 (m). The cumulative power of discrimination (PD) and the exclusion probability when both parents are known (PE2) approached 100% ($\geq 99.999\%$) across all populations, while the probability of identity (PID) ranged from $0.16 \cdot 10^{-17}$ (kn) to $9.19 \cdot 10^{-17}$ (z/sztum).

Conclusions: The analysed panel of 17 STR markers exhibits high discriminatory power and polymorphism across all investigated groups. The low F_{ST} value indicates genetic stability within the populations, confirming the high efficacy of this panel for routine parentage verification and biodiversity monitoring of horses in Poland.

Polymorphism of the PRNP gene in Old-type Polish Merino sheep

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Słowa kluczowe: PRNP, genotyping, polymorphism, scrapie, sheep

Scrapie is one of the prion diseases and belongs to the group of transmissible spongiform encephalopathies (TSEs). Caused by prions (PrP^{Sc}), scrapie is connected with the PRNP gene. It has been shown, that ovine PRNP gene polymorphism influence the scrapie susceptibility. The VLRQ allele significantly increases susceptibility to classical scrapie, while the ALRR allele confers genetic resistance to the disease. The ALHQ and AFRQ alleles, on the other hand, are considered to be associated with the occurrence of atypical scrapie. The aim of the study was to investigate the polymorphism of the PRNP gene in Old-type Polish Merino sheep breed. We have genotyped four codons of the PRNP gene (136, 141, 154, 171) in 1064 Old-type Polish Merino sheep breed. The samples are from the routine PRNP genotyping program for sheep and originated from 16 flocks. The DNA was isolated using Sherlock AX (A&ABiotechnology). Then samples were genotyped using TaqMan MGB probes and StepOne Plus Real-time PCR System (Applied Biosystems). We found 10 different genotypes in studied group. The most frequent genotypes were ALRR/ALRQ (41,2%), ALRR/ALRR (38,63%) and ALRQ/ALRQ (10,71%). ALRR, associated with classical scrapie resistance, was the most frequent allele in the Old-type Polish Merino sheep breed (62,03%). The most undesirable VLRQ allele, connected to classical scrapie risk, occurred at a frequency of 0,42%. Alleles ALHQ and AFRQ, associated with atypical scrapie, had frequency 2,16% and 1,97%, respectively. About 85% of the animals belonged to the NSP1 and NSP2 risk groups, which have the lowest risk of classical scrapie occurrence. Based on the results obtained, it can be concluded that the Old-type Polish Merino breed appears to be relatively resistant to classical scrapie. The study was financed by Ministry of Agriculture and Rural Development Multiannual Programme, 03-18-09-09 (A9).

Z35.P

Microsatellite panel for roe deer profiling

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Słowa kluczowe: STR panel, microsatellites, roe deer

Microsatellite markers (short tandem repeats, STRs) remain one of the most widely applied molecular tools for individual identification, parentage testing, population genetic studies, and wildlife forensics due to their high polymorphism, codominant inheritance, and reproducibility. In roe deer (*Capreolus capreolus*), microsatellite-based genotyping has been successfully used for population monitoring and forensic investigations, particularly in cases of illegal harvesting and wildlife crime. The aim of the study was to revalidate the roe deer STR panel. We used 11 autosomal STR markers and one sex determination marker in the panel for roe deer profiling: INRA222, MAF70, CSSM66, SPS115, T26, RM188, RT1, INRA177, RT13, ETH225, OarAE129, AMEL. All the markers were amplified in one Multiplex PCR reaction using TypeIT Microsatellite PCR Kit (Qiagen). The PCR products were electrophoresed with presence of GeneScan™ 500 LIZ dye Size Standard in the SeqStudio Flex Genetic Analyzer (Applied Biosystems by Thermo Fisher Scientific). Then, each marker was amplified separately (monoplex PCR) for chosen homozygous samples. We have examined the markers in 100 roe deer and determine the number of alleles in each marker. We have establish allelic ladders using selected homozygous individuals. We have sequence some of the alleles to calculate the exact number of tandem motif repeats. We have characterized some of the alleles of the STR panel in the studied group. The study allowed to increase the accuracy of STR markers genotyping and the results can be used as valuable tool for identifying new alleles and mutations in the panel. Research is continuing. The study was financed by Statutory project no 01-18-26-11 and 01-18-31-11

Z36.P

Genetic diversity of the last European roller (*Coracias garrulus*) population from Poland

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Keywords: *Coracias garrulus*, European Roller, mitochondrial genes, microsatellites

The European Roller (*Coracias garrulus*) is a bird species characterized by its distinctive and colourful plumage, dominated by blue and blue-green hues. Once widespread throughout Poland, the species is now on the brink of extinction. The intensification and modernization of agriculture, leading to a reduction in insect abundance and nesting-site availability, are considered the main factors responsible for its critical conservation status. For many years, the Kurpie Plain supported the largest and most stable population of the European Roller in Poland, with more than 25 breeding pairs recorded there in 2015. Currently, the Polish population is extremely small, with very few birds left at Kurpie region. The aim of this study was to assess the genetic diversity of the last remaining population of the European Roller in Poland and compare it with historical samples, using molecular genetic data. Genetic diversity was evaluated based on fragments of the mitochondrial cytochrome c oxidase subunit I gene (COI), the mitochondrial DNA control region (mtDNA CR), and microsatellite markers. Additionally, samples collected in Ukraine were also analysed. Altogether, the obtained genetic data were compared with sequences available in GenBank to establish the phylogeographic position of the nearly extinct Polish population, thereby improving our understanding of the species' population genetics across Europe, and to provide a scientific basis for the development of active conservation strategies. Preliminary results suggest that the Polish population of the European Roller represents a unique gene pool that differs significantly from other European populations studied to date. These findings highlight the high conservation value of the Polish population and emphasize the risk of losing a unique component of the species' genetic diversity if the European Roller becomes extinct in Poland.

Z37.P

Human-Mediated Dispersal and Environmental heterogeneity shape genetic variation of *Mytilus* spp. in South American Coastal Hybrid Zones

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Słowa kluczowe: mussels, population genetics, SNP, genetic-environmental association

In South America, nowadays three *Mytilus* taxa are recognized: native *M. chilensis* and *M. platensis* and invasive *M. galloprovincialis*. However, the application of molecular tools to investigate these populations remains limited. Using SNP markers, we characterized species composition and patterns of admixture within the hybrid zone between *Mytilus chilensis* and *M. platensis* in South America. Bayesian and non-Bayesian clustering, together with reassignment analyses, revealed a complex structure of the natural hybrid zone encompassing the Strait of Magellan, Isla Grande de Tierra del Fuego, and the Falkland Islands. This zone can be divided into three regions. The first, located along the Atlantic coast, is dominated by *M. platensis* and hybrids. The second, within the Strait of Magellan, comprises individuals of both species alongside mussels exhibiting varying degrees of hybridization. The third region, including the Strait near Punta Arenas, fjords of Isla Grande de Tierra del Fuego, and the Beagle Channel, is characterized predominantly by *M. chilensis* with a low proportion of hybrids. In contrast, analyses of *Mytilus* populations in Uruguay revealed a clear genetic discontinuity across the Río de la Plata, separating northern and southern populations. The Uruguayan coast is mainly inhabited by *M. chilensis* and hybrids with *M. platensis*, suggesting an anthropogenic introduction, as the nearest natural populations of *M. chilensis* occur far to the south, while Argentine coasts are dominated by pure *M. platensis*. Although environmental variables explained a small fraction of the overall genetic variation, four factors: total cloud cover, photosynthetically available radiation, ocean temperature, and silicate concentration may contribute to shaping genetic patterns. Notably, a single locus (BM57A), annotated as a subunit of mitochondrial complex I, emerged as a candidate for environment-mediated selection. These findings highlight the importance of human-mediated dispersal in shaping marine biodiversity and underscore the need for monitoring programs to assess ecological consequences.

Assessment of Genetic Variability in the Polish Hunting Spaniel Population

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Słowa kluczowe: Polish Hunting Spaniel, Genetic Variability, microsatellite

The Polish Hunting Spaniel (PHS) is a relatively young breed. While its history dates back to the 19th century, the breed was recreated in the 1980s thanks to the efforts and breeding work of Dr. Andrzej Krzywiński. To be recognized as a domestic dog breed by the FCI, the Polish Hunting Spaniel must demonstrate sufficiently high genetic variability. Therefore, the aim of the study was to determine the genetic variability of the Polish Hunting Spaniel population. The biological material consisted of topcoat hairs and peripheral venous blood collected intravitaly from 216 dogs. DNA was isolated using a column method. The study of genetic variability of the Polish Hunting Spaniel population utilized 22 microsatellite sequences, located on 21 different autosomes and three on the Y chromosome. STR sequences were amplified in three multiplexes. The following programs were used for the analyses: Cervus 3.0.3, ALFREQ, GENETIX 4.05, and STRUCTURE v 2.3.4. The average number of alleles per locus was 7.18. The observed (HO) and expected (HE) heterozygosity values were similar (HO = 0.646, HE = 0.641), indicating a lack of significant genetic imbalance. Most markers were highly polymorphic and suitable for genetic analysis. FCA analysis confirmed genetic similarity between males and females. However, STRUCTURE analysis revealed variation within the population, with the possibility of distinguishing 3-4 subpopulations. Analysis of microsatellite sequences on the Y chromosome – 4 haplotypes were identified. The level of genetic variability in the Polish Hunting Spaniel population indicates the possibility for The Polish Kennel Club to seek 'provisional breed recognition' from the FCI.

Z39.P

Transcriptome analysis revealed differential gene expression in mammary gland lymph nodes of sheep with opposite SRLV infection status and different TMEM154 E35K genotypes

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Słowa kluczowe: SRLV, transcriptome, TMEM154, sheep

Host genetic factors influence sheep susceptibility to the infection with small ruminant lentiviruses (SRLV). The TMEM154 E35K variation is associated with infection risk in sheep, with the KK genotype conferring reduced susceptibility compared to those with EK or EE genotypes. The degree of the protective effect varies according to breed and SRLV genotype, suggesting the involvement of additional contributing factors. This study aimed to compare mammary gland lymph node (MGLN) transcriptomes in sheep with opposite SRLV serological infection status and different TMEM154 E35K genotype. German Merinoland ewes (>3 years of age) were screened serologically for SRLV infection and genotyped for TMEM154 E35K. Based on these criteria, three groups were defined: (1) SRLV-positive, TMEM154 EK (posEK, n=3); (2) SRLV-positive, TMEM154 KK (posKK, n=5); (3) SRLV-negative, TMEM154 KK (negKK, n=5). Following RNA sequencing, the analysis of differentially expressed genes (DEGs) and functional enrichment were conducted in DESeq2 ($|\log_2FC| \geq 1$; $FDR \leq 0.5$) and DAVID tools, respectively. The highest number DEGs (54 up, 32 down) was found between negKK and posEK ewes. These genes were enriched in several GO terms, including antimicrobial humoral immune response mediated by antimicrobial peptide, innate immune response, extracellular space, and KEGG pathways, including neutrophil extracellular trap formation and IL-17 signaling pathway. Moreover, 15 (3 up, 12 down) genes were differently expressed between negKK and posKK ewes. These DEGs were enriched in different GO terms, including those related to antimicrobial humoral immune response mediated by antimicrobial peptide, extracellular region and space, and endothelial cell migration. A comparison between posKK and posEK revealed a single DEG. In conclusion, gene expression in MGLN of German Merinoland ewes was influenced by the combined effect of SRLV infection status and the TMEM154 E35K genotype. Funding: National Science Centre, Poland (UMO-2020/39/I/NZ9/01304), German Research Foundation (DFG, LU 1439/4-1). Bioinformatics analysis: Poznań Supercomputing and Networking Center resources.



R1.O

Hidden diversity within the *Medicago truncatula* HapMap collection: new chromosome-scale genomes reveal extensive genomic variation and question species boundaries

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Słowa kluczowe: *Medicago*, genetic variation, HapMap, symbiotic islands, genomic assembly

Exploring natural genetic diversity provides a powerful framework for dissecting complex biological processes, including host–microbe interactions, environmental adaptation, and trait evolution. The model legume *Medicago truncatula* has been instrumental for understanding nitrogen-fixing symbiosis, indeterminate nodule development, and the genetic regulation of plant–rhizobium interactions. The establishment of the *Medicago* HapMap collection, comprising hundreds of accessions sampled across the Mediterranean Basin, has transformed the species into a powerful system for population genomics and genome-wide association studies. This resource captures extensive phenotypic and genetic diversity and has enabled the identification of loci controlling symbiosis, development, and adaptation-related traits. Despite its importance, genomic resources remain limited. Only a few chromosome-scale reference genomes are currently available, restricting our ability to assess structural variation, gene content diversity, and the evolutionary relationships among HapMap accessions. Recent phylogenomic studies have further suggested that some accessions within the collection may represent misclassified or introgressed lineages, raising the possibility that the HapMap resource contains taxa beyond *M. truncatula* sensu stricto. Such findings have important implications for the interpretation of association studies and for understanding the evolutionary history of the genus *Medicago*. To further explore the genetic diversity of this important dataset, we generated chromosome-scale assemblies for three diverse ecotypes (HM257, HM266 and HM315) using long-read sequencing technologies, effectively doubling the number of high-quality genomes available for this species. By combining genetic, epigenetic and transcriptomic data, we revealed substantial variation among these ecotypes, affecting, among others, symbiosis-related genes and gene expression in symbiotic nodules. Our results highlight the need for a more comprehensive genomic characterization of HapMap accessions and support the view that the collection harbors previously underappreciated genetic and potentially taxonomic diversity.

R2.O

Evolutionary Conservation of Pericentromeric Crossover Hotspots in *Arabidopsis thaliana*

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Słowa kluczowe: crossover, meiosis, hotspot, pericentromeres, *Arabidopsis*

Meiotic recombination generates genetic diversity through reciprocal DNA exchange between homologous chromosomes, known as crossovers (COs). Together with chromosome segregation, COs create new allele combinations and reshape parental genomes. In most eukaryotes, COs are concentrated within narrow recombination hotspots. Although meiotic recombination is generally suppressed within centromeres, individual hotspots in the pericentromeric regions of *Arabidopsis thaliana* can exhibit exceptionally high crossover activity. While recombination hotspots are often considered evolutionarily dynamic, the conservation of individual pericentromeric hotspots across genetically divergent *Arabidopsis* genomes remains unclear. To address this question, we combined a fluorescence-based recombinant enrichment strategy with high-resolution sequencing to investigate meiotic recombination around centromere 3 in *Arabidopsis*. We generated detailed crossover maps from more than 2,000 recombinant plants derived from hybrids involving the highly diverged African relict accession Tanz-1 and the accessions Col-0 and Ler-0. Comparison of hotspot activity and location across Col×Ler, Col×Tanz, and Ler×Tanz hybrids revealed that crossover hotspots within the pericentromeric region were remarkably conserved despite substantial sequence divergence. We further analysed a pericentromeric interval (ChP) containing three recombination hotspots, including the highly active hotspot Coco. Using 25 hybrids generated by crossing ChP-Col with diverse *Arabidopsis* accessions, we observed considerable variation in recombination frequency across the interval. High-resolution seed-typing of five selected hybrids enabled precise mapping of crossover distributions. Despite extensive sequence polymorphism, hotspot positions remained highly conserved, whereas differences in hotspot activity were primarily associated with local structural variation. To our knowledge, this study provides the first high-resolution analysis of the evolutionary conservation of individual recombination hotspots within a plant pericentromeric region. Our findings show that hotspot positions, and often their relative activity, remain stable despite extensive sequence divergence, revealing an unexpected robustness of meiotic recombination landscapes and providing new insights into the mechanisms governing hotspot evolution. These results may also inform efforts to manipulate recombination in genetically constrained genomic regions for crop improvement.

R3.O

Identification of QTLs for yield, thousand-seed weight, and seed coat proportion using a newly developed genetic map of narrow-leaved lupin.

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Słowa kluczowe: narrow-leaved lupin, genetic map, QTL, thousand-seed weight, yield, seed coat proportion

Narrow-leaved lupin is a high-protein legume used in the feed industry, but it also has potential for human consumption. Furthermore, it plays a significant role as a rotation crop, improving soil conditions for the cultivation of other plants, particularly cereals. Narrow-leaved lupin is grown mainly in Australia. Poland ranks second in the world in terms of lupin cultivation area. Breeding of this species should be supported by biotechnological tools, particularly molecular markers useful for selecting genotypes with desirable traits. Therefore, the aim of the study was to develop a genetic map incorporating QTL loci for traits such as thousand-seed weight, seed coat percentage, and yield. Reducing the seed coat weight in conjunction with thousand-seed weight and yield is of key importance for the use of narrow-leaved lupin in various sectors of the bioeconomy. Reducing the proportion of the seed coat will increase the edible portion, improve digestibility, and enhance the crop's utility. The genetic map was developed based on the genotyping of recombinant inbred lines derived from a cross between the Graf variety and a thin-seed-coat mutant (AU-11257-19/1). Genotyping was performed using sequencing (SeqSNP) and the DArT-seq method. A total of 3,092 unique loci were identified, representing all 20 chromosomes and their corresponding 20 linkage groups, 154 scaffolds, and 640 loci not assigned to the genome sequence. Furthermore, the map enabled the assignment of 129 scaffolds to the genome sequence. The map currently contains 2,407 loci, with a total length of 2,883.16 cM. For the analyzed traits, QTLs were localized in 13 linkage groups, while for thousand-seed weight, they were localized in 12 linkage groups. The results of QTL mapping for the studied traits showed a high level of collinearity with the narrow-leaved lupine genome sequence, as well as reproducibility of results across replicates and years.

R4.O

From Genome-Wide Association Analysis to Plant Breeding Practice: Development and Validation of PCR Markers for Marker-Assisted Selection of Blackleg Resistance in Rapeseed

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Słowa kluczowe: Brassica napus, blackleg, DArTseq, GWAS, marker assisted breeding, PCR

High-throughput genotyping technologies, such as DArTseq, enable the identification of molecular markers associated with agriculturally important traits. However, their practical application in plant breeding requires conversion into simple, cost-effective genotyping assays that can be performed in standard molecular laboratories. A particular challenge is the development of assays for SNP markers, whose analysis typically relies on specialized detection methods. The aim of this study was to develop PCR-based markers for marker-assisted selection (MAS) of blackleg resistance in rapeseed (*Brassica napus* L.) by converting SilicoDArT and SNP markers identified through a genome-wide association study (GWAS) using DArTseq technology. Based on phenotypic evaluation and genome-wide association analysis, markers associated with blackleg resistance were selected for conversion into PCR assays. Conventional PCR assays were developed for SilicoDArT markers, whereas allele-specific ARMS-PCR assays were designed for SNP markers. The developed assays were optimized and validated using DNA sequencing and breeding materials differing in their levels of resistance. The resulting PCR markers enable rapid, reproducible, and cost-effective genotyping of selected loci associated with blackleg resistance. The developed tool provides a practical solution for marker-assisted selection and facilitates the translation of genomic discoveries into routine rapeseed breeding programs.

R5.O

Development and characterization of interspecific hybrids between autotetraploid bilberry (*Vaccinium myrtillus* L.) and highbush blueberry (*Vaccinium corymbosum* L.), and identification of genes associated with phenolic compound accumulation in bilberry fruits using NGS

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Słowa kluczowe: *Vaccinium* sp., interspecific hybrids, anthocyanins, crossbreeding barrier, NGS

Bilberry (*Vaccinium myrtillus* L.) is a wild species with fruits rich in bioactive compounds, particularly anthocyanins, which are present in both the skin and flesh at levels approximately three times higher than in highbush blueberry. Bilberry is not commercially cultivated and its fruits are harvested only from natural habitats, which is costly and harmful to forest ecosystems. In contrast, highbush blueberry (*Vaccinium corymbosum* L.) is widely cultivated in Poland, but cultivars with purple flesh are not available. Developing *Vaccinium* interspecific hybrids with anthocyanin-rich flesh could enhance fruit nutritional value and support food and pharmaceutical applications. To overcome the postzygotic crossing barrier (triploid block) between diploid bilberry and tetraploid highbush blueberry, we obtained tetraploid bilberry plants capable of crossbreeding with highbush blueberry. We expect the resulting hybrids to inherit high anthocyanin content fruit flesh, while the identification of genes and molecular markers associated with anthocyanin accumulation may facilitate the early selection of desirable genotypes. Over four years, the hybrid status of more than 100 seedlings was verified using molecular markers. Most detected alleles were of maternal origin, whereas the loss of paternal alleles likely resulted from genetic or epigenetic changes associated with interspecific hybridization. All seedlings are currently undergoing phenotypic evaluation, as they exhibit traits characteristic of highbush blueberry, and their hybrid status will be further confirmed by repeated molecular analyses in subsequent growing seasons. Our research also investigates the expression of genes associated with anthocyanin accumulation in the fruit flesh, peel, and leaves of bilberry and highbush blueberry. Analysis of more than 128,000 sequences identified nearly 1,500 annotated contigs, most of which were homologous to genes from Ericaceae species. Functional annotation revealed candidate genes involved in flavonoid biosynthesis, secondary metabolism, fruit ripening, and gene regulation. These candidate genes will be validated in tetraploid bilberry to identify molecular markers associated with anthocyanin accumulation in fruit flesh.

R6.O

Molecular Echoes of Time: Non-coding RNA Signatures of Seed Ageing

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Słowa kluczowe: seed, aging, non-coding RNA, miRNA, siRNA, lncRNA, gene bank

Seed longevity is a key determinant of ex situ conservation efficiency, yet the regulatory processes underlying loss of viability during long-term storage remain insufficiently understood. Here, we present an integrated analysis of coding and noncoding RNA dynamics in a unique experimental system of barley (*Hordeum vulgare* L. cv. Damazy) seeds derived from a single 1972 harvest batch and characterized the differences in viability after decades of storage. Low- and high-viability, and regenerated reference seeds were analyzed across dry and early imbibition stages using RNA-seq, small RNA-seq, degradome profiling, and lncRNA annotation. The material provided an exceptional opportunity to separate the effects of seed aging from genetic background variation. miRNA profiling revealed 62 known and 234 novel miRNAs, including those in the conserved miR159, miR168, and miR166 families, with 28 miRNAs displaying viability- and germination-stage-dependent expression. Degradome-supported target analysis revealed the involvement of these miRNAs in developmental regulation, hormone signaling, nucleic acid metabolism, and cytoplasmic organization. siRNA profiling revealed 85,728 differentially expressed siRNAs, predominantly 21- and 22-nt, with viability-specific targeting of genes involved in transcription, membrane trafficking, cell cycle regulation, stress responses, RNA processing, and translation. Complementary transcriptome and lncRNA analyses revealed 800 differentially expressed lncRNAs and 37,891 mRNAs, with lncRNA-associated target genes enriched in lipid metabolism, amino acid regulation, polyamine transport, and cellular reactivation processes. Taken together, these findings show that seed aging is accompanied by coordinated disruption and reconfiguration of RNA-mediated regulation during germination. The identified miRNA, siRNA, lncRNA, and mRNA signatures provide candidate molecular biomarkers of seed vigor and may support improved monitoring and management of long-term conserved cereal germplasms

R7.O

Weighted gene co-expression network analysis (WGCNA) reveals candidate genes related to phosphorus-deficiency tolerance in rye (*Secale cereale* L.)

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Słowa kluczowe: *Secale cereale*, inbred lines, hydroponic-growing system, comparative transcriptomics, WGCNA, phosphorus-deficiency tolerance

Phosphorus (P) is a fundamental nutrient for plant development, and its low availability in agricultural lands negatively affects crop production. Rye's resilience to low-nutrient stresses makes it a good model for studying mechanisms of tolerance to phosphorus deficiency. Our research aims to dissect the genetic basis of P-deficiency tolerance by comparing rye inbred lines (ILs) with contrasting responses to P-limiting conditions. In our study, we screened various rye ILs under P-deficient conditions and measured fresh and dry weight and inorganic phosphorus content in leaves and roots. The results showed that the L9 leaves contain higher levels of inorganic P (Pi) than the K3 under P deficiency. We performed a comparative transcriptomic analysis of these two ILs at 14 and 21 days. The Principal Component Analysis (PCA) revealed that the genotype explains 62% and 64% of the variation in leaf and root samples, respectively. Each genotype displayed a specific response to P deficiency, and K3 exhibited a higher number of differentially expressed genes (DEGs) than L9 leaves and roots under low-P conditions, with the exception of L9 roots at 21 days. The Over-Representation Analysis (ORA) showed enrichment of distinct pathways in L9 and K3 leaves, with L9 displaying an over-representation of biological processes associated with Pi-deficiency response and stress tolerance. Conversely, the root DEGs of both ILs were enriched in pathways associated with phosphorus starvation and stress responses. The WGCNA identified modules associated specifically with L9 under P-deficiency. The L9-specific modules contain positive regulators of the Pi-starvation response and several transporters that might play a role in the translocation of inorganic phosphorus. Our results will allow us to select candidate genes conferring high-Pi content in the L9 leaves during low-P regimes. **Acknowledgments** This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

R8.O

Histone acetyltransferase expression dynamics during wheat microspore embryogenesis reveal genotype-dependent and homeolog-specific regulatory patterns

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Słowa kluczowe: microspore embryogenesis; histone acetylation; chromatin remodeling; homeolog-specific expression; doubled haploids; wheat

Microspore embryogenesis (ME) is an important biotechnological tool supporting doubled haploid production in wheat breeding; however, its efficiency remains strongly genotype-dependent. Chromatin remodeling mediated by histone acetylation is considered one of the key mechanisms controlling cellular reprogramming during androgenesis. The aim of this study was to investigate the relationship between androgenic responsiveness, trichostatin A (TSA) treatment, and the temporal dynamics of histone acetyltransferase gene activity during wheat anther culture. Four winter wheat genotypes differing in embryogenic potential were analyzed, including responsive doubled haploid lines (DH1/2 and PO19) and recalcitrant hybrid lines (K20290 and K393). The effects of TSA on embryo-like structure (ELS) formation, green plant regeneration, and green plant frequency were evaluated. Simultaneously, transcription profiles of TaHAG3, TaHAC4 and TaHAM were analyzed at multiple culture stages using both universal (ABD) and homeolog-specific primers. TSA treatment exerted limited but genotype-dependent effects on androgenic response. In responsive genotypes, TSA improved either ELS induction or green plant regeneration depending on treatment conditions. Transcriptional analyses revealed pronounced temporal and genotype-dependent regulatory patterns associated with embryogenic competence. Responsive lines showed earlier activation of histone acetyltransferase genes during culture progression, whereas recalcitrant genotypes exhibited delayed induction, with transcription maxima frequently shifted to late culture stages. Particularly strong genotype-dependent differences were observed for TaHAM activation timing. Homeolog-specific analysis demonstrated largely coordinated expression profiles for TaHAG3 and TaHAM, whereas TaHAC4 displayed pronounced subgenome imbalance, especially involving the A-genome copy. The obtained results suggest that successful androgenic induction in wheat depends not only on the activation of histone acetylation-related pathways, but also on the precise temporal coordination of chromatin remodeling during early stages of microspore embryogenesis.

R9.O

Translational landscape of impaired photomorphogenesis in *elo3-6* and *urm11 urm12* mutants

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Słowa kluczowe: Translation, Elongator complex, *Arabidopsis thaliana*, Ribosome profiling

Magdalena Jarosz-Ostrówkamagdalena.jarosz@upwr.edu.pl605729318Photomorphogenesis in *Arabidopsis thaliana* involves coordinated morphological changes, such as cotyledon expansion and inhibition of hypocotyl elongation, driven by massive transcriptional reprogramming. The Elongator complex is one of the regulators of photomorphogenesis – the Elongator-deficient mutant *elo3-6* exhibits photomorphogenic defects, including an elongated hypocotyl and hyponastic cotyledons. The Elongator controls gene expression epigenetically in the nucleus and in the cytoplasm by catalyzing modifications of the wobble uridine in the anticodon loop of specific tRNAs, which can be further thiolated by URM proteins. These modifications optimize mRNA decoding accuracy and translation rates. While transcriptional regulation of photomorphogenesis is well-characterized, the significance of translational control remains less understood. To capture these translational dynamics, we employed ribosome profiling on wild-type (WT), *elo3-6*, and *urm11 urm12* seedlings. Defects in tRNA modifications, most likely by disrupting codon–anticodon pairing, reduced the translational efficiency of numerous transcripts, including those encoding chaperones and proteins involved in light responses and cell wall organization. In *elo3-6*, increased mRNA-ribosome binding was predominantly detected for transcripts related to chloroplast function. This likely indicates ribosome pausing on those transcripts rather than accelerated translation. To determine how defective tRNA modifications affect ribosome movement, we analyzed the reading frame periodicity of ribosome footprints. Surprisingly, both *elo3-6* and *urm11 urm12* mutants exhibited a significantly higher proportion of periodic reads than the WT, with a stronger effect observed in *elo3-6*. This suggests that disrupted codon-anticodon interactions delay elongation. While slower movement reduces ribosomal frameshifting (thereby enhancing detected periodicity), it likely compromises overall translation fidelity by increasing amino acid misincorporation. In conclusion, Elongator- and URM-dependent tRNA modifications are essential for proper protein synthesis during early seedling growth by fine-tuning the translation of chaperones and chloroplast-related transcripts. Additionally, the compromise between translation speed and accuracy is derailed in the absence of functional Elongator and URM proteins.

R10.O

Wheat small interfering RNAs induced by inoculation with pathogenic and beneficial fungi

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Słowa kluczowe: small RNAs, RNA interference, *Triticum aestivum*, *Fusarium culmorum*, *Trichoderma atroviride*, plant-fungus interaction

Common wheat (*Triticum aestivum* L.) is one of the most widely cultivated crops worldwide, yet its production is limited by pathogens that reduce yield and grain quality. To mitigate these losses, biological control and integrated pest management (IPM) strategies are increasingly being developed. Promising approaches include host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS), both of which exploit RNA interference (RNAi) pathways in plants. In RNAi, small non-coding RNAs regulate gene activity at the post-transcriptional level by repressing translation or inducing transcript cleavage. Among these molecules, small interfering RNAs (siRNAs) are 18–24-nucleotide double-stranded RNAs derived from longer double-stranded precursors. siRNAs may therefore be used to trigger highly selective and targeted defence responses in plants or their pathogens. Therefore, identifying wheat siRNAs responsive to both pathogenic and beneficial fungal inoculations is important for understanding the molecular mechanisms underlying plant–fungus interactions and for developing targeted RNAi-based crop protection strategies. This study aimed to identify wheat siRNAs associated with inoculation by the pathogenic fungus *Fusarium culmorum* and the beneficial fungus *Trichoderma atroviride*. Two wheat cultivars differing in susceptibility to *Fusarium* were inoculated with *F. culmorum*, *T. atroviride*, or both fungal species. Small RNA sequencing (sRNA-seq), transcriptome sequencing (RNA-seq), and degradome sequencing were used to characterise molecular responses. Differentially expressed small RNAs were identified, and phasiRNA- and heterochromatic siRNA-producing loci were predicted using sRNAminter. In total, 258 siRNA clusters were identified across the experimental conditions, of which 30 were differentially expressed in at least one condition. Additionally, 98 putative PHAS loci and approximately 15000 putative heterochromatic siRNA-producing loci were identified. These results highlight candidate siRNA loci potentially involved in wheat responses to pathogenic and beneficial fungi and provide a foundation for future RNAi-based crop protection strategies. The research was conducted and funded as part of the NCN OPUS24 2022/47/B/NZ9/01282 project

R11.O

The Conserved MiaA/MiaB Pathway Supports Chloroplast Translation and Cold Acclimation in *Arabidopsis thaliana*

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Słowa kluczowe: tRNA, chloroplast, translation

Transfer RNAs (tRNAs) are extensively decorated with chemical modifications that support codon recognition, proper secondary and tertiary structure formation, and aminoacylation. These modifications are introduced by specific tRNA-modifying enzymes. In chloroplasts, however, only a limited number of such enzymes have been identified and functionally characterized. The aim of this work was to identify chloroplast tRNA modification enzymes in the model plant *Arabidopsis thaliana*. Based on similarity to well-described systems, mainly *Escherichia coli* and *Saccharomyces cerevisiae*, we identified 37 putative chloroplast-targeted tRNA-modifying enzymes in *Arabidopsis*. We expressed all candidate proteins as yellow fluorescent protein fusions in *Arabidopsis* seedlings and confirmed chloroplast localization for 31 of them. This set included mainly pseudouridine synthases, methyltransferases, and enzymes predicted to participate in wobble uridine and adenosine 37 modifications. We then focused on enzymes responsible for $\text{ms}^2\text{i}^6\text{A37}$ formation, a modification located next to the anticodon and important for codon recognition, reading-frame maintenance, and translation efficiency. In *E. coli*, $\text{ms}^2\text{i}^6\text{A37}$ is formed in two steps: MiaA first isopentenylates A37, producing $\text{i}^6\text{A37}$, which is then methylthiolated by MiaB. We identified chloroplast-targeted *Arabidopsis* orthologues of MiaA and MiaB and obtained mutants in the corresponding genes. The *miaa* mutants displayed a pale-green phenotype, slower growth, and reduced photosynthetic performance compared with wild-type plants. RNA sequencing revealed downregulation of genes related to photosynthesis and chlorophyll biosynthesis, together with upregulation of genes involved in protein quality control. The *miaa* mutants were also more susceptible to cold stress, suggesting impaired chloroplast translation. Direct analysis using puromycin labelling showed reduced accumulation of newly synthesized proteins in *miaa*, especially under cold conditions. In contrast, *miab* mutants did not differ markedly from wild type, suggesting that $\text{i}^6\text{A37}$ is the key modification for chloroplast translation under the tested conditions. Our results show that the MiaA/MiaB pathway is conserved in chloroplasts and that MiaA-dependent tRNA modification supports chloroplast translation, photosynthetic performance, and cold acclimation.

R12.O

Comparative Analysis of Three-Dimensional Chromatin Organization in Carrot: Identification and Characterization of Topologically Associating Domains

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Słowa kluczowe: Hi-C, *Daucus carota*, topologically associating domains

The three-dimensional organization of chromatin within the nucleus has become an important area of research in genetics. Studies in animal systems have demonstrated that local chromatin structures, including topologically associating domains (TADs) and chromatin loops, can influence gene expression, DNA replication, and genome stability. In plants, the spatial organization of chromatin remains less extensively studied. However, current evidence indicates that local chromatin architecture may contribute to phenotypic variation through the regulation of nearby genes. Several studies investigating plant TADs have highlighted substantial structural differences compared with those described in animal genomes. Nevertheless, similar patterns of gene transcription and epigenetic marks at TAD boundaries in both kingdoms suggest that these regions are generally associated with active and accessible chromatin. Here, we present the first study investigating chromatin interactions in carrot (*Daucus carota* L.), with a particular focus on topologically associating domains (TADs). High-throughput chromosome conformation capture (Hi-C) data were generated for two homozygous genotypes, DH1 and B493, enabling the construction of high-resolution genome-wide contact matrices. TADs were identified using multiple algorithms and computational tools, with the number of predicted domains ranging from 19 to 6273, depending on the resolution. Conservation of TADs between the two genotypes was assessed, and the genomic features associated with conserved TAD boundaries were analyzed to better understand their potential functional significance. Ongoing work focuses on identifying sequence motifs enriched at TAD boundaries that may contribute to chromatin domain formation and maintenance. The research was financed by the Polish National Science Center (NCN) (projects Preludium-Bis2022/47/O/NZ9/00290).

R13.O

A mutation in the brassinosteroid biosynthesis gene CsCYP90B1 disrupts vegetative and reproductive development in cucumber (*Cucumis sativus* L.)

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Słowa kluczowe: cucurbits, dwarfism, gene mapping, phytohormones, plant growth and development

In plants, the steroid hormone brassinosteroids (BRs) play pivotal roles in plant development, growth, and stress response. Mutations in genes encoding enzymes involved in the BR biosynthesis pathway result in dwarf or compact plant architecture. Here, we characterized a cucumber line super compact (scp), previously described by Niemirowicz-Szczyt et al. (1996), which displays extreme dwarfism due to the absence of main shoot elongation and a lack of female flower production. Genome-wide mapping using the F2:3 population developed from a cross between the L500 wild-type line and the scp line, revealed a single nucleotide substitution in the cucumber cytochrome P450 90B1 ortholog (CsCYP90B1), which is responsible for the altered growth. A T-to-C substitution in the CsCYP90B1 gene results in a serine-to-proline substitution in the cytochrome domain. RT-qPCR expression profiling revealed differential regulation of BR biosynthesis genes in the scp line. Treatment with exogenous 24-epibrassinolide restored the phenotype of the scp line. Functional validation of the CsCYP90B1 gene using CRISPR/Cas technology is currently in progress. Our research highlights the role of the CsCYP90B1 gene in regulating plant architecture and demonstrates the effect of a single nucleotide change on brassinosteroid biosynthesis and plant growth. This research was financially supported by the Polish Ministry of Agriculture and Rural Development under the project "Basic research for biological progress in crop production", Task 33 "Identification of genes controlling plant growth architecture of cucumber (*Cucumis sativus* L.)". Niemirowicz-Szczyt K, Rucińska M, Korzeniewska A (1996) An induced mutation in cucumber: super compact. *Cucurbit Genet Coop Rep* 19:1-3.

R14.O

Development and characteristics of intergeneric *Brassica rapa* L. subsp. *pekinensis* × *Sinapis alba* hybrids of early generations as a new germplasm for the breeding.

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Słowa kluczowe: intergeneric hybrids; insects' cross-pollination; DNA content; phenotype; seeds

Intergeneric hybridization between inbred lines of Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*; $2n = 20$) and white mustard (*Sinapis alba* L.; $2n = 24$) was conducted to generate novel germplasm resources for *Brassica* vegetable breeding. Insect-mediated pollination effectively facilitated crosses between cytoplasmic male sterile (CMS) *B. rapa* lines and male-fertile *S. alba* plants, resulting in 48 intergeneric F_1 hybrids and 17 F_2 sublines. Among the F_1 hybrids, seven were male-fertile and 41 were male-sterile, indicating the presence of nuclear fertility-restorer genes derived from *S. alba*. Segregation for cytoplasmic male fertility/sterility was also observed in the F_2 generation, with fertile and sterile plants occurring in an approximately 1:1 ratio. The nuclear DNA content of the parental *B. rapa* lines ranged from 1.05 to 1.07 pg, whereas *S. alba* contained 1.12 pg DNA per nucleus. In contrast, the hybrids exhibited increased and highly variable genome sizes. Nuclear DNA content ranged from 1.57 to 2.26 pg in F_1 plants and from 1.29 to 2.25 pg in F_2 progeny. Chromosome numbers in F_1 hybrids varied from 28 to 39, reflecting substantial genomic instability and recombination. Morphological evaluation revealed predominantly intermediate or transgressive phenotypes relative to the parental genotypes, suggesting the emergence of novel trait combinations with potential breeding value. Hybrid status was confirmed using SSR markers, which detected parental alleles from both species and revealed unique genetic profiles not present in either parent. The study also demonstrated the high reproductive capacity of early-generation hybrids. Both F_1 and F_2 plants were capable of producing viable progeny through open pollination, self-pollination, and cross-pollination, highlighting their reproductive stability and utility for further breeding.

R15.O

Multiple layers of rye's (*Secale cereale* L.) phosphorus deficiency response: genetics, epigenetics and beyond

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Słowa kluczowe: rye, phosphorus deficiency, transcriptomics, methylome profiling, proteomics, genetic diversity

Rye (*Secale cereale* L.) is an important cereal crop in Poland and neighbouring countries. It is characterised by a very good tolerance of adverse conditions, including nutrient deficiency. Phosphorus deficiency response occurs at multiple levels (molecular, physiological, morphological) and changes over time. Both general and species specific response mechanisms exist. Genes underlying P-deficiency response have been identified in several plants, including arabidopsis and rice. Our aim is to identify the genetic basis of phosphorus tolerance in rye. In our research we focused on the initial stages of vegetative development and performed RNAseq, methylome profiling (DARtmetseq) and quantitative proteomics (LC-MS/MS) on rye inbred lines showing contracting response to phosphorus deficiency (K3 and L9). Root and leaf samples of hydroponically grown plants were collected on days 14 and 21. We have also performed genome-wide discovery and characterisation of rye phosphate transporter genes (ScPht). Using qRT-PCR we have shown that phosphorus deficiency influences the expression levels of ScPht1;6, ScPht2 and ScPht3;3. Furthermore, based on low-coverage whole-genome resequencing of diverse rye accession putative functional variants in ScPhts were identified. We have also demonstrated that P deficiency impacts root anatomical and architectural traits differently in rye inbred lines representing different genetic backgrounds. We will present an overview of the results of our holistic approach, highlighting gene pathways influenced by P deficiency, and report on progress in functional characterisation of selected candidate genes, involving subcellular localisation and complementation assays in mutant yeast strains. This research was funded by the National Science Centre, Poland, projects no. 2020/37/B/NZ9/00738 and no. 2024/55/B/NZ9/00950

R16.O

Studies of the effects of temperature and duration of vernalization on flowering time, frost tolerance and the development of the three lupins (*Lupinus angustifolius* L., *Lupinus albus* L., *Lupinus luteus* L.)

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Słowa kluczowe: vernalization, flowering time, frost tolerance, lupins, phenotyping

IntroductionGlobal climate change threatens food production through frequent extreme weather events that damage crops. Application of climate-smart breeding is necessary to meet the increasing requirements for plant-based protein. Lupins are capable of thriving in diverse environments and an excellent protein, fiber, and vitamins source. Furthermore, being a Europe native, Lupins are an attractive alternative to soybean. Lastly, they are an excellent research subjects due to their requirement of vernalization to promote flowering, despite lacking a few major vernalization-controlling genes. **Scientific goal**The main goal of this study is to investigate and evaluate the effects of temperature and duration of vernalization on flowering time and plant development, frost tolerance, as well as the quality and quantity of yield in the three lupin crops: *L. angustifolius*, *L. albus* and *L. luteus*. Additionally, the study aims to examine how vernalization influences the photoperiod requirements for these three species. **Research project hypothesis**Type of vernalization, the appropriate temperature, and the duration of vernalization will affect the time of flowering and the maturation of pods. The vernalization treatment should increase the frost tolerance and improve features of the yield structure. **Materials and methods**Three representative genotypes per species were selected: a late-flowering vernalization-responsive line, an intermediate line, and an early-flowering line that is non-responsive to vernalization. The effects of two vernalization temperatures (4°C and 8°C), two photoperiods (8 h and 14 h), treatment duration (1- 3 weeks), and vernalization of seeds versus young seedlings on flowering time will be assessed. After the vernalization treatment, plants will be assessed for frost tolerance. For each treatment, leaf samples will be collected for RNA-seq analysis. Differential gene expression analysis will be performed using the obtained sequences to identify candidate genes for further analysis using RT-qPCR. Future plans include a field experiment and an analysis of seed composition under different vernalization treatments. **Funding:** National Science Centre, PRELUDIUM23, 2024/53/N/NZ9/03463

R17.O

Hyperspectral Imaging as a Tool for Phenotypic Screening of Sugar Beet (*Beta vulgaris* L.) Genotypes Under Stress Conditions

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Słowa kluczowe: high-throughput phenotyping, hyperspectral imaging, drought stress, sugar beet (*Beta vulgaris* L.), vegetation indices

In the era of rapid advances in genomics, precise phenotypic assessment is essential for linking genetic information to observed plant traits. Using high-throughput digital phenotyping enables effective and unbiased evaluation of plants. It also identifies potential markers, for example for abiotic stress tolerance. Understanding the mechanisms of tolerance to water-deficient stress remains a major challenge in modern plant genetics and biotechnology, especially in the context of climate change. The aim of the present study was to evaluate the suitability of non-invasive hyperspectral imaging techniques for phenotyping sugar beet (*Beta vulgaris* L.) plants under controlled drought stress conditions. The plant material included sugar beet genotypes from Polish breeding programs. The plants were sown in pots compatible with the PlantScreen™ Modular System (PSI spol. s r.o.). Plants were grown under controlled greenhouse conditions. The experiment had a control group and a drought-stressed group with reduced watering. Hyperspectral cameras were used for phenotypic analysis in the VNIR and SWIR spectral regions. The WATER1 index was used in SWIR imaging, enabling monitoring of changes in water content in tissues. Significant differences between the groups were observed 10 days into the stress period, confirming the sensitivity of this method in detecting water deficit. VNIR imaging allowed for the analysis of three vegetation indices: PRI [photosynthetic efficiency], MCARI1 [chlorophyll content], and SIPI [carotenoid-to-chlorophyll ratio]. In the control plants, a systematic increase in index values was observed, while in the test group, the values remained stable or showed a downward trend. The results confirm the usefulness of hyperspectral imaging as a tool for early screening of plants for drought tolerance. The spectral indices used enable the detection of subtle physiological changes preceding visible symptoms of stress, which confirms the potential for their application in research aimed at improving plant tolerance to water-deficient conditions.

R18.O

Expression changes of candidate genes for freezing tolerance in wheat under de-acclimated conditions

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Key words: Wheat, active de-acclimation, freezing tolerance, cold acclimation

Active de-acclimation induced by transient mid-winter warming reduces freezing tolerance in winter wheat and is accompanied by changes in stress- and hormone-related pathways. The aim of this study was to verify RNA-seq-derived candidate genes and identify expression patterns associated with contrasting tolerance to active de-acclimation.

Winter wheat accessions classified as tolerant or susceptible to de-acclimation were analysed at three stages: before cold acclimation, after cold acclimation, and after de-acclimation. RT-qPCR was used to examine genes related to ABA and gibberellin metabolism, phenylpropanoid metabolism, ethylene biosynthesis, cell wall modification, molybdate transport, RNA processing, and transcriptional regulation. Leaf ABA content was determined at the same time points.

Expression of all analysed genes changed after de-acclimation, confirming their involvement in the molecular response to this process. However, most candidates, including PAL, ETO1-like, licheninase, molybdate transporter, PPR5, and WRKY, showed treatment-dependent patterns only weakly associated with tolerance status. The clearest agreement with differences between tolerant and susceptible accessions was observed for ABA hydroxylase. Its expression increased during cold acclimation in both groups, but after de-acclimation it declined to approximately the pre-acclimation level in susceptible accessions, whereas tolerant accessions maintained expression close to that observed after cold acclimation. These group-specific differences were supported by changes in leaf ABA content measured at corresponding stages, indicating coordinated regulation of ABA metabolism. Gibberellin oxidase also differentiated tolerant and susceptible accessions: expression generally remained stable or increased during cold acclimation in tolerant accessions, but decreased in susceptible ones, followed by a further decline after de-acclimation.

The results confirm that all selected genes participate in the transcriptional response to active de-acclimation, while ABA hydroxylase shows the strongest association with tolerance. Gibberellin oxidase represents an additional promising marker of differential de-acclimation response.

Funding: This study was funded by the Polish Ministry of Agriculture and Rural Development, grants no. DHR.hn.802.11.2024 and DHR.hn.802.9.2025.

R19.O

Comparative proteome analysis of the response of two rye inbred lines (L9 and K3) to phosphate deficiency stress

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Słowa kluczowe: rye, phosphorus-deficiency stress, proteomics, LCMS/MS

Rye (*Secale cereale* L.) is an exceptional cereal crop to study nutritional deficiency stresses due to its excellent tolerance to poor-nutrient soils. Phosphorus is a critical driver of plant growth and development, yet its bioavailability in soils frequently limits crop productivity. The underlying mechanisms conferring rye's high adaptability to phosphate deficiency remain unknown. In this study, we utilized a quantitative proteomics approach to systematically profile the changes in the proteome of two rye inbred lines to gain insights into the specific metabolic pathways and regulatory networks governed by phosphorus-deficiency stress. We conducted the proteomics profiling of two rye inbred lines (L9 and K3) displaying contrasting inorganic Pi levels in their leaves under phosphate deficiency. The plants were grown in 0.2 mM KH₂PO₄ control (+P) and 0.01mM KH₂PO₄ (with additional 0.19mM KCl) phosphate-deficient conditions (-P) in a hydroponic system for 14 and 21 days. Total protein extracts from roots and shoots were extracted using an optimized SDS-phenol extraction method followed by chloroform/methanol precipitation. Subsequently, the protein pellets were digested with trypsin, labeled with Tandem Mass Tags (TMT), and quantitatively profiled using high-resolution Liquid Chromatography-Tandem Mass Spectrometry. The Principal Component Analysis (PCA) revealed that the variation between the roots and shoots was explained by genotype. We identified a total of 5617 high-confidence proteins utilizing the *Secale cereale* L. and *Triticum aestivum* databases. The comparative analysis of the two lines showed a significant shift in the proteome under phosphate starvation. The Gene Ontology analyses revealed that the differentially expressed proteins (DEPs) belong to pathways involved in oxidative stress, phosphate starvation, and stress responses, as well as phosphate transport and metabolic adaptation. Acknowledgment: This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Center, Poland.

R20.O

Structural variation of the Receptor-Like Protein (RLP) Gene Family in the *Arabidopsis thaliana* genome revealed by Oxford Nanopore sequencing and de novo assemblies

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Słowa kluczowe: Long-read sequencing, de novo assembly, genome variation, plant receptor genes, *Arabidopsis*

Receptor-Like Proteins (RLPs) form a family of genes involved in plant development and response to biotic and abiotic stresses. However, among the 57 RLP genes annotated in *Arabidopsis thaliana*, only a limited number have been functionally characterized. As immune-related genes often display extensive natural variation, detailed analysis of genomic diversity at RLP loci may help identify candidate genes, alleles, and structural variants relevant for plant defense mechanisms. We utilised an emerging long-read sequencing technique (Oxford Nanopore Technology) to investigate structural variation affecting RLP genes in eight diverse *A. thaliana* accessions. We established a protocol for high-molecular-weight genomic DNA extraction from leaves, generated de novo genome assemblies, and performed their quality assessment under three categories: contiguity, completeness, and correctness. The assemblies showed high quality within chromosome arms, although repeat-rich regions such as centromeres and telomeres remained fragmented. We annotated genes and transposable elements in individual assemblies and examined variation patterns in RLP loci. We revealed numerous small- and large-scale variants, including gene deletions, duplications, insertions, and rearrangements derived from transposable elements. Notably, the region spanning RLP47–RLP50 showed an interspersed duplication in one accession and a transposable element-derived insertion in another accession, indicating that RLP loci can undergo substantial structural remodeling within the species. We also took advantage of DNA methylation detection directly from nanopore data and evaluated methylation levels in transposable elements. These findings suggest that many RLP genes display considerable intraspecific diversity. Extending this analysis to a worldwide *A. thaliana* population may provide a foundation for functional studies and improve our understanding of how structural variation contributes to plant immune mechanisms.

R21.O

Sequential chitosan-fungicide application improves early physiological response and chlorophyll fluorescence in Fusarium-infected barley

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Słowa kluczowe: chitosan, fungicides, *Fusarium graminearum*, *Hordeum vulgare*

The development of innovative strategies to control fungal pathogens is crucial for sustainable agriculture. Pure chitosan directly inhibits *Fusarium graminearum* growth and mycotoxin accumulation. It also acts as an elicitor, inducing systemic acquired resistance (SAR) via the salicylic acid (SA) pathway and stimulating biomass gain. In vitro, combining chitosan with fungicides (especially azoxystrobin) demonstrates strong synergistic antifungal efficacy. This study evaluates this combined strategy in planta through a sequential treatment: an initial chitosan application to prime barley defenses against *Fusarium* infection, followed by the application of specific fungicides (azoxystrobin, tebuconazole, metconazole, difenoconazole). This integrated approach optimizes protection and enables reduced synthetic fungicide use. The research also verifies the genotype's influence on this two-step protection method. Two spring barley cultivars were tested: the susceptible 'Amaretto' and resistant 'Orlando'. Chitosan priming prior to *Fusarium* inoculation visibly reduced fungal development in 'Amaretto', while 'Orlando' showed less intensive phenotypic differences. These findings were confirmed by qPCR analysis determining fungal biomass in the plant tissue. In parallel, chlorophyll fluorescence kinetics revealed significant stimulation of the photosynthetic apparatus. Increases in PI_{total} (performance index), DF_{total} (total driving force), and S_m/t_{Fm} parameters indicate higher vitality, increased energetic efficiency, and effective cellular protection against early infection-induced oxidative stress. These results validate the early molecular-physiological response of barley. They confirm the biophysical efficacy of chitosan priming, demonstrating realistic possibilities for reducing chemical protection while maintaining high crop health. Research was funded by the Ministry of Agriculture and Rural Development, grant "Dotacja Celowa", task 5.4.

R22.O

miR167c-3p-calmodulin-like (CML) module associated with the resistant flax response to *Fusarium oxysporum*

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Słowa kluczowe: MicroRNA, Linum, Flax

Fusarium oxysporum wilt is a major constraint on flax (*Linum usitatissimum*), yet closely related cultivars differ markedly in susceptibility. Because resistant and susceptible lines share the same pathogen but reach opposite outcomes, they are a natural system for separating resistance-linked responses from generic infection responses. microRNAs (miRNAs) are central post-transcriptional regulators of plant immunity, but the miRNA-target modules that distinguish resistant from susceptible flax remain poorly defined. We compared the resistant cultivar Nike with the susceptible cultivar Regina at an early infection stage (48 hours post-inoculation), integrating small RNA sequencing, mRNA transcriptome profiling, computational target prediction, and stem-loop RT-qPCR of both miRNAs and their targets. Differential expression was analysed separately for each cultivar to avoid cross-cultivar normalisation artefacts. From the infection-responsive miRNAs we focused on miR167c-3p, selected for its consistent differential expression, its stronger repression in the resistant cultivar by small RNA sequencing, and its high-confidence, seed-complementary targeting of two calmodulin-like (CML) genes. In the resistant cultivar, infection repressed miR167c-3p and induced both CML targets, a coherent de-repression signature reproduced independently by RNA sequencing and qPCR. The agreement between a genome-wide sequencing read-out and targeted qPCR strengthens confidence that this anti-correlation reflects genuine regulation rather than a single-platform artefact. The coordinated miR167c-3p-CML response was most pronounced in the resistant cultivar, suggesting that engagement of the module is associated with resistance rather than with infection alone. CML proteins are calcium sensors implicated in plant immune signalling, so their de-repression following miR167c-3p down-regulation is a plausible early component of the resistant response. These findings identify the miR167c-3p-CML module as a candidate marker of resistance in flax and a target for functional validation.

R23.O

Development of SNP marker for gene Pc39 determining oat resistance to *Puccinia coronata*

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Słowa kluczowe: RNA-seq, DEG (differential gene expression), *Avena sativa*, crown rust

Efficient molecular markers for the Pc39 gene were developed based on the sequence of ATP-sulfurylase 2, a gene identified by RNA-seq as differentially expressed between near-isogenic resistant and susceptible *Avena sativa* lines inoculated with an incompatible *Puccinia coronata* isolate. Transcript levels of ATP-sulfurylase 2 (AVESA.00001b.r3.4Cg0000059) were significantly higher in Pc39-carrying lines than in susceptible plants; notably, this elevated expression was constitutive, occurring regardless of infection status, indicating that it results directly from the presence of the Pc39 allele. A molecular marker was subsequently designed based on a unique SNP-type polymorphism identified within the ATP-sulfurylase 2 sequence, using comparative sequence data from the PanOat resource available through GrainGenes. Primers were designed to amplify the gene in both Pc39 carriers and non-carriers used in the RNA-seq analysis; however, owing to the polyploid nature of the gene, it was not possible to design primers spanning its entire sequence. Sequencing of selected PCR products and alignment with reference genomes nonetheless enabled primer design for the intronic region between exon 1 and exon 2, which was distinguished by an approximately 100 bp insertion absent in susceptible lines. These primers were validated in a segregating population for this gene as well as in cultivars carrying Pc39. The resulting marker constitutes a ready-to-use tool for the molecular identification of Pc39 in oat breeding programs.

R24.O

Development of a CRISPR/Cas9-Based Genome Editing Strategy Targeting an ABA Hydrolase Gene in Flax (*Linum usitatissimum* L.)

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Słowa kluczowe: CRISPR/Cas9, flax, genome editing, ABA metabolism, *Agrobacterium tumefaciens*, sgRNA

Development of a CRISPR/Cas9-Based Genome Editing Strategy Targeting an ABA Hydrolase Gene in Flax (*Linum usitatissimum* L.) Background: Abscisic acid (ABA) plays a central role in plant responses to environmental stresses and developmental regulation. Manipulating hormone levels in plants is inherently due to the pleiotropic roles of phytohormones in growth and development; therefore, we targeted a gene involved in ABA catabolism rather than biosynthesis, as severe hormone deficiencies often result in developmental abnormalities or lethality. Genes involved in ABA metabolism represent promising targets for functional studies and crop improvement. The aim of this study was to develop a CRISPR/Cas9-based genome editing strategy targeting an ABA hydrolase gene in flax (*Linum usitatissimum* L.) and to establish an experimental workflow for the generation and molecular characterization of edited plants. Methods: Target-specific single guide RNAs (sgRNAs) were designed using bioinformatic tools based on predicted editing efficiency and minimization of potential off-target effects. Candidate sgRNAs were evaluated in vitro through Cas9 cleavage assays to verify their ability to recognize and cleave the selected target sequence. Validated sgRNAs were incorporated into CRISPR/Cas9 expression constructs and introduced into *Agrobacterium tumefaciens*. Flax explants were transformed using *Agrobacterium*-mediated transformation and regenerated under selective culture conditions. Molecular screening of regenerated plants is being performed using PCR-based approaches followed by sequence analysis of the target locus to detect CRISPR/Cas9-induced mutations. Results: The study established a workflow encompassing target identification, sgRNA design, in vitro validation, vector construction, *Agrobacterium*-mediated transformation, plant regeneration, molecular screening of edited lines. Regenerated plants are currently being analyzed to determine editing efficiency and characterize induced genetic modifications. Conclusions: The developed approach provides a framework for targeted genome editing in flax and will enable functional investigation of genes involved in ABA metabolism. The obtained methodology may facilitate future studies aimed at improving stress-related traits and expanding the application of genome editing technologies in this economically important crop species.

R1.P

MicroRNAs as post-transcriptional components of abscisic acid- and epibrassinolide-driven drought responses in barley

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Słowa kluczowe: barley, drought regulation, miRNA, phytohormones

Plant stress signaling and regulation depend on phytohormones with abscisic acid (ABA) being a central messenger. Brassinosteroids (BRs) are recognized as multifunctional plant regulators, modulating other hormonal pathways and contributing to stress tolerance. However, how they shape cereals drought responses remains unclear. We investigate more precisely mechanisms underlying ABA- and BR-mediated drought responses and their cross-regulation in barley. We propose that pre-stress application of ABA and epibrassinolide (epiBL) modulates the post-transcriptional regulatory layer during drought, shaping growth–stress balance. By comparing different concentrations of these hormones (ABA10, ABA100, epiBL1, epiBL10) against controls, we characterized temporal and dose-dependent shifts in endogenous hormonal balance, and microRNA regulation across drought stages. Treatments induced suppression of bioactive gibberellin GA1 and indole-3-acetic acid, favouring a restrained growth. However, increase in GA3 under ABA10 and epiBL1 suggested a redirection of gibberellin biosynthesis, maintaining minimal growth. Cytokinin accumulation varied across time points in response to ABA and epiBL. Jasmonic acid (JA) was the most responsive stress hormone, exhibiting an antagonistic early-drought pattern: ABA100 established a suppressed entry state, whereas epiBL10 triggered JA burst. Salicylic acid accumulation changed in a treatment- and time-dependent (T1-T4) manner under ABA and epiBL, whereas endogenous ABA levels decreased in response to epiBL during prolonged drought. Hormone priming altered drought-responsive microRNA profiles, with miR168, miR172, miR396 supporting ABA–BR coupling at the post-transcriptional level. miR168 abundance fluctuated moderately under drought in water-treated plants, whereas hormone treatments enhanced its accumulation. miR172 responded markedly to ABA100 and epiBL1, displaying a decline from T1 to T3 followed by recovery at T4, mirroring the temporal pattern of miR168. In turn, miR396 levels remained consistently elevated in all hormone-treated plants relative to the control. These findings demonstrate that ABA–BR interplay extends to post-transcriptional layer, where miRNA-mediated regulation contributes to the coordination of growth- and stress-associated hormone programs. This work was funded by the National Science Centre Poland grant number 2021/41/B/NZ9/02576.

Structure of a genomic region governing monogerm in sugar beet

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Słowa kluczowe: *Beta vulgaris*, gene expression, polymorphism, seed material, structural variants

Monogerm in sugar beet is an agriculturally desirable trait as it removes the requirement for manual thinning. According to classical genetics studies, it is conditioned by a single M gene. A recessive homozygote mm governs the formation of a single flower while in multigerm beets (M*) inflorescences are placed in the axils of the leaf bracts. Previously, using whole genome sequencing of bulk segregant analysis (WGS-BSA) we localized the M locus at the distal region of the long arm of sugar beet chr. 4, spanning ca. 3 Mb. Here, in order to identify candidate genes, we performed RNA-seq and identified differentially expressed genes (DEGs) in developing inflorescences at two time points, differentiating monogerm and multigerm plants. In T1 (very young inflorescences) and T2 (unopened flower buds stage), we identified three and 15 DEGs, respectively, of which two localized within the above-mentioned region on chr. 4, showed higher expression in multigerm plants, both in T1 and T2. We used Oxford Nanopore Technology to produce long gDNA reads for six monogerm and six multigerm plants. It indicated that in multigerm plants the genes likely resided within tandemly duplicated regions. It points at possible significance of structural variation at the M locus as a possible genetic determinant of monogerm. However, as the current sugar beet reference genome EL10.2 is not fully contiguous and the region in question contains gaps, a more thorough analysis will be required to confirm these findings. The research was funded by the Ministry of Agriculture and Rural Development of the Republic of Poland within the framework of the program for basic research supporting biological progress in agriculture, task no. 23 (2021-2026).

R3.P

The impact of the MutLy complex in the effect of heterozygosity on meiotic recombination in *Arabidopsis thaliana*

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Słowa kluczowe: MSH2, MLH1, meiosis, crossover, MMR system

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Meiotic recombination is essential for proper chromosome segregation and the generation of genetic diversity in sexually reproducing organisms. In plants, crossover (CO) distribution is highly non-random and is shaped by chromosomal context, chromatin state, and local sequence divergence between homologs. It has been observed in *Arabidopsis thaliana* that heterozygous regions juxtaposed with homozygous regions display elevated crossover frequencies compared with the same regions in full F1 hybrids (Ziolkowski et al., 2015; Szymanska-Lejman et al., 2023). This phenomenon, termed the juxtaposition effect, suggests that local polymorphism context can modulate meiotic recombination, but the molecular mechanisms underlying this effect remain poorly understood. The mismatch recognition factor MSH2 is responsible for detection of polymorphisms during meiotic recombination and has been shown to be required for the juxtaposition effect (Blackwell et al., 2020), whereas MLH1, as a component of the MutLy (MLH1–MLH3) complex, is a key factor in the maturation of Class I crossovers. This project aims to dissect the mechanistic basis of the juxtaposition effect by combining genome-wide and hotspot-specific analyses of meiotic recombination in *Arabidopsis*. First, whole-genome crossover maps generated previously for *mlh1* and newly generated for *msh2 mlh1* mutant hybrid backgrounds will be compared with CO maps for wild type and *msh2* to determine how mismatch detection and Class I crossover resolution shape recombination landscape architecture. In parallel, recombination frequencies within selected recombination hotspots will be measured using seed reporter fluorescent lines, allowing direct assessment of the local juxtaposition effect in *mlh1* and *msh2 mlh1* mutant backgrounds. Together, this work will clarify how polymorphisms are detected and influence meiotic recombination, providing mechanistic insight into how local genomic context shapes crossover formation. Understanding how MSH2 and MLH1 regulate crossover distribution may inform future strategies for targeted manipulation of meiotic recombination in plant breeding.

Genetic resistance to clubroot in Polish breeding lines of winter oilseed rape

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Słowa kluczowe: clubroot, pathotype, Plasmodiophora brassicae, winter oilseed rape

The aim of the study was to determine the genetic resistance of winter oilseed rape, WOSR (*Brassica napus* L.) to clubroot, a disease caused by the protist *Plasmodiophora brassicae* Woron., which belongs to the infrakingdom Rhizaria. Greenhouse trials were conducted to evaluate 18 breeding lines obtained from Polish plant breeding companies. Inoculation was performed using eight defined pathotypes of *P. brassicae*, including those capable of overcoming the 'first-generation resistance' derived from WOSR cultivar 'Mendel'. The first stage of the study involved propagating the inoculum on susceptible cultivars of Chinese cabbage (*B. rapa* subsp. *pekinensis*) cv. 'Granaat' and 'Hilton', followed by resistance screening tests. The evaluation was carried out in batches of 21 forms, including the 18 lines from breeders, alongside control cultivars: 'Cromat' (carrying the resistance of cv. 'Mendel'), 'Create' (a new oilseed rape cultivar resistant to the previously known pathotypes that overcome the resistance of cv. 'Mendel'), and the susceptible *B. rapa* cv. 'Granaat'. The tested oilseed rape breeding materials exhibited enhanced resistance to clubroot caused by *P. brassicae*; however, this resistance was not uniform across all lines or against all applied pathotypes. In the breeding materials from Plant Breeding Strzelce, Branch Borowo, resistance to pathotype 1 or to both pathotypes 1 and 3 was predominant. In contrast, the materials from Branch Małyszyn primarily showed resistance to pathotype 2, occasionally in combination with pathotype 1 or 3, but never with both simultaneously. Resistance to all three tested pathotypes was demonstrated by the doubled haploid line OI 21 DH 2212 from Branch Borowo. Among the materials from PB Smolice, genotype SM 03-25 exhibited the highest resistance, showing resistance to pathotypes 1 and 2, but susceptibility to pathotype 3. The obtained plant materials can be used in subsequent crosses to develop lines with comprehensive resistance to all three tested pathotypes.

Preparatory studies for mitochondrial transplantation in triticales

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Słowa kluczowe: cytoplasmic male sterility, mitochondria, triticales

All analyses were performed within the project aimed at the transplantation of mitochondria to transfer cytoplasmic male sterility in triticales. This main research goal requires both to optimize the protoplast/callus cultures and to select proper male-fertile acceptor materials. Protoplasts were isolated from aseptically grown seedlings of three triticales cultivars. In this part of the study, grain infection by microorganisms proved to be a serious problem. Of the 11 tested grain disinfection variants, only one allowed the development of aseptic seedling cultures, and only for one analyzed cultivar. Maceration of leaves in an enzymatic mixture containing 1% cellulase and 0.25% macerozyme allowed the release of approximately one million good-quality protoplasts from one gram of tissue. Observations of the obtained protoplast cultures suggest that their development is favored by immobilization in an alginate matrix, higher osmotic concentration of the medium (0.6 M), and its supplementation with both phytosulfokine and SAHA. The same triticales cultivars were analyzed with respect to callus development using immature zygotic embryos as explants for induction. All three applied media stimulated callus development at a similar level. It was the time between anthesis and embryo isolation that had the greatest impact on callus development, with older embryos being less efficient in this process. In 2025, for the selection of acceptor accessions, 40 triticales families were test-crossed with two male-sterile lines with the Pampa cytoplasm. In the current year, plants obtained from the F1 seeds were subjected to phenotyping through both visual inspection of anthers and grain setting in isolated spikes. Preliminary observations based on anther appearance indicate full sterility of approx. 20% of the tested F1 progenies. These results will be verified after harvest based on the number of grains formed from self-pollination. The financial support of the Polish Ministry of Agriculture and Rural Development is acknowledged (grant no. DHR.hn.802.14.2025)

R6.P

Coordinated expression of cell wall-related genes during flax seedling development

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Słowa kluczowe: *Linum usitatissimum*, gene expression, cell wall biosynthesis, transcriptional coordination, functional modules, correlation network, seedling development

The plant cell wall is a dynamic structure whose formation and remodeling require coordinated regulation of genes involved in polysaccharide biosynthesis, lignification, cell wall organization, and signaling. Despite the importance of flax (*Linum usitatissimum* L.) as a fiber crop, transcriptional relationships among cell wall-related genes during early seedling development remain poorly understood. Expression of genes associated with cell wall biosynthesis, remodeling, organization, signaling, and stress responses was analyzed in four Polish flax cultivars representing oil (Linola, Szafr) and fiber (Modran, Nike) flax types. Seedlings were sampled at 12 and 28 days after sowing, and gene expression was quantified using RT-qPCR. Genes were grouped into functional modules representing major biological processes, including cellulose, hemicellulose, pectin, lignin, cell wall integrity signaling (CWI), antioxidant system, and secondary wall NAC transcription factors. Principal component analysis (PCA), hierarchical clustering, fold-change analysis, correlation analysis, and network reconstruction were performed. PCA and hierarchical clustering demonstrated that developmental stage was the primary factor shaping gene expression patterns, whereas differences between fiber and oil cultivars were less pronounced. Most cell wall-related modules exhibited higher activity in 12-day-old seedlings and decreased expression during development. The strongest reductions were observed for lignin biosynthesis, pectin remodeling, pectin degradation, and secondary wall NAC transcription factor modules. Correlation analysis revealed a highly interconnected transcriptional network centered around pectin-related processes. Strong positive correlations were observed among pectin biosynthesis, pectin modification, lignin biosynthesis, cell wall integrity signaling, and cell wall organization modules. Early flax seedling development is characterized by intensive and coordinated expression of cell wall-related genes. Pectin-associated pathways occupy a central position within the transcriptional network, linking cell wall organization, integrity signaling, and lignification processes.

R7.P

Advancing the Understanding of Organellar Genome Regulation: The Role of Long-Read Sequencing in Multi-Level Modification Mapping.

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Słowa kluczowe: DNA methylation, RNA methylation, poly(A) length, non-adenine residues, organellar genomes

The widespread application of long-read sequencing technologies was utilized to revolutionize the approach to nuclear and organellar genome assembly, and issues related to, among others, repetitive sequences were successfully resolved. Despite the progress that was made in de novo assembly techniques, the epigenetic and post-transcriptional regulation of organellar gene expression was recognized as remaining poorly understood. In this study, it was hypothesized that a comprehensive analysis of DNA and RNA modifications constitutes a key, hitherto uncharacterized, mechanism of gene expression regulation in organelles. The research was primarily aimed at evaluating the potential of long-read technologies for the multi-level mapping of organellar genome modifications, and the impact of these modifications on the stability and efficiency of plastid and mitochondrial transcription was determined. To verify this hypothesis, an integrated approach was employed, in which direct RNA and direct DNA sequencing (Oxford Nanopore Technologies) were combined with high-throughput RNA-seq (Illumina). By this method, DNA methylation patterns were precisely mapped, RNA methylation was identified, and the length of poly(A) tails as well as non-adenine residues within those tails were analyzed in detail. Correlations between epigenetic modifications and the dynamics of post-transcriptional processes in organellar genomes were revealed by the obtained results. It was demonstrated that long reads were effectively used for the multidimensional characterization of mitochondrial and plastid function, and specific regulatory mechanisms that determine the fate of organellar transcripts were identified. New evidence was provided by this study, by which the integration of long-read sequencing data was established as an essential step toward a full understanding of the molecular dynamics of organellar genomes.

R8.P

Physiological, biochemical and molecular changes in *Brassica napus* L. under drought and salinity at various developmental stages

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Słowa kluczowe: oilseed rape, physiology, biochemical parameters, drought, salinity, PP2C

As sessile organisms, plants are continuously exposed to a wide range of biotic and abiotic stressors. Our research focuses on investigating the mechanisms underlying the plasticity of oilseed rape (*Brassica napus* L.) responses to drought and salinity. Due to its allopolyploid origin and three rounds of whole-genome duplication, *B. napus* is an excellent model for studying the functional divergence of duplicated genes and their contribution to enhanced adaptive potential. As a winter crop, it faces water deficit and increased soil salinity during seed germination and root development in autumn, while increasingly mild winters contribute to physiological and spring soil drought, leading to yield losses. Our current research aims to characterize the dynamics of physiological and biochemical changes in oilseed rape under drought and salinity at various developmental stages. Analyses were conducted on three-week-old seedlings and during vernalization. Gas exchange parameters, including net photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO₂ concentration, and water-use efficiency, were measured. Furthermore, photosynthetic pigment content, stress-related markers (proline and malondialdehyde-MDA), and antioxidant enzyme activities were determined. Changes in transcriptional profiles were evaluated for selected stress-responsive genes (P5CS, chaperone proteins and ion transporter genes) and six *BnaABI1* homologues encoding protein phosphatase type 2, which act as negative regulators of ABA-dependent signaling. The results showed that both stress factors induced significant stage-dependent changes in the photosynthetic apparatus and plant water status. At both developmental stages, reduced stomatal conductance and transpiration rate served as the first line of defense against water loss and decreased the net photosynthetic rate. This physiological response was accompanied by enhanced oxidative stress, reflected by increased MDA levels and compensatory proline accumulation. At the molecular level, stress-responsive genes were generally up-regulated. Notably, the *BnaABI1* homologues exhibited distinct functional divergence: while some duplicates were downregulated under stress conditions, others showed constitutive expression. This work was funded by the National Science Centre, Poland (grant number 2023/51/B/NZ9/01614).

R9.P

Methylome changes under low phosphorus in roots and shoots of rye (*Secale cereale* L.) inbred lines with contrasting phosphorus-deficiency tolerance.

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Słowa kluczowe: *Secale cereale* L., methylation profiling, phosphate deficiency, Methyl Sensitive DArT sequencing.

Phosphorus (P) is an essential nutrient needed for important cellular and metabolic processes. These include energy metabolism, nucleic acid synthesis, membrane lipid production, and enzyme regulation. Because of this, low P availability in soils limits plant growth and crop yields. When plants experience P deficiency, they undergo significant changes in gene expression and metabolic adjustments. However, the role of epigenetic regulation, particularly DNA methylation changes, in response to P deficiency is not well understood in rye (*Secale cereale* L.), a stress-resistant cereal related to wheat and barley. To investigate epigenetic responses to phosphorus deficiency, rye inbred lines were screened under hydroponic conditions, and two contrasting genotypes, K3 and L9 were selected for further study. DNA methylation was profiled using methylation-sensitive DArT sequencing (DArTmetseq). Differentially methylated cytosines (DMCs) and differentially methylated regions (DMRs) were identified in CpG, CHG, and CHH sequence contexts by comparing control and phosphorus-deficient plants at 14 and 21 days after treatment in roots and leaves. The chromosomal distribution of DMCs and DMRs across the whole genome was examined to assess their occurrence within annotated gene regions. The results have revealed substantial variation in DNA methylation patterns among organs, time points, and genotypes. Both K3 and L9 exhibited methylation changes in response to P deficiency with differences in the abundance and distribution of DMCs and DMRs between genotypes suggesting distinct epigenetic responses to phosphorus deficiency. Root and leaf samples also displayed contrasting methylation profiles, indicating organ-dependent regulatory mechanisms. Variation in DMCs and DMRs patterns was also observed across sampling time points. Overall, this study provides new insights into DNA methylation dynamics in rye under phosphorus deficiency and identifies candidate genomic regions that may contribute to adaptation to low-phosphorus environments. This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

Dynamic Transcriptomic and miRNA Reprogramming during Female Flower Development in the Gynoecious Cucumber Line 2gg

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Słowa kluczowe: female flower development; cucumber (*Cucumis sativus*); transcriptome; miRNA; gene regulation; hormonal signaling; RNAseq

Background: Female flower development in cucumber (*Cucumis sativus* L.) is a complex process regulated by coordinated transcriptional and post-transcriptional mechanisms. To elucidate the molecular basis of this process, we analyzed transcriptome and miRNA dynamics during successive stages of female flower bud development in the gynoecious cucumber line 2gg. **Results:** Integrated RNA-seq and small RNA-seq analyses revealed extensive stage-specific reprogramming of gene expression and miRNA accumulation. Early developmental stages were associated with photosynthesis- and metabolism-related pathways, whereas later stages showed activation of genes involved in hormone signaling, transcriptional regulation, stress responses, and floral organ differentiation. Network analyses identified AP2 transcription factors, auxin-responsive genes, and phenylpropanoid biosynthesis enzymes as central regulatory hubs. miRNA profiling uncovered dynamic regulatory modules targeting genes involved in auxin, ethylene, and cytokinin signaling. Integration of mRNA and miRNA datasets indicated that coordinated miRNA–target interactions contribute to gynoecium specification and maturation. **Conclusions:** Our results demonstrate that female flower bud development in cucumber is governed by tightly coordinated transcriptional and post-transcriptional regulatory networks. The identified regulatory hubs and miRNA–target pairs provide new insights into the molecular mechanisms underlying gynoecium development and female reproductive organ formation. This research was funded by a project from the National Science Center UMO2020/37/B/NZ9/00586

R11.P

Aegilops sharonensis cytoplasm: investigating the source of male sterility in triticales

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Słowa kluczowe: triticales, cytoplasmic male sterility, pollen development, fertility restoration

Cytoplasmic male sterility is an inability of a plant to produce functional pollen. This trait can be determined by spontaneous mutations in the mitochondrial genome, but it also can be a consequence of interspecies/intergenic crosses, when a cytoplasm from one species cooperates with a nucleus of another species. Sterilizing cytoplasm has been used in hybrid seed production to obtain male-sterile maternal lines. For triticales, *Triticum timopheevii* cytoplasm has been considered a source of male sterility. However, the phenotypic effect of male sterilization is not stable in different environmental conditions. *Aegilops sharonensis* cytoplasm is proposed as an alternative sterilizing source for triticales; however, there is a lack of information regarding the mechanism underlying this phenomenon. The aim of the study was to identify the pollen developmental stage crucial for its dysfunction and to establish the ability of triticales with *Ae. sharonensis* cytoplasm to restore male fertility. The study materials were male-sterile (A-Salvo) and male-fertile (A-DAD) triticales lines with *Ae. sharonensis* cytoplasm and their maintainers with normal cytoplasm (N-Salvo, N-DAD). The F1 progeny obtained by manual pollination of A-Salvo with the A-DAD line, as well as subsequent F2 and BC1 generations were also examined. Anthers for pollen microscopy observation were collected at different growth stages (BBCH 39–65), while the final assessment of male fertility was performed after harvesting, based on the number of grains set per spike. Microscopy observations revealed that pollen development in male-sterile triticales line with *Ae. sharonensis* cytoplasm was disrupted at the microspore stage (BBCH 52). Cells had an irregular shape and were devoid of starch. Finally, at BBCH 65, non-viable pollen was detected, whereas in the maintainer line, full viability was observed. However, phenotypic assessment of male fertility restoration in the F1, F2 and BC1 generations enables us to further detailed investigation.

R12.P

Investigation of male fertility restoration in wheat with the *Triticum timopheevii* cytoplasm

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Słowa kluczowe: wheat, CMS, male fertility restoration, Rf genes

Restoration of male fertility is crucial for hybrid seed production in cereals. Hybrid varieties are obtained by pollination of a specific male sterile line with a specific restorer line. However, the ability to restore male fertility strictly depends on the interaction between mitochondrial and nuclear genomes. Genes that are responsible for male sterilization are located in the mitochondrial genome, whilst genes that manage restoration – Rf genes – are located in the nucleus. So far, nine Rf genes have been described in wheat with the *T. timopheevii* cytoplasm. In European wheat germplasm, Rf3 is the most common (Geyer et al. 2016). Our preliminary research also pointed out at one gene with strong fertility restoration effect. The main goal of this research was to identify new genes associated with male fertility restoration in wheat. A wheat male-sterile line with the *T. timopheevii* cytoplasm, a male-fertile restorer line, and two bulked samples each of ten F2 plants representing male-sterile and male-fertile phenotypes were used for the study. A total cellular RNA was isolated from leaves followed by custom construction of cDNA sequencing libraries and custom Illumina sequencing. Sequence polymorphisms were identified using the *T. aestivum* genome assembly (IWGSCCS RefSeq v2.1) as a reference. The QTL-seq approach allowed identification of polymorphisms associated with fertility restoration. The most pronounced effect was observed for polymorphisms from chromosome 1B. References: Geyer M, Bund A, Albrecht T, Hartl L, Mohler V (2016) Distribution of the fertility-restoring gene Rf3 in common and spelt wheat determined by an informative SNP marker. *Mol Breed* 36:167.

R13.P

Spatiotemporal Expression Dynamics of Phosphate Transporter Genes in Rye (*Secale cereale* L.) Under Phosphorus Deficiency

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Słowa kluczowe: *Secale cereale*, phosphate transporters, phosphate deficiency, spatiotemporal expression

Phosphate transporter (Pht) genes play a central role in phosphorus uptake, translocation, and intracellular distribution, thereby contributing to phosphorus (P) homeostasis and plant adaptation to phosphorus deficient conditions. Several ScPht gene families have been identified in rye (*Secale cereale* L.), but their spatiotemporal expression patterns in response to phosphorus deficiency remain poorly understood. In this study, two experimental designs using the inbred line Lo7 were employed. In the first experimental setup, seedlings were grown in hydroponics in full nutrient conditions for 7 days and then transferred to control (0.2 mM KH₂PO₄) and P-deficient (0.01mM KH₂PO₄ with additional 0.19mM KCl) media for 5 and 7 days. In the 2nd setup, seedlings were directly transferred to the control and P-deficient media. Expression analysis of selected putative rye phosphate transporter genes was performed by qRT-PCR using the reference genes Actin V3 and GAPDH and the 2^{-ΔΔCt} method. Our results suggest functional divergence in phosphorus acquisition and internal phosphate redistribution, with stronger transcriptional activation likely occurring at later stages of P deficiency. This study provides new insights into the spatiotemporal regulation of phosphate transporter genes in rye and contributes to a better understanding of the molecular mechanisms underlying adaptation to phosphorus deficiency. Funding: This research was funded by the National Science Centre, Poland, project no. 2024/55/B/NZ9/00950.

R14.P

Genetic diversity of rye (*Secale cereale* L.) inbred lines with respect to phosphorus deficiency response and root architecture traits

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Słowa kluczowe: *Secale cereale* L., phosphorus deficiency, genetic diversity, phosphorus deficiency response related genes, root system architecture

Phosphorus (P) is one of the essential elements for plant growth and development. Acquisition of P by plant roots is a crucial characteristic determined by genetics background. The role of root system architecture response to the phosphorus deficiency is well documented. Rye is characterized by good tolerance of various stresses, including low P content. This characteristic is conditioned by rye's unique genetic background and, therefore, morphological variability. Several rye lines were characterized under P deficiency and normal conditions. Their tolerance to P deficiency was estimated based on P content in root and shoot. Two contrasting lines (K3 and L9) were chosen based on this analysis and subjected to the transcriptome, methylome and proteome analysis. Here we extend the investigations by an analysis of root architecture and morphological variability under control and phosphorus deficiency conditions. The root system architecture parameters (total root length, network area, average diameter, etc.) were studied by image analysis in RhizoVision. The rapid anatomics tool (RAT) was used to generate images of dissected root parts. Different effect sizes of phosphorus deficiency were observed for different inbred lines and various root traits. Most lines have shown an increase in their root length, area, etc., and decrease in average diameter. Microscopy of root sections confirmed their variability in structure and root cells organization. The results of phenotyping variability of rye lines will be also used in studies to integrate transcriptomic data and properties of the root system architecture through weighted co-expression network analysis (WGCNA).

R15.P

Expression analysis of PPR genes tentatively associated with the restoration of male fertility in rye (*Secale cereale* L.)

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Słowa kluczowe: rye; CMS; fertility restoration; PPR genes; real-time PCR

Cytoplasmic male sterility (CMS) and fertility restoration are key elements of the system used for seed production of hybrid rye cultivars. Despite extensive research, the molecular mechanisms underlying sterility and male fertility restoration remain not fully explained. This study aimed to verify the expression levels of selected candidate restorer genes formerly identified during RNA-seq analyses in male-sterile forms and forms with restored fertility. Real-time RT-PCR analysis included two CMS rye inbreds, 541C and 541P, and their corresponding fertility restored near-isogenic lines, NIL541C-20 and NIL541P-5. Transcripts of five genes encoding PPR proteins (pentatricopeptide repeat proteins), designated as 4-356, 4-018, 4-625, 4-838 and 7-626, were analysed. The expression analysis revealed clear differences in the accumulation of most of the examined transcripts, including both quantitative and qualitative differences between the male-sterile and male-fertile lines. Gene 4-356 showed more than five-fold higher expression in the fertility-restored lines compared with the CMS lines, regardless of cytoplasm type. For gene 4-018, a significant quantitative difference was observed in pair of lines with P-cytoplasm where transcript accumulation was approximately 21-fold higher in the fertile line, but amplification was detected in only three of five fertile plants. In the pair with C-cytoplasm, no amplification products were detected in sterile plants, showing the qualitative character of the difference in expression. Similar qualitative expression patterns were observed for genes 4-625 and 4-838, whose amplification products were present in all fertility-restored plants but absent from both CMS lines. In contrast, gene 7-626 showed comparable expression in sterile and fertile forms of both analysed cytoplasm. The results indicate that 4 genes encoding PPR proteins, may be significantly associated with the mechanism of male fertility restoration in rye and represent valuable targets for further studies. Acknowledgments: This research was supported financially by Polish Ministry of Agriculture and Rural Development under the Programme of Basic Research for Biological Progress in Plant Production (task 10).

R16.P

Two rye glaucousness genes are localized on chromosome 7RL

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Słowa kluczowe: DArTseq, epicuticular wax, genetic mapping, genotyping by sequencing (GBS), glaucousness, *Secale cereale*

Identifying genes controlling epicuticular wax deposition (glaucousness) is important for understanding plant adaptation to environmental conditions. Previous studies in rye near-isogenic lines (NILs) have shown that drought response and photosynthetic traits vary depending on the genotype, suggesting that both genetic background and wax structure influence plant performance. We report the mapping of two rye loci controlling the non-glaucous phenotype. The genes, designated waxM and waxR, were identified and localized to the distal region of chromosome 7RL using high-density DArTseq-based genetic maps constructed in two F2 mapping populations. Comparative mapping analysis revealed co-localization of both loci within the same genomic interval. An association between non-glaucous phenotype and increased leaf rolling was observed. Significant differences in selected traits, including tiller number (TN) and thousand grain weight (TGW), were detected between glaucous and non-glaucous plants, although these effects varied across populations and growing seasons. Single QTLs for FD and RL were detected on 7RL. Both loci were mapped to the distal part of chromosome 7RL, suggesting the presence of a genomic region involved in wax deposition. The mapped interval is syntenic with wheat chromosomes 2BS and 2DS, where the wax inhibitor loci lw1 and lw2 are located near the EST marker BE498358, indicating that waxM and waxR may represent orthologous or functionally related genes. The co-localization of leaf rolling and wax-related loci suggests tight genetic linkage between these traits. The lack of consistent associations between glaucousness and most agronomic traits indicates that variation in epicuticular wax does not universally affect plant performance, although it may influence yield-related traits such as TGW under specific environmental conditions.

R17.P

Complex transcriptome analysis of *F. culmorum*, *T. atroviride* and wheat consortium

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Słowa kluczowe: Wheat, RNA-seq, *Fusarium culmorum*, *Trichoderma atroviride*, cross-kingdom interactions

Bread wheat is one of the most vital food crops in the world - it feeds over 30% of the world population. Wheat is susceptible to many pathogenic fungi like *Fusarium culmorum*, which induce fusarium head blight disease reducing grain yield and quality. One of the common plant symbiont - *Trichoderma atroviride* - is a well known natural biocontrol agent for its fungicide abilities. What is more, *T. atroviride* strengthens the plant by colonizing the roots by making them thicker and longer, and improves defense mechanisms. In this study we delve into complex cross- and within-kingdom interactions on RNA level. Two genotypes of bread wheat (*Triticum aestivum*) - Muszelka and Ambicja - non-axenic seedlings were treated with *Fusarium culmorum*, *Trichoderma atroviride* and a mix of both spores. Leaves and roots of the plant were collected in the early stage (up to 24 hours after inoculation) and late stage (from 3 to 7 days after inoculation) of fungi treatment. Total RNA were isolated and sequenced. Differential gene expression analysis was conducted with distinction based on treatment (*Fusarium*, *Trichoderma* and *Fusarium + Trichoderma*), time phase (early and late) and organ (leaves and roots). This complex approach helps visualize how, when and where in the plant defense mechanisms occur and what genes are related to them. Conducted analysis of the fungal RNA highlights genes participating in *Trichoderma atroviride* response to *Fusarium culmorum* and vice versa. Gene ontology and KEGG pathways analysis was conducted to further examine specific genes' role in complex cross- and within-kingdom interactions.

R18.P

Transient expression-based analysis of cucumber CsDET2 subcellular localization in *Arabidopsis thaliana*

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Słowa kluczowe: brassinosteroids, *Cucumis sativus*, DE-ETIOLATED 2, steroid reductase, phytohormones

Brassinosteroids are essential plant hormones that regulate growth, development, and stress responses. DET2 encodes a steroid 5 α -reductase involved in brassinosteroid biosynthesis; however, the subcellular localization of cucumber DET2 remains unconfirmed. This study aimed to determine the intracellular localization of the CsDET2 protein using transient expression in *Arabidopsis thaliana*. The coding sequence of cucumber DET2 was fused to RFP (CsDET2-RFP) and introduced into *Agrobacterium tumefaciens* GV3101. Transient expression was performed using the modified Fast-Agro method (Li et al., 2009) in an *Arabidopsis* N-YFP-HDEL line carrying endoplasmic reticulum-anchored YFP (ER-YFP). Fluorescence signals were analyzed by confocal laser scanning microscopy. The CsDET2-RFP fusion protein produced a distinct fluorescence pattern within transformed cells. This pattern suggests that CsDET2 associates with the endoplasmic reticulum and is consistent with its proposed role in the general sterol and brassinosteroid biosynthesis pathway. The results demonstrate the usefulness of the Fast-Agro approach for functional characterization of cucumber proteins and provide preliminary insight into the cellular localization of DET2 in plant cells. Li JF, Park E, von Arnim AG, Nebenführ A (2009) The FAST technique: a simplified *Agrobacterium*-based transformation method for transient gene expression analysis in seedlings of *Arabidopsis* and other plant species. *Plant Methods* 5:6.

R19.P

Mapping the bushy gene regulating growth habit in cucumber (*Cucumis sativus* L.)

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Słowa kluczowe: BSA-seq, chemical mutagenesis, bushy

Cucumber (*Cucumis sativus* L.) naturally exhibits an indeterminate, prostrate growth habit suitable for greenhouse production, whereas in field conditions for mechanical harvesting, compact cultivars are favored, as they allow denser planting. One of the commercially successful bushy cultivars, "Dar," possesses the bushy (bu) gene, which alters plant phenotype by significantly shortening internodes and changing growth architecture to a more compact form. Originally, the bu gene was identified during a chemically induced mutagenesis experiment, and it was assessed as a single recessive gene, but its molecular basis wasn't a subject of research (Kubicki et al., 1986). This study aimed to identify the genomic location and molecular function of the bu gene. To achieve this, a bulked-segregant analysis (BSA-seq) was conducted on DNA bulks from extreme phenotypes from an F2 population derived from a B10 × W-19 cross, and the locus was mapped to chromosome 6. Subsequent mapping using PCR-based markers narrowed the region, from which a list of candidate genes underlying bu have been identified and preliminarily characterized. One of those possesses a nonsense mutation (premature STOP codon), which might result in a truncated protein leading to a loss of its function, which is predicted to take part in brassinosteroid sensing. These findings provide a molecular tool for marker-assisted selection in cucumber breeding programs focused on different growth habits. This research is funded by National Science Center – NCN with the project PRELUDIUM 24 no. 2025/57/N/NZ9/02804 with the title: "Identification and characterization of the bushy gene determining compact growth in cucumber" Reference: Kubicki B, Soltysiak U, Korzeniewska A. Induced mutations cucumber (*Cucumis sativus* L.) IV. A Mutant of the bush type of growth. *Genetica Polonica*. 1986;27(3-4).

R20.P

The characteristics of genetic resources involved in the resistance of sugar beet plants to *Cercospora beticola* Sacc.

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Słowa kluczowe: Beta vulgaris, Cercospora leaf spot, SilicoDART, DARTseq, GWAS, marker-assisted selection.

Cercospora leaf spot (CLS), caused by the fungal pathogen *Cercospora beticola* Sacc., is one of the most devastating foliar diseases affecting sugar beet crops worldwide. This disease significantly reduces root yield, sugar content, and technological quality, with losses reaching up to 55% of root mass and 34% of total sugar yield. Increasing pathogen pressure, the emergence of fungicide-resistant pathogen populations, and restrictions on the use of plant protection products have intensified the need to develop sugar beet varieties with durable genetic resistance to *C. beticola*. Therefore, the identification and characterization of genetic resources associated with *C. beticola* resistance is of particular importance for modern breeding programs. The aim of this study was to identify genomic regions associated with resistance to Cercospora leaf spot in sugar beet using high-throughput genotyping technologies and genome-wide association studies (GWAS). The experimental material consisted of several hundred breeding genotypes with a broad spectrum of responses to *C. beticola* infection. Genome profiling was performed using SilicoDART markers generated using the DARTseq platform. High-density genome scanning was employed, yielding 2.5 million sequencing reads per sample, enabling comprehensive coverage of the sugar beet genome. The analyses revealed numerous genomic regions significantly associated with disease resistance. Mapping of associated SilicoDART markers on the sugar beet reference genome demonstrated that *C. beticola* resistance is controlled by an oligogenic architecture encompassing several loci with moderate effect. The strongest marker-trait associations were identified on five chromosomes, with three to five significant SilicoDART markers detected in each genomic region. The identified SilicoDART marker set provides valuable information on the genetic basis of CLS resistance and represents a useful resource for marker-assisted selection (MAS). Their implementation in breeding programs could accelerate the development of sugar beet varieties with improved resistance to *C. beticola*, thus contributing to sustainable crop production and improved disease management strategies.

R21.P

Gynogenesis in tomato – morphological, histological and transcriptomic analysis of callus derived from unpollinated ovule explants

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Słowa kluczowe: *Solanum lycopersicum*, gene expression, ovule culture

A major advantage of haploidization is the reduced time required to produce true homozygous plants by genome doubling of haploids, resulting in doubled haploids (DH) used to accelerate breeding programs. Haploidization of tomatoes (*Solanum lycopersicum* L.) is very challenging. Initial efforts started in the 1970s focused on inducing androgenesis, but only limited callus formation was achieved, with no regeneration. Attempts at gynogenesis led to plant regeneration, but mostly from somatic tissues. This study aimed to induce haploid development from female gametophyte in three tomato accessions and perform morphological, histological, and transcriptomic characterization of the obtained development. Ovaries were excised immediately after flower anthesis, then disinfected, and isolated ovules were cultured on induction medium. Callus formation occurred in all genotypes with various frequencies (24-68% of ovules). Callus obtained after 8 weeks on induction medium was transferred to regeneration media. Flow cytometry of obtained calluses revealed variation in ploidy, as haploid, diploid, and mixoploid samples were detected. Scanning electron microscopy and histological and analyses of the samples collected from the culture at different stages were performed. Analyses revealed the origin of callus development from ovules and showed differences between embryogenic and non-embryogenic ovule-derived calluses. These latter were also selected for transcriptomic profiling. Analysis revealed 6209 differentially expressed genes (DEGs) in the embryogenic callus compared to the non-embryogenic callus, with 3805 genes upregulated and 2404 downregulated. Among the upregulated genes, several were identified as being involved in cell wall remodeling, redox metabolism, and hormonal signaling.

R22.P

Expression quantitative trait loci mapping in white lupin (*Lupinus albus* L.) response to anthracnose

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Słowa kluczowe: lupin, anthracnose, e-QTL

Legumes are among the most important plant protein sources for human and livestock. Among them, white lupin is especially valuable, due to its ability to mobilize mineral phosphorus by cluster root, in addition to nitrogen fixation by symbiotic bacteria in the root nodules. The seeds of white lupin are rich in protein and have moderate content of high quality fat, however its cultivation worldwide was impeded by wide spread of hemibiotrophic pathogenic fungus, *Colletotrichum lupini*, causing devastating disease, anthracnose. Worldwide scientific efforts on germplasm screening resulted in selection of several lines with increased resistance. One of them, P27174 line from Ethiopia, was used to create a mapping population by crossing with a susceptible Ukrainian breeding line Kiev Mutant. In this study 189 recombinant inbred lines (RILs) from this population were subjected to anthracnose resistance survey in controlled environment in randomized design (8 replicates). After 25 days of growth two batches of plants were sprayed with *C. lupini* conidial spores suspension (one batch with derma-roller leaf scarification directly before inoculation to facilitate fungal infection). Control plants were mock-inoculated with water. After inoculation, all variants were kept in 90% humidity for 48 hours. Leaf tissue samples were collected 48 and 96 hours after inoculation to profile early stages of the defense response. To evaluate anthracnose progression, plants were scored 7, 14 and 21 days after inoculation based on the presence of disease symptoms on the scale from 1 (immune) to 9 (extremely susceptible). The results of phenotypic observations will be used for quantitative trait loci (QTL) mapping whereas collected plant tissue will be subjected to RNA isolation, RNA sequencing and expression-QTL mapping. Based on the co-localization of QTL and e-QTL peaks genes involved in anthracnose resistance will be identified. The study was supported by National Science Centre, Poland (OPUS23, project no. 2022/45/B/NZ9/01397).

R23.P

Expression analysis of genes encoding pathogenesis-related (PR) proteins in wheat (*Triticum aestivum* L.) progeny with chromatin introgression from related species

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Słowa kluczowe: leaf rust, resistance breeding, gene pools, PR genes

Triticum aestivum L. is an important species in cereal production, with significant yield potential and importance for human and animal nutrition. Leaf rust, caused by the fungus *Puccinia triticina* Eriks (Pt), is a damaging disease that results in a significant decrease in common wheat yields. During an infection, plants activate an immune response that begins at the cellular level and causes visible changes in the plant. An important element of the cellular resistance response cascade is the family of genes that encode PR (pathogenesis-related) proteins, which recognize effector molecules during gene-pathogen interactions and enhance their expression as part of the plants defense mechanism. The adaptability and development of pathogens are major challenges, which require continuous research of resistance genes. One of the approaches used to improve resistance is the use of Crop Wild Relatives (CWRs), which provide a source of genetic diversity for Lr genes conferring resistance to *P. triticina*. The aim of this study was to analyse the expression of four genes encoding PR proteins in hybrid forms containing foreign chromatin segments derived from *Thinopyrum* and *Secale cereale* species. The expression of genes encoding PR proteins was analysed by RT-qPCR in hybrid lines carrying different combinations of the Lr19, Lr24, Lr26, Lr38, and Lr63 resistance genes. Analysis of gene expression was performed before and after inoculation: 0, 6, 12, 24, 48, and 72 hours post-inoculation (hpi). Results indicated that the PR2, PRA2, PR5, and PR9 genes are associated with a rapid resistance response to Pt, with the highest expression levels observed at 6 hpi. Moreover, the PR2 and PR9 genes show high expression levels in plants displaying pyramidization of the Lr genes. The results indicate the importance for further study into the resistance response associated with PR genes and their interactions with the resistance genes to leaf rust.

R24.P

Evaluation of reference genes for RT-qPCR normalization in leaf rust-infected wheat (*Triticum aestivum* L.)

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Słowa kluczowe: gene expression, housekeeping genes, biotic stress, *Puccinia triticina*

Puccinia triticina (Pt) is a pathogenic fungus that causes leaf rust, one of the most damaging diseases that significantly reduces common wheat yields. An accurate analysis of gene expression during the infection process can enhance our understanding of the molecular mechanisms underlying its pathogenicity. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is a highly effective molecular technique widely used to detect and quantify for gene expression levels. However, it requires validated reference genes to normalize the obtained data and to minimize nonbiological variability between samples. The optimal approach is to use validated endogenous reference genes whose expression remains stable across all experimental treatments. Selecting and validating reference genes for RT-qPCR data normalization is crucial for obtaining reliable, accurate, and reproducible gene expression results. This study aimed to examine the stability of twenty potential reference genes in hybrid wheat lines at five time points (0, 6, 12, 24, and 48 hours after inoculation; hpi). Biotic stress was induced in adult plants through inoculation with Pt fungal spores under controlled conditions. In this study, twenty potential reference genes were selected and the specificity of the primers were assessed by melting curves of PCR products. The expression stability of selected reference genes were evaluated using commonly used algorithms such as geNorm, Norm Finder, BestKeeper, and cycle threshold (Ct) values, then ranked from most to least suitable for normalization. Out of the twenty reference genes tested, ten were excluded due to the presence of nonspecific amplification products, as confirmed by agarose gel electrophoresis and melting curve analysis, as well as high values $C_q > 40$. This study provides a comprehensive list of reference genes for reliable normalization using the RT-qPCR method to study genes with differential expression the response to biotic stress induced by the Pt.

R25.P

Activity of antioxidant enzymes in the leaves of oat near-isogenic lines Pc60/pc60 in response to *Puccinia coronata* infection

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Słowa kluczowe: antioxidant enzymes, catalase, peroxidase, superoxide dismutase, *Avena sativa*, crown rust

The aim of this study was to evaluate the activity of selected antioxidant enzymes – catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) – in the leaves of oat (*Avena sativa* L.) seedlings of the Pc60/pc60 near-isogenic lines in response to *Puccinia coronata* infection. Plant material for the biochemical analyses originated from a host–pathogen test; enzyme activity was determined spectrophotometrically in 36 samples collected at eight time points after infection (0–96 hours), using the methods of Aebi (1984), Lück (1962) and McCord and Fridovich (1969), and the results were expressed as specific activity relative to protein content (Bradford 1976). Analysis of variance showed a significant effect of plant form (resistant/susceptible) on the activity of all three enzymes ($p \leq 0.001$), whereas time after infection (HPI) and the form $\times$ HPI interaction significantly affected only CAT activity ($p \leq 0.05$ and $p \leq 0.01$, respectively). Resistant forms showed significantly higher CAT activity than susceptible forms regardless of HPI, with a maximum at 12 HPI (424.21 Δ ABS/g protein) and the largest difference between forms at 12 and 96 HPI (4.4- and 4.3-fold, respectively). SOD activity was likewise consistently higher in resistant forms (mean 4.49 vs. 2.76 Δ ABS/g protein), with the largest difference observed at 96 HPI (2.3-fold). An opposite trend was found for POD, whose activity was higher in susceptible forms at all stages of infection (mean 1223.78 vs. 905.62 Δ ABS/g protein), with the largest difference at 9 HPI (nearly 2-fold). These results indicate that elevated and more rapidly induced CAT and SOD activity, together with reduced POD activity, may constitute an important component of the resistance mechanism of the Pc60 gene against *Puccinia coronata* infection.

R26.P

Unraveling the molecular basis of Trichoderma-induced immunity in common barley (*Hordeum vulgare*)

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Słowa kluczowe: common barley, fusarium head blight, Trichoderma, plant induced resistance, sustainable agriculture, biocontrol

This study addressed the need for sustainable strategies to mitigate Fusarium head blight (FHB) in barley, caused by *Fusarium graminearum*, which had been associated with yield losses and mycotoxin contamination. Biological control using *Trichoderma* spp. had been considered a promising alternative; however, the molecular basis of their interactions with the host and pathogen remained insufficiently understood. The objective of the study was to investigate the effects of three *Trichoderma* species (*T. asperellum*, *T. atroviride*, *T. gamsii*) on the germination and early growth of brewing barley infected with *F. graminearum*, and to identify transcriptional responses associated with biocontrol activity. The *Trichoderma* isolates had been obtained from local field samples, cultured in vitro, and identified at the species level based on genomic data and comparative analysis with GenBank resources. Barley seeds were treated with conidial suspensions of *F. graminearum*, *Trichoderma* spp., or their combinations. Total RNA was isolated from roots of barley seedlings and used for transcriptomic profiling. cDNA libraries were prepared and sequenced using Oxford Nanopore technology. Differential gene expression analyses were conducted to compare *Trichoderma* + *Fusarium* versus *Fusarium*-only treatments to identify markers of immune priming, and *Trichoderma*-only versus non-inoculated controls to characterize strain-specific effects linked to induced systemic resistance. The results demonstrated that *Trichoderma* spp. modulated host responses and mitigated the negative impact of *F. graminearum* on seed germination and seedling development. Distinct transcriptional signatures associated with defense activation and stress responses were identified, indicating host-mediated resistance mechanisms. These findings contributed to understanding the molecular basis of *Trichoderma*-mediated biocontrol and supported their application in sustainable barley protection strategies.

R27.P

An Efficient and Universal Protoplast Isolation Protocol Suitable for single-cell RNA sequencing

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Słowa kluczowe: Protoplast isolation, Single-cell RNA sequencing, Transcriptome,

Plant growth and development depend on the varied expression of genes in different cell types. Compared to traditional RNA sequencing from pooled samples, single-cell RNA sequencing based on droplet technology (scRNA-seq) enables the creation of transcriptomic profiles of individual cells within heterogeneous tissues. The scRNA-seq method provides a high-resolution atlas of cellular characteristics and significantly expands our understanding of the interactions between individual cells and their microenvironment. In plants, the key step in preparing material for scRNA-seq is obtaining a high-quality suspension of live protoplasts by removing the cell wall. The effectiveness of this process depends on the species, tissue type, and the composition of the enzymatic mixture and digestion conditions. The aim of this study was to develop a universal protocol for isolating protoplasts for scRNA-Seq analysis. The study used seeds and leaves from beans (*Phaseolus vulgaris*), corn (*Zea mays*), and barley (*Hordeum vulgare*). Protocol optimization included optimizing enzymatic digestion, filtration, and cell purification, which allowed for the preparation of a suspension of live protoplasts suitable for single-cell sequencing. The isolated protoplasts from barley embryos and leaves were used to prepare libraries using the Chromium GEM-X Single Cell 3' Gene Expression Universal Kit v4 (10x Genomics) and sequenced on the Illumina NovaSeq platform. The resulting data will be used to assess library quality and to identify cell populations and gene expression profiles in barley leaf tissue. The developed protocol serves as a basis for further single-cell transcriptomic studies of barley and can be used to analyze plant responses to environmental factors and to identify the mechanisms regulating the functioning of specific cell types.

CRISPR/Cas9 Technology for Gene Function Analysis and Wheat Improvement

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Słowa kluczowe: CRISPR/Cas9, bread wheat, homeologs, TaCKX9, TaPslPK1, stripe rust

Bread wheat (*Triticum aestivum* L.) is an allohexaploid species comprising three subgenomes (A, B, and D). Multiple homeologous gene copies complicate gene function analysis while enabling targeted modification of one, two, or all three homeologs using genome editing. This study aimed to apply the CRISPR/Cas9 system to investigate the function of TaCKX9 and to edit the yellow rust susceptibility gene TaPslPK1, a potential target for improving disease resistance, in spring and winter bread wheat cultivars.

For TaCKX9, a single guide RNA (sgRNA) targeting a conserved region shared by the A, B, and D homeologs was designed. Genome-edited plants were obtained in spring wheat cultivars Fielder and Kontesa. Molecular analysis of the T₁ generation confirmed small insertions and deletions affecting different combinations of the three homeologs. Five independent mutant lines were identified in cv. Fielder and four in cv. Kontesa. Preliminary observations of the T₁ generation revealed considerable phenotypic variation among the edited lines. Further analyses will clarify genotype–phenotype relationships.

In parallel, TaPslPK1 was edited in the spring wheat cultivar Fielder and in selected Polish winter wheat cultivars and breeding lines. Mutations were identified in T₀ plants of the cultivars Fielder, Belissa, and Elektra, whereas no edits have been detected in the remaining genotypes to date. Among the tested winter wheat genotypes, Elektra showed high responsiveness to transformation and genome editing, comparable to that of the spring wheat cultivar Fielder, a widely used genotype for wheat transformation. These results suggest that Elektra may be a suitable genotype for CRISPR/Cas9-mediated genome editing in winter wheat.

The mutant lines provide valuable material for future studies on TaCKX9 function, mutation inheritance, and the effects of TaPslPK1 disruption on wheat susceptibility to stripe rust.

Funding: National Science Centre (NCN), OPUS 19: UMO-2020/37/B/NZ9/00744, (A N-O); Ministry of Agriculture and Rural Development, Targeted Grant, Task 4.3, (A N-O).

R28.P

From Genome-Wide Association Analysis to Plant Breeding Practice: Development and Validation of PCR Markers for Marker-Assisted Selection of Blackleg Resistance in Rapeseed

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Słowa kluczowe: Brassica napus, blackleg, DArTseq, GWAS, marker assisted breeding, PCR

High-throughput genotyping technologies, such as DArTseq, enable the identification of molecular markers associated with agriculturally important traits. However, their practical application in plant breeding requires conversion into simple, cost-effective genotyping assays that can be performed in standard molecular laboratories. A particular challenge is the development of assays for SNP markers, whose analysis typically relies on specialized detection methods. The aim of this study was to develop PCR-based markers for marker-assisted selection (MAS) of blackleg resistance in rapeseed (*Brassica napus* L.) by converting SilicoDArT and SNP markers identified through a genome-wide association study (GWAS) using DArTseq technology. Based on phenotypic evaluation and genome-wide association analysis, markers associated with blackleg resistance were selected for conversion into PCR assays. Conventional PCR assays were developed for SilicoDArT markers, whereas allele-specific ARMS-PCR assays were designed for SNP markers. The developed assays were optimized and validated using DNA sequencing and breeding materials differing in their levels of resistance. The resulting PCR markers enable rapid, reproducible, and cost-effective genotyping of selected loci associated with blackleg resistance. The developed tool provides a practical solution for marker-assisted selection and facilitates the translation of genomic discoveries into routine rapeseed breeding programs.



M.O

Targeting PNPase-Dependent RNA Homeostasis in Mycobacteria - Implications for Post-Transcriptional Gene Regulation

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Słowa kluczowe: RNA metabolism, PNPase, degradosome, tuberculosis, persistence

Mycobacterium tuberculosis adapts to hostile host environments through complex regulatory networks controlling gene expression at both transcriptional and post-transcriptional levels. Among these, RNA metabolism has emerged as a critical determinant of bacterial adaptation, persistence and antimicrobial tolerance. However, the contribution of RNA degradation pathways to mycobacterial physiology remains incompletely understood. This study aimed to investigate the RNA degradosome as a source of novel therapeutic targets, with particular emphasis on polynucleotide phosphorylase (PNPase), a central enzyme involved in RNA turnover. The molecular organization of the mycobacterial RNA degradosome was investigated by characterizing interactions between PNPase and the DEAD-box RNA helicase RhIE using size-exclusion chromatography, native PAGE, microscale thermophoresis and biolayer interferometry. Functional validation of PNPase as a therapeutic target was performed through biochemical screening of small-molecule inhibitors followed by enzymatic characterization, antimycobacterial activity testing, hypoxia survival assays, regrowth experiments and evaluation of recovery of antibiotic-resistant colonies. PNPase formed a stable complex with RhIE, supporting the existence of a functional RNA degradation machinery involved in post-transcriptional regulation in mycobacteria. Several compounds inhibited PNPase activity and displayed antimycobacterial effects. Inhibition of RNA metabolism reduced bacterial survival under oxygen-limited conditions and markedly impaired recovery of antibiotic-resistant

colonies following antibiotic selection, indicating compromised adaptive capacity under stress. These findings suggest that disruption of RNA homeostasis interferes with regulatory pathways required for persistence and long-term survival. The presented results identify RNA metabolism as a fundamental component of the genetic regulatory network governing mycobacterial adaptation. Functional perturbation of PNPase demonstrates that post-transcriptional RNA processing represents a promising target for the development of novel antitubercular therapies and provides new insight into the molecular mechanisms underlying bacterial persistence.

M1.O

Bacterial Epigenetics as a Regulatory Layer in Development and Secondary Metabolism of *Streptomyces coelicolor* A3(2)

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Słowa kluczowe: bacterial epigenetics; DNA methylation; *Streptomyces coelicolor* A3(2); secondary metabolism; biosynthetic gene clusters; 5mC; 6mA; 4mC

Bacterial genome function is regulated not only by DNA sequence, but also by epigenetic modifications that can influence DNA-protein interactions, gene expression, genome defence, replication, repair, and adaptation to environmental conditions. Among the most important bacterial epigenetic marks are DNA methylation modifications, including N6-methyladenine (6mA), 5-methylcytosine (5mC), and N4-methylcytosine (4mC). These modifications are introduced by DNA methyltransferases and may contribute to restriction-modification systems as well as broader regulatory processes. This presentation focuses on the possible role of DNA methylation in the developmental and metabolic regulation of *Streptomyces coelicolor* A3(2), a model actinomycete characterized by a complex mycelial life cycle and the ability to produce specialized metabolites, including coelimycin (CPK), undecylprodigiosin (RED), actinorhodin (ACT), and calcium-dependent antibiotic (CDA). Because morphological differentiation and secondary metabolism are tightly coordinated in *Streptomyces*, this genus provides an attractive model for investigating how methylation patterns may be linked with developmental transitions and antibiotic biosynthesis. Genome-wide methylation patterns were analysed at three developmental time points: 18 h, 27 h, and 50 h, representing early growth, the transition between primary and secondary metabolism, and later stages associated with visible actinorhodin production. Particular attention was given to methylation changes in biosynthetic gene clusters and in genes encoding pathway-specific regulators, core biosynthetic enzymes, and other proteins involved in secondary metabolism. The analysis revealed relatively stable methylation patterns between 18 h and 27 h, followed by pronounced changes at 50 h, including increased 5mC and 4mC methylation and decreased 6mA methylation. These results suggest that bacterial DNA methylation in *S. coelicolor* A3(2) is developmentally dynamic and may contribute to late-stage genome regulation, metabolic reprogramming, and specialized metabolite production.

M2.O

Full-length 16S rRNA gene microbiome profiling in morbid obesity using the novel Oxford Nanopore Microbial Amplicon Barcoding Kit reveals associations with metabolic and inflammatory markers

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Słowa kluczowe: gut microbiota; morbid obesity; Oxford Nanopore sequencing; 16S rRNA gene sequencing; metabolic biomarkers; inflammation

Introduction. Gut microbiota alterations are increasingly linked to obesity, insulin resistance, and metabolic inflammation. Long-read 16S rRNA sequencing enables comprehensive taxonomic profiling of the intestinal microbiota. The aim of this pilot study was to explore associations between gut microbiota composition and metabolic parameters in bariatric patients using Oxford Nanopore Technologies (ONT) sequencing. **Methods.** The study included 62 patients with morbid obesity (mean age 38.8 ± 9.6 years; mean BMI 40.5 ± 4.2 kg/m²); women accounted for 84% of the cohort. Stool samples collected at hospital admission underwent full-length 16S rRNA gene sequencing using the Oxford Nanopore MinION platform and the novel Microbial Amplicon Barcoding Kit 24 V14 (SQK-MAB114.24). Demultiplexed reads filtered to 1300–1700 bp were taxonomically classified using the EPI2ME Labs wf-16S pipeline, incorporating Kraken2 and Minimap2 against the NCBI_16S_18S database, with taxonomic assignments reported to the species level. Digital PCR was used to validate sequencing results and quantify bacterial 16S rRNA gene copy numbers per sample. Associations with $p < 0.05$ were considered statistically significant. **Results.** Sequencing generated high-quality datasets with a low proportion of unclassified reads (~0.5%). Gut microbiota profiles were dominated by Bacillota and Bacteroidota. Significant associations were observed between the TyG index and Negativicutes, glucose levels and Bacteroidia, CRP levels and Mollicutes/Anaerolineae, and PCSK9 levels and Erysipelotrichia. Marked inter-individual heterogeneity in the microbiome was observed among bariatric patients. **Conclusions.** Oxford Nanopore full-length 16S rRNA sequencing with the novel ONT kit enabled robust and reproducible microbiome profiling in bariatric patients. Exploratory analyses identified several potentially relevant associations between gut microbiota composition and metabolic and inflammatory markers. These findings warrant validation in larger, independent cohorts. **Funding:** Medical Research Agency, Poland (ABM), grant no. 2023/ABM/02/00004, Regional Centre for Digital Medicine (RCMC).

Molecular and evolutionary insights into contact-dependent growth inhibition (CDI) systems associated with virulence plasmids of opportunistic bacteria of the genus *Cronobacter*

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Słowa kluczowe: *Cronobacter* spp., virulence plasmids, contact-dependent inhibition (CDI) systems, plasmid evolution

Cronobacter spp. are opportunistic food-borne pathogens capable of causing life-threatening infections in neonates and infants. Their pathogenic potential is enhanced by virulence plasmids of the pESA3 family, which are stable and characteristic genomic features of these bacteria. Genomic analysis of *Cronobacter sakazakii* MK_1 (sequence type ST145), a food-born strain recovered from raw milk, revealed that its virulent plasmid pCS-MK1_P1 (131,742 bp) carries a large (~17 kb) gene cluster encoding a putative contact-dependent growth inhibition system (CDIMK1). CDI systems mediate antagonistic interbacterial interactions by producing and secreting a large polymorphic effector protein (toxin), which inhibits the growth of neighbouring bacteria. The CDIMK1 locus comprises three genes encoding a putative membrane transporter protein (CdiB), the toxin (CdiA), and immunity protein (CdiI), which protects the host cell from the toxin's activity. Additionally, the locus contains putative remnants of two other distinct CDI systems, each consisting of an orphan cdiI gene preceded by the terminal portion of a cdiA gene (cdiA-CTO –cdiI cassettes). These cdiA remnants contain internal promoters that, by driving expression of additional immunity genes, may protect the host bacterial cell against a broader repertoire of toxins produced by other bacteria. Functional characterization revealed that the CDIMK1 both confers a growth inhibition phenotype against some Enterobacteriaceae and stimulates biofilm formation in MK_1. We further show that CDI loci are widely distributed across the genus *Cronobacter*, particularly in non-*C. sakazakii* strains. Funding: National Science Centre, Poland (grant no: DEC-2022/47/B/NZ8/03264)1. Franco AA et al. 2011. Appl. Environ. Microbiol. (doi: 10.1128/aem.03023-10) 2. Korsak D. et al. 2025. Front. Microbiol. (doi: 10.3389/fmicb.2025.1713963)

M4.O

The influence of natural selection on the DNA gyrase genes of *Mycobacterium tuberculosis*

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Słowa kluczowe: *Mycobacterium tuberculosis*, tuberculosis, drug-resistance, evolutionary patterning

Tuberculosis remains one of the most serious infectious diseases worldwide. It represents a major clinical and epidemiological challenge, particularly in the context of the increasing drug resistance of *Mycobacterium tuberculosis* strains. In 2024, it caused approximately 1.23 million deaths globally. A key element in the search for new therapeutic strategies is the identification of enzymes essential for bacterial survival and replication. One such molecular target is DNA gyrase, responsible for controlling DNA topology, which is a molecular target of fluoroquinolones currently used in tuberculosis treatment. This study aimed to use evolutionary patterning to identify new inhibitors of *M. tuberculosis* DNA gyrase and to improve the diagnosis of drug-resistant cases. Evolutionary patterning is based on analyzing the variability of gene sequences within a bacterial population and identifying codons under natural selection. This approach makes it possible to detect sites under positive selection, which are characterized by sequence variability that may result from selective pressure and be associated with the development of drug resistance. It also enables the identification of sites under negative selection, which are conserved and may represent promising binding sites for new antituberculosis drugs. We verified our bioinformatics data by constructing mutants, recombinant proteins, and in vitro assays. Through in silico analyses and experimental validation, we identified five new substitutions in DNA gyrase genes associated with the emergence of fluoroquinolone resistance. This may be useful in the diagnosis of drug-resistant tuberculosis with genetic methods. In addition, we identified seven conserved sites that may serve as promising binding sites for new antituberculosis drugs. At least two new potential inhibitors of gyrase were proposed that will be investigated further. Acknowledgments This work is part of the research project financed by the National Science Center of Poland, grant number 2019/34/E/NZ6/00221

M5.O

PknB-dependent phosphorylation of CslA links beta-glucan synthesis to tip growth regulation in *Streptomyces*

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Słowa kluczowe: ell morphogenesis, cell wall, CslA, DivIVA, phosphorylation, PknB, polarisome, *Streptomyces*, Tip Organising Centre (TIPOC)

How filamentous bacteria coordinate tip growth with cell envelope integrity remains poorly understood. *Streptomyces* grow by extending and branching hyphal tips, with new growth zones established on lateral cell walls through the action of polarisome complexes. DivIVA, the central polarisome component, is essential for this process — yet the regulatory networks that tune its activity remain largely uncharacterised. We exploited the toxic effects of DivIVA overproduction in *Streptomyces albus* to design a forward genetic screen for suppressors of growth inhibition. This approach identified *pknB*, encoding a serine/threonine kinase, as a locus whose deletion restores viability under DivIVA overproduction conditions. To understand how PknB connects to the tip growth machinery, we performed phosphoproteomic profiling of a *pknB* deletion strain, which revealed altered phosphorylation of CslA — a cellulose synthase that produces a beta-glucan polymer at the cell envelope. Phosphoablation of the PknB target site in CslA impaired beta-glucan synthesis and increased lysozyme sensitivity, pointing to a role for this modification in maintaining envelope robustness. Strikingly, CslA overproduction suppressed the colony growth defects caused by DivIVA-induced hyperbranching without resolving the underlying morphological phenotype, indicating that enhanced beta-glucan deposition compensates for, rather than corrects, polarisome imbalance. Our results establish a PknB–CslA phosphorylation axis as a critical interface between cell growth signalling and cell envelope construction in *Streptomyces*, with implications for understanding morphogenesis in antibiotic-producing actinomycetes.

M6.O

Structure-function characteristics of CemR regulators in *Campylobacter jejuni* and *Arcobacter butzleri*

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Słowa kluczowe: Redox regulation, CemR, *Campylobacter*, Oxidative stress, Protein–DNA interactions

Pathogens of the *Campylobacter* class, including *Helicobacter pylori*, *Campylobacter jejuni*, and *Arcobacter butzleri*, are continuously exposed to host-derived reactive oxygen species (ROS). Their survival depends on redox-sensitive regulatory proteins that detect thiol oxidation and coordinate adaptive responses. CemR from *H. pylori* is an established thiol-based redox switch. Its homologs, CjCemR and AbCemR, share high structural similarity and conserved cysteine residues, suggesting a common evolutionary origin. However, whether they employ analogous molecular mechanisms of redox regulation has remained unclear. Our study demonstrates that CjCemR and AbCemR both rely on cysteine-dependent regulation, but through distinct mechanisms. In CjCemR, in vitro mPEG thiol trapping identified two reactive cysteines, while mutant analyses indicated unequal contributions of individual residues to protein function. One cysteine mutant displayed impaired DNA binding comparable to the cysteine-free variant, suggesting a key role in redox regulation. In vivo, diamide treatment confirmed oxidation of CjCemR cysteines, and thiol trapping implicated Cys233 as a regulatory residue. In contrast, the cysteine-free AbCemR mutant increased paraquat sensitivity more strongly than complete protein deletion, suggesting a dominant-negative effect on oxidative stress responses. In vitro, DNA binding by wild-type AbCemR depended on its redox state, whereas the cysteine-free variant lost redox responsiveness, demonstrating the essential role of cysteine residues in regulation. Structural and biochemical analyses further suggest that AbCemR Cys230 contributes to protein stability through Zn²⁺ coordination, whereas CjCemR regulation appears independent of divalent metal ions. Combining in vitro and in vivo approaches revealed that CemR homologs represent conserved yet mechanistically diverse redox switches in *Campylobacter*. Despite their structural similarity, these proteins have evolved distinct strategies for sensing oxidative stress, highlighting the functional diversification of homologous redox regulators and providing new insight into bacterial adaptation to the host environment. This work provides the first comparative characterization of CemR homologs from *C. jejuni* and *A. butzleri*, revealing that evolutionarily conserved proteins have diverged into distinct redox-sensing mechanisms.

M7.O

No room for two polyketides - coelimycin molecules suppress actinorhodin biosynthesis in *Streptomyces coelicolor* A3(2)

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Słowa kluczowe: Gene expression regulation, Specialized metabolites, Regulatory networks, Polyketide biosynthesis

Streptomyces bacteria possess one of the most complex gene regulation machineries in the bacterial kingdom, allowing them to coordinate production of multiple compounds at discrete timing. Navigating through these regulatory networks to unlock the biosynthetic potential is challenging. Expression of silent biosynthetic gene clusters (BGCs) occurs only under strict culture conditions and, as in the case of coelimycin (CPK), can be coordinated via Quorum Sensing by gamma-butyrolactones (GBLs). Moreover, some of the specialized metabolites can act as ligands affecting gene expression regulation for instance via transcription factors (TFs) or riboswitches. Present study investigates the role of coelimycin in the well-established model organism *S. coelicolor* A3(2). The interplay between CPK and ACT polyketides production were studied. We show that CPK and ACT biosynthesis are exclusive processes. Induction of CPK synthesis by external application of GBLs inhibited ACT production in the strain overexpressing AtrA. AtrA activates expression of ActII-orf4, the direct activatory protein of ACT BGC. Inhibition of ACT by CPK-induction was not observed under direct overexpression of ActII-orf4. Based on comparative analysis of several mutant strains, we conclude that intracellular CPK intermediates inhibit the cascade of ACT induction at the level of AtrA activity. Additionally, proteomics analysis of AtrA-overexpression in the strain lacking GBLs and preCPK reveals that, besides being ACT inducer, AtrA is a pleiotropic regulator that activates arsenopolyketide BGC, yet inhibits the undecylprodigiosin and calcium-dependent antibiotic BGCs. AtrA also downregulates primary metabolism of carbohydrates, lipids and amino acids, along with tricarboxylic acid cycle.

M8.O

The mycobacterial nucleoid-associated protein NapM exhibits stress-induced septal localization and modulates cell envelope gene expression

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Słowa kluczowe: organizacja chromosomu, mycobacterium, stres, podział komórkowy,

Nucleoid-associated proteins (NAPs) organize the bacterial chromosome and influence transcription, yet the functional diversity of NAPs across the genus *Mycobacterium* remains poorly defined. We characterized NapM, a mycobacterial-specific NAP of the PadR family, in *Mycobacterium smegmatis*, combining phenotypic analysis, RNA sequencing, lipidomics, fluorescence microscopy, and protein–protein interaction assays. Deletion of napM significantly increased cell length, while NapM overproduction shortened cells, without affecting cell width or division symmetry. Genome-wide RNA sequencing revealed that loss of NapM dysregulates nearly one-sixth of the *M. smegmatis* transcriptome, with strong downregulation of genes governing peptidoglycan and polar lipid (PIM, LM, LAM) biosynthesis, and of divIVA, a master divisome regulator. Lipidomic profiling confirmed these changes biochemically, showing accumulation of unacylated phosphatidylinositol dimannoside. Consistent with impaired envelope homeostasis, Δ napM cells showed increased sensitivity to cell-envelope-targeting antibiotics, while NapM overproduction sensitized cells to peptidoglycan synthesis inhibitors. Unexpectedly, NapM was diffusely distributed under standard growth, unlike canonical chromosome-bound NAPs, but relocalized sharply to the forming septum upon envelope-damaging stress. Co-immunoprecipitation and bacterial two-hybrid assays identified direct interactions between NapM and the divisome components SepIVA, CwsA, and DivIVA, and loss of NapM destabilized septal DivIVA accumulation under stress. NapM is a dual-function regulator that maintains cell envelope homeostasis through global transcriptional control under optimal growth, while reinforcing divisome integrity through direct protein interactions under stress — a condition-dependent behavior not previously described for mycobacterial nucleoid-associated proteins.

M9.O

Bacteria resistant to β -lactam antibiotics isolated from horses

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Słowa kluczowe: antibiotic resistance, β -lactamases, horses, NGS sequencing, biochemical tests

Bacterial antibiotic resistance is a global public health problem recognized by the WHO. Excessive and inappropriate use of antibiotics promotes the selection and spread of resistant strains in human and animal populations. Interspecies transmission between animals and humans plays an important role in the process. Horses may serve as reservoirs of commensal and pathogenic bacteria capable of horizontal transfer of resistance genes, which increases with close contact with humans and enhances infection risk. The aim of this study was to characterize ampicillin-resistant bacteria isolated from the oral mucosa of horses, identify mechanisms of β -lactam resistance, and assess the role of plasmids in the spread of resistance genes. Fifty-one bacterial strains isolated from the oral cavity of six healthy horses were analyzed using microbiological and molecular methods. Bacteria were assigned to species using morphological and biochemical assays. Minimum inhibitory concentration (MIC) for ampicillin was determined using the microdilution method, and tests for β -lactamase (ESBL, AmpC, KPC, and MBL) production were performed using disc diffusion kits. Plasmid DNA was isolated using alkaline lysis, and plasmid replicon types were assigned by multiplex PCR. Four strains were additionally subjected to whole-genome sequencing (NGS). Both commensal and potentially pathogenic bacteria were identified, including *Escherichia/Shigella*, *Pseudomonas fluorescens/putida*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, and *Moraxella osloensis*. All isolates showed high resistance to ampicillin, with MIC values as high as 10240mg/L. Using phenotypic resistance tests, β -lactamases, including ESBLs and carbapenemases (mainly KPC), as well as MBL and AmpC enzyme activity, were detected. Isolated plasmids belong to IncF, IncK, IncB/O, and IncW groups. Whole-genome sequencing identified four strains as *Pseudomonas moraviensis*, *Pseudomonas* sp. MH9.3, *Pseudomonas orientalis*, and *Luteimonas* sp. S4-F44 and did not confirm some phenotypic and biochemical results, highlighting limitations of conventional methods. The findings emphasize the need for genomic approaches in the comprehensive characterization of resistance mechanisms.

M10.O

Plasmidome diversity and plasmid-encoded effectors may contribute to pathogenic repertoire of the *Legionella* genus

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Plasmids represent an important but still poorly characterized component of genome plasticity in the genus *Legionella*. Although the biology of *Legionella* has been extensively studied in the context of the Dot/Icm type IVB secretion system and its large repertoire of translocated effector proteins, the contribution of extrachromosomal replicons to effector diversification remains insufficiently understood. Here, we combined comparative plasmidomics, effector prediction and experimental validation to investigate plasmid-associated virulence potential in *Legionella*. The analysis was based on a curated dataset of publicly available complete *Legionella* genomes carrying plasmids, and newly sequenced plasmids from environmental isolates. In total, the analyzed plasmidome comprised approximately 150 plasmids, with replicon sizes ranging from 13 to 373 kbp. Plasmids were first characterized using nucleotide similarity, clustering of backbone regions, and identification of key classification features, including replication, partitioning, and mobility-related modules. These analyses revealed considerable diversity in plasmid backbone regions, indicating the presence of multiple *Legionella* plasmid groups, including replicons shared among different species. Subsequently, coding sequences located on plasmids were screened for candidate Dot/Icm effector proteins. Six plasmid-encoded effectors were selected for detailed analysis and experimental testing. These proteins were characterized using sequence- and structure-informed features, including similarity to known effectors, C-terminal translocation signal properties, secondary structure and domain architecture. Particular attention was paid to conserved or functionally interpretable domains that may suggest interactions with host-cell pathways. Experimental assays performed for these candidates provided evidence that at least some plasmid-encoded proteins show properties consistent with Dot/Icm-dependent effector activity and may be translocated to host cells. Together, these results indicate that *Legionella* plasmids are not only diverse extrachromosomal replicons, but may also contribute to the expansion and diversification of the effector repertoire. Plasmid-associated effectors may therefore represent an underexplored link between mobile genetic elements, environmental adaptation and host-pathogen interactions in *Legionella*.

M1.P

Oxford Nanopore-based metagenomic survey of bacterial community restructuring and functional predictions in the rhizosphere of invasive *Impatiens parviflora*

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Słowa kluczowe: *Impatiens parviflora*; rhizosphere microbiome; long-read metagenomics; microbial ecology; plant invasion

Impatiens parviflora is a widespread invasive plant in temperate European forests, yet its influence on rhizosphere microbial communities remains poorly understood. This study applies Oxford Nanopore long-read shotgun sequencing combined with taxonomy-based functional inference to provide initial metagenomic insights into taxonomic shifts and predicted functional potential of bacterial communities associated with this invader. Rhizosphere soil was collected from eight *I. parviflora*-invaded and eight non-invaded control plots in a mixed coniferous forest in northern Poland. DNA was extracted and subjected to Oxford Nanopore shotgun sequencing. Functional potential was inferred from taxonomic composition using the FAPROTAX database. Differences in alpha diversity, taxonomic composition, and predicted functional categories between invaded and control soils were assessed statistically. Bacterial richness was significantly reduced in invaded soils, while Shannon and Simpson diversity indices showed no significant changes, indicating selective loss of rare taxa. At the order level, Bacillales were significantly enriched in the invaded rhizosphere, whereas Burkholderiales and Propionibacteriales were depleted. FAPROTAX-based inference revealed consistently lower predicted abundances of metabolic processes in invaded plots, including pathways related to nitrogen cycling and degradation of complex plant polymers. The observed taxonomic shifts and concordant reductions in inferred functional potential generate testable hypotheses about plant–soil feedbacks that may contribute to the ecological success of *I. parviflora*. The selective enrichment of specific bacterial groups and the predicted decline in nitrogen-cycling processes suggest potential mechanisms by which this invader may alter nutrient dynamics to its advantage. This study highlights the value of long-read metagenomics combined with functional inference for exploring microbial dynamics in plant invasion ecology.

M2.P

The role of PstP phosphatase in the regulation of growth and coordination of the cell cycle in *M. smegmatis*

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Słowa kluczowe: mycobacteria, DivIVA, ParA, PstP phosphatase, chromosome segregation, polar growth

Mycobacterium are characterized by a unique lipid-rich cell envelope that contributes to their ability to survive unfavorable environmental conditions and increases their tolerance to antibiotics. A distinctive feature of mycobacterial cells is their polar and asymmetric growth. Proper growth and progression of the cell cycle require tight coordination between chromosome segregation and cell wall synthesis. Two proteins play central roles in these processes: ParA, involved in chromosome segregation, and DivIVA, which determines polar cell elongation. However, the mechanisms regulating the ParA–DivIVA interaction remain poorly understood. High-throughput transposon mutagenesis (Tn-seq) performed in wild-type and Δ parA strains of *Mycobacterium smegmatis* identified genes showing genetic interactions with parA. A significant fraction of these genes encodes proteins associated with the cell envelope, with particular attention drawn to msmeG_0033, encoding the phosphatase PstP. Mutations in pstP are synthetically lethal in Δ parA background, suggesting a critical role for PstP under conditions of impaired chromosome segregation and disrupted cell cycle coordination. PstP is the major serine/threonine phosphatase in mycobacteria and is responsible for the dephosphorylation of numerous cell cycle-associated proteins, including DivIVA, indicating its potential involvement in the regulation of polar growth and cell division. The aim of this project is to determine how PstP contributes to growth regulation and cell cycle coordination and how it influences the ParA–DivIVA interaction in *Mycobacterium smegmatis*. To functionally validate the results obtained from transposon mutagenesis, and confirm genetic interactions between pstP, parA, and divIVA, strains carrying pstP deletions in different genetic backgrounds are analyzed under various growth conditions [DJ4.1]. In addition, a bacterial two-hybrid system is used to investigate protein–protein interactions, focusing on potential interactions of PstP with ParA and DivIVA. Conservation of the ParA–DivIVA interaction is also examined in *Mycobacterium marinum*. The results will provide new insights into fundamental aspects of mycobacterial biology and may identify potential therapeutic targets against pathogens such as *Mycobacterium tuberculosis*.

M3.P

Transposon mutagenesis identifies genetic interactions between parA and genes involved in cell wall biosynthesis

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Słowa kluczowe: Mycobacterium smegmatis, transposon mutagenesis, cell wall, ParA, essential genes, chromosome segregation

Mycobacterium tuberculosis, the bacterium that causes tuberculosis, is responsible for approximately 1.5 million deaths annually. Mycobacteria are bacteria that are highly resistant to external factors due to their thick and complex cell wall. For this reason, many of the anti-tuberculosis therapies used to date target processes related to cell wall synthesis. The second most common target is the cell cycle, particularly processes related to DNA dynamics. In mycobacteria, as in other bacteria, the ParA ATPase is responsible for chromosome segregation and likely also participates in the coordination of the cell cycle through interactions with other proteins. However, this coordination remains poorly understood, which is why the search for genes that may be involved in it and also interact genetically with parA appears crucial. M. smegmatis was used as a model organism in studies of the mycobacterial cell cycle. To identify genes that may interact genetically with parA, a transposon mutagenesis experiment (Tn-seq) was performed. Tn-seq identified the set of genes involved in cell envelope biosynthesis that may be essential for the M. smegmatis strain lacking parA but not for the wild-type strain. Derivative strains were generated in which the selected genes were silenced using the CRISPR/dCas9 technique, and their growth was then examined under optimal and stress conditions. These analyses confirmed that silencing genes whose products are associated with cell envelopes inhibits the growth of strains lacking ParA. The identified genetic interactions may help elucidate the checkpoints regulating the cell cycle of mycobacteria. In summary, Tn-seq proved to be a powerful method that allows for the simultaneous analysis of the functions of thousands of genes, enabling the identification of genes essential for bacterial fitness.

M4.P

The Effect of *Fusarium graminearum* on the Expression of *Debaryomyces hansenii* Genes

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Słowa kluczowe: biological control, *Fusarium* head blight, wheat, *Debaryomyces hansenii*, transcriptome.

The Effect of *Fusarium graminearum* on the Expression of *Debaryomyces hansenii* Genes
The hemibiotrophic pathogen *Fusarium graminearum* is the causative agent of *Fusarium* head blight (FHB). Current European Union policy calls for a reduced fungicidal plant protection programs. Consequently, there is a need to expand the range of microbial preparations with fungistatic activity. The foliar application of *Debaryomyces hansenii* yeast, a biological control agent (BCA), reduces pathogen pressure. However, its efficacy is limited in some cases for reasons that have not been fully elucidated to date. The aim of this greenhouse experiment was twofold: first, to determine the effect of *D. hansenii* applied to the leaves of durum wheat inoculated with *F. graminearum* on its health status; and second, to analyze the transcriptome of BCA applied to the spikes. Spike samples were collected 48 hours after BCA application and 24 hours after spike inoculation with the pathogen. The application of *D. hansenii* reduced the spread of FHB by 36%, compared with the unprotected treatment with *F. graminearum* inoculation. The low efficacy of biological protection could result from the aggressive and rapid growth of the pathogen, which significantly reduced the expression of 357 *D. hansenii* genes already 24 hours after inoculation, compared with the transcriptome of *D. hansenii* cells cultured in a liquid medium. These genes encode enzymes involved in the tricarboxylic acid (TCA) cycle and the functionally related metabolic pathways, including propionate metabolism, and glyoxylate and dicarboxylate metabolism. Significant changes were also observed in pathways associated with carbon metabolism, glycolysis/gluconeogenesis, pyruvate metabolism, 2-oxyacid and lipid metabolism, as well as the biosynthesis of amino acids and secondary metabolites, and the mitogen-activated protein kinase (MAPK) pathway. The foliar application of BCA may be useful for controlling wheat pathogens; however, due to its low efficacy, it should be supplemented with fungicide protection.

M5.P

Novel Multifunctional Plasmid Toolkit for Gene Expression Studies in *Enterococcus faecium*

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Słowa kluczowe: *Enterococcus faecium*, reporter genes, gene regulation, synthetic biology

Enterococcus faecium is one of the most significant opportunistic pathogens responsible for healthcare-associated infections, as well as the global spread of antibiotic resistance. Despite its clinical relevance, the availability of versatile genetic tools dedicated to enterococci remains limited. To address this challenge, we developed a novel family of multifunctional plasmids based on the broad-host-range shuttle vector pREG45, designed for comprehensive analyses of gene expression and regulatory networks in enterococcal species. The newly constructed plasmid toolkit combines two complementary reporter systems with different variants within a unified experimental framework. First, transcriptional reporter vectors carrying either an optimised luxAB cassette, which requires the addition of an exogenous aldehyde substrate for light production, or the complete luxABCDE operon, which enables autonomous bioluminescence. Second, optimised fluorescent reporter variants based on mCherry and mTFP provide fluorescence signal suitable for population-level analyses and fluorescence microscopy. The additional advantage of these vectors is that they can easily be switched from a low to a high copy number per cell, which is useful for certain applications. Furthermore, the toolkit comprises multiple vector variants carrying different antibiotic resistance markers. This feature is particularly important for enterococci, which exhibit substantial strain-to-strain variability in antibiotic susceptibility profiles, often limiting the applicability of a single selection marker. The major innovation of this project lies in the creation of a versatile and modular platform that enables quantitative investigation of transcriptional and translational regulation in living enterococcal cells. Unlike currently available tools, the developed plasmids offer broad experimental flexibility and can be easily adapted to characterize promoters and regulatory networks involved in stress adaptation, antibiotic resistance, and biofilm development, as well as to monitor bacterial responses to environmental signals in real time.

M6.P

Can we edit the genome of Wolbachia? Strategies for genetic modification of an intracellular bacterium.

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Słowa kluczowe: Wolbachia, Intracellular bacteria, Genome editing, CRISPR/Cas9, Insect cell culture

Wolbachia are one of the most widespread intracellular symbionts found in arthropods and some nematodes. They inhabit host-derived vacuoles which protect them from host immunity in both somatic and germline tissues. Wolbachia are transmitted maternally and induce strong antiviral effect in some of its insect hosts. The introduction of Wolbachia into mosquito populations is being trialed to reduce transmission of arboviruses such as dengue, Zika and yellow fever. However, despite its significant potential, Wolbachia has not yet been genetically modified using currently available genome engineering methods. This lack of genetic tools remains one of the major limitations in Wolbachia research. We aimed to address this limitation and explored multiple strategies for genetic modification of Wolbachia, including CRISPR-based approaches. Using electroporation, transformation and lipofection of plasmid DNA, donor DNA and Cas9-based RNP complexes supplemented with single-stranded DNA-binding protein, we attempted to establish stable populations of modified Wolbachia in an insect cell line system. Our results highlight key technical challenges and biological barriers associated with delivery and selection, and provide a foundation for the development of effective genetic tools for this symbiont.

M7.P

Comparative genome characterisation of *Mycobacterium bovis* BCG PCM 2097 and PCM 2098

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Słowa kluczowe: *Mycobacterium tuberculosis* variant *bovis*, BCG, SNP, phylogenetic analysis

Mycobacterium bovis is a group of bacteria belonging to the *Mycobacterium tuberculosis* complex. They are aerobic, Gram-positive rod-shaped bacteria characterized by a thick cell wall composed mainly of lipids and mycolic acids. The natural reservoirs of *Mycobacterium bovis* include wild animals such as deer, wild boar, and badgers, as well as domestic animals such as cattle, goats, and cats. They are also isolated from humans. *Mycobacterium tuberculosis* is the etiological factor of tuberculosis. According to the WHO, 1.23 million people died of tuberculosis in 2024. *Mycobacterium bovis* BCG strain is a component of the tuberculosis vaccine obtained by attenuating the *Mycobacterium bovis* strain. Work on the production of the vaccine contributed to the creation of about 50 different substrains of *Mycobacterium bovis*, including BCG-Pasteur, Moreau, Tokyo and Danish. The study investigated two strains of *Mycobacterium bovis* BCG deposited in the Polish Collection of Microorganisms under accession numbers PCM 2097 and PCM 2098. PCM strains were sequenced on the Illumina MiSeq system, and genomes were mapped to the reference genome *M. bovis* AF2122/97 and compared with the Moreau (ASM2875223v1), Pasteur (ASM944v1), Danish (ASM515610v1), Russia (ASM127455v1), and Tokyo (ASM158038v1) isolates. Preliminary SNP distance analysis among the studied genomes reveals population structure of broad but coherent clusters characterized by moderate genomic diversity, with pairwise SNP distances ranging approximately from 22 to 115 SNPs across most comparisons. The closest relationships are observed between PCM 2097 and *M. bovis* BCG Moreau (29 SNPs) and between PCM 2098 and *M. bovis* BCG Pasteur (22 SNPs), suggesting tighter phylogenetic links within this cluster. Research findings indicate potential subclusters within a larger evolutionary lineage; however, definitive conclusions about phylogenetic relationships require confirmation via whole-core genome alignment-based phylogenetic reconstruction.

M8.P

NanoRNases in *Mycobacterium tuberculosis* as Promising Molecular Targets for the Development of New Antituberculosis Therapies

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Słowa kluczowe: *Mycobacterium tuberculosis*; nanoRNA; Orn(Rv2511); NrnA(Rv2837c)

Tuberculosis, caused by *Mycobacterium tuberculosis* (MTB), remains a major global public health challenge despite the availability of effective treatment regimens. According to the WHO, tuberculosis incidence remains high, and drug-resistant strains are a particular concern. Therefore, alongside improved diagnostics and therapies, new drugs with different mechanisms and novel molecular targets are needed. A key process in bacterial cell function is the control of short RNA-derived products. During RNA maturation and degradation, very short oligonucleotides, known as nanoRNAs, are generated. These molecules can affect RNA and DNA metabolism, for example by initiating transcription and shifting transcription start sites, thereby altering the transcriptomic profile. Their accumulation may also disturb nucleotide pool balance. For this reason, bacteria use specialized enzymes that degrade nanoRNAs into mononucleotides. Literature data and *in silico* analyses indicate that two enzymes may perform this function in *M. tuberculosis*: oligoribonuclease Orn, encoded by Rv2511, and Rv2837c, a DHH–DHHA1 family protein referred to as NrnA. The putative involvement of Rv2837c in both c-di-AMP metabolism and short oligoribonucleotide substrate hydrolysis suggests a possible link between RNA degradation products and nucleotide signaling. In our study, utilizing homologous and site-specific recombination processes through the construction of directed mutants, we verify the essentiality of each of the aforementioned genes, as well as both genes simultaneously, in *Mycobacterium tuberculosis*. We have also expressed and purified the recombinant proteins Orn and NrnA, and evaluated their enzymatic activity. The purified enzymes will be used to develop a high-throughput *in vitro* screening assay to identify inhibitors of these enzymes. From a drug discovery perspective, Orn and Rv2837c are promising molecular targets. Both proteins contain metal-dependent active sites and defined substrate-binding pockets, while available structural data may support inhibitor design and interpretation of screening results.

M9.P

Influence of DNA extraction method on ITS2-based fungal identification

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Słowa kluczowe: fungi, microbiome, ITS, sequencing

Background: The chicken intestine is colonized by beneficial bacteria that contribute to host homeostasis. Although the role of fungi is less understood, they are likely to have similar functions. The identification of fungi from the chicken caecum using ITS2 (Internal Transcribed Spacer) sequencing remains challenging. Since the ITS region enables identification of not only fungi but also plants and protists, the selection of specific primers is crucial. Additional issues are the presence of inhibitors, high diversity of fungal taxa, and their resistant cell walls, which make them more difficult to extract than bacteria. Therefore, efforts should focus on selecting the best DNA extraction method. The aim of this study is to compare four extraction approaches for fungi taxonomic identification. Materials and methods DNA was extracted from chicken caecum (n=4) using four different approaches: Maxwell Tissue DNA Kit, Promega (P); QIAamp PowerFecal Pro DNA Kit, Qiagen (Q), and QIAamp with additional enzymatic treatment of lyticase (Q-L) and proteinase (Q-P). The ITS2 region was amplified with fITS7/ITS4 primers, and Nextera indexes were added. Samples were sequenced on Illumina MiSeq (2x250). Data analysis was prepared in Qiime2 with UNITE database.

Results: Based on the alpha-rarefaction plot, the highest number of observed features was in the Q-L approach, and the lowest was in the P approach. Similar results were observed in alpha-diversity analyses: observed_features, Shannon_entropy, and Pielou_evenness. Proteinase does not seem to significantly influence the mycobial diversity compared to the Q approach. Some fungal genera were identified exclusively in one tested approach.

Conclusions: DNA extraction and enzyme treatment influence fungal identification using ITS2 analysis. Every method introduced bias; thus, no method can recover every taxon present in the sample. However, the highest sample diversity and taxon recovery were observed with the Q approach using lyticase treatment. Thus, this method seems to be a good choice for following up mycobiome studies.

M10.P

Nutrient-dependent remodelling of the proteome and lysine acetylation in *Rhizobium leguminosarum* bv. *trifolii* TA1: a preliminary study

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Słowa kluczowe: lysine acetylation; acetylation; rhizobia; GNAT; exopolysaccharide; nutrient adaptation

Lysine acetylation is increasingly recognised as a regulatory layer connecting bacterial metabolism with environmental adaptation, including symbiotic interactions with plants. Previous transcriptomic studies showed extensive responses of *Rhizobium leguminosarum* bv. *trifolii* TA1 to nutrient limitation and acidic stress, while a genome-wide screen identified numerous putative GNAT-family acetyltransferases, including stress-responsive candidates. This prompted us to ask whether nutrient availability is reflected at the level of protein acetylation. RtTA1 was cultivated in nutrient-rich 79CA or defined M1 minimal medium. Global proteomes and anti-acetyl-lysine-enriched fractions were analysed by DIA mass spectrometry. Signals from acetylated lysines were interpreted together with changes in corresponding protein abundance. The first biological replicate yielded more than 5,500 protein groups in each condition and revealed extensive proteome remodelling in M1. The strongest response involved nitrogen acquisition and assimilation, including urea and ammonium transport, PII-dependent regulation and glutamine synthesis, together with changes in central carbon metabolism. Enrichment increased detection of acetylated lysine (Kac) sites more than threefold and revealed widespread, often multisite acetylation. In total, 5,670 non-contaminant Kac sites were identified, including 3,209 shared between conditions. Integration with the global proteome showed that many apparent Kac changes reflected altered protein abundance, whereas others represented site-specific regulation. Prominent candidates included RpoD K464, HtpG K220 and ClpP K153. Among 61 detected GNAT protein groups, most changed only moderately, suggesting selective rather than coordinated regulation. All 22 analysed proteins encoded by genes within the Pss-I exopolysaccharide biosynthesis region were present in both conditions; 15 carried at least one Kac site, with PssZ K256 and PssP K749 emerging as interesting candidates. These preliminary data indicate that nutrient availability reshapes both metabolism and lysine acetylation in RtTA1. Ongoing replicate analyses and functional studies will test selected GNAT proteins and Kac sites in stress adaptation, exopolysaccharide regulation and symbiosis with plants.

M11.P

Novel M97 proteases from Bacteroidota phylum

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Słowa kluczowe: proteases, Bacteroidota

We have recently identified a gene coding for a novel periplasmic M97 protease, zuzalysin, in the genome of *Porphyromonas gingivalis*, a distant homologue of toxilysin from *Escherichia coli*. The gene was cloned and characterized both biochemically and structurally, revealing a calcium-dependent enzyme that very slowly degrades folded proteins. Moreover, structural analysis of recombinant zuzalysin demonstrated a remarkable tendency to form oligomeric assemblies. However, despite these intriguing in vitro properties, the biological significance of zuzalysin in *P. gingivalis* remains unclear. To address this knowledge gap, we generated an isogenic zuzalysin deletion mutant, as well as double-mutant strain lacking both zuzalysin and the periplasmic HtrA protease. These strains were subjected to temperature stress, acidic stress, and nutrient-limitation conditions. Furthermore, analysis of bacterial genomic databases revealed that proteases of this type are widespread across the Bacteroidota phylum. Most species possess a single homologue; however, in some genera we observed extensive expansion of this protein family, with up to 22 paralogues per genome. Interestingly, some of these expanded homologues differ from *P. gingivalis* zuzalysin: they changed the subcellular localization and some lost the catalytic motif while retaining the overall catalytic-domain architecture. This finding suggests that these paralogues may have acquired additional regulatory functions.

M12.P

Insight into the interactome and regulon of orphan histidine kinase CpkM in *Streptomyces coelicolor* A3(2)

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Słowa kluczowe: Two-component systems, Protein-protein interaction, Omics, Regulatory Networks, Regulone, Interactome

Histidine kinases in *Streptomyces* have been demonstrated to influence a number of key processes, including the production of specialized metabolites, as well as morphological development and cell survival responses. *Streptomyces* are soil-dwelling bacteria of significant industrial importance, serving as a source of antibiotics and other therapeutic agents. Expression of the biosynthetic gene clusters (BGCs) is regulated by a variety of regulatory systems, including two-component systems comprising histidine kinases (HKs) and cognate response regulators (RRs). Diverse environmental stimuli can trigger HKs to autophosphorylate and subsequently transfer the phosphoryl group to RR. This post-translational modification alters the properties of RRs, for example, activating DNA binding activity, thereby regulating transcription activation. Usually, a cognate response regulator locus is placed next to a HK gene, yet in *S. coelicolor* 33 of 100 HKs do not have such cognate RRs, therefore they are named "orphan histidine kinases". CpkM is one of the orphan kinases. Its locus is directly between the gamma-butyrolactone (GBL) BGC, and coelimycin (CPK) BGC. CpkM expression is correlated with the whole cpk cluster under influence of quorum sensing system responsive to GBLs. The objective of this study was to elucidate the function of CpkM in the physiology of *S. coelicolor* A3(2). The work consist of protein-protein interaction studies, proteomics, in vivo promoter activity luciferase assay and a detailed phenotypic analysis of mutants on diverse media. In the present study we show that CpkM positively regulates development and antibiotic synthesis. Δ cpkM mutant exhibits delayed aerial mycelium formation. Furthermore, lowered yields of polyketides production were detected. The observed phenotypic changes are supported by proteomics and promoter-activity data. Possible RRs of CpkM were initially selected via interaction in the two-hybrid system.

M13.P

Sialic acid specificity of Erythrocyte Binding Antigen 140 orthologues in human- and ape infective *Laverania* species

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Słowa kluczowe: malaria, *Plasmodium*, EBA-140 ligand, erythrocyte invasion

There are an estimated 260 million malaria cases and up to 600,000 deaths per year caused by *Plasmodium* parasites, among which the most malignant species, *P. falciparum*, belongs to the *Laverania* subgenus. *Plasmodium* parasites within the *Laverania* subgenus infect both humans and African great apes, and are largely host-specific. The factors that control host-specificity are likely multifactorial, but the ability of merozoites to distinguish among erythrocytes through the specificity of invasion ligands is thought to be one possible mechanism. The Erythrocyte Binding Antigen-140 (EBA-140) is one of the ligands involved in erythrocyte recognition, belongs to the erythrocyte binding-like (EBL) protein superfamily, and the genomes of all *Laverania* species contain its orthologue. The human-infected *P. falciparum* EBA-140 ligand has been shown to bind to the erythrocyte sialoglycoprotein Glycophorin C, while the chimpanzee-infected *P. reichenowi* ligand has been shown to recognise its truncated form Glycophorin D, both in a sialic acid-dependent manner. Using the CRISPR-Cas9 method, we generated transgenic *Plasmodium falciparum* lines in which the PfEBA-140 binding region was replaced with the corresponding region from *P. praefalciparum*, the closest ancestor of *P. falciparum*. Since *P. praefalciparum* naturally infects gorillas rather than humans, these chimeric lines provide a valuable model for investigating host-specific receptor recognition. We also generated *P. falciparum* EBA-140 knock-out lines. The modified protein was colocalised using immunofluorescence microscopy, and the lines were used in invasion and erythrocyte binding assays. We observed no significant differences between modified lines and wild-type lines in both assays, which indicates that EBA-140 is not a key ligand in the erythrocyte recognition by *P. falciparum*, as even knockout of EBA-140 does not alter the level of invasion. The process of erythrocyte invasion is complex and involves a number of different proteins and their receptors, which can function interchangeably and complement each other when one is missing.

M14.P

Autoregulation of the biosynthetic gene cluster by intracellular coelimycin precursors in *Streptomyces coelicolor* A3(2)

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Słowa kluczowe: Gene expression regulation, Secondary metabolism, Specialized metabolites, Regulatory networks, Polyketide biosynthesis

Coelimycin (CPK) producer, soil filamentous bacterium *Streptomyces coelicolor* A3(2), is a model organism for the genetic studies of the bacterial genus comprising producers of the majority of currently known antibiotics and other secondary/specialized metabolites (SMs) of medical and industrial importance. *Streptomyces* genomes contain dozens of biosynthetic gene clusters (BGCs), the majority of which remain „silent” under standard laboratory conditions. Coordination of the BGCs expression with the colony development and ensuring appropriate response to environmental pressures is provided by a multilevel regulatory networks made of hundreds of regulatory proteins, ncRNA, small signalling compounds such as gamma-butyrolactones, as well as more complex metabolites, which can be ligands of transcription factors or riboswitches. CPK biosynthesis starts with the formation of an unsaturated 12-carbon polyketide chain, which then undergoes several post-polyketide enzymatic modifications, first inside the cell, leading to an amine derivative, which is exported by the CpkF membrane pump. In the next step, extracellular enzymes perform double epoxidation giving rise to a bis-epoxide compound with slight antibiotic activity (abCPK). Non-enzymatic reactions of abCPK with N-acetylcysteine or glutamate present in the medium lead to the formation of coelimycins P1 or P2, respectively, which are both yellow pigments (yCPK). Here, we present the evidence that intracellular CPK precursor(s) (preCPK) is involved in a negative feedback loop repressing the CPK BGC. This conclusion was based on the comparison of the promoter activities in the luciferase based reporter system in two mutant strains: Δ cpkF lacking only the extracellular CPK variants due to the transporter gene deletion and Δ cpkC unable to produce preCPK due to the knockout of one of the main polyketide synthase subunits.

M15.P

Evolutionary dynamics of secondary metabolite gene clusters in *Fusarium graminearum*

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Słowa kluczowe: *Fusarium graminearum*, secondary metabolites, positive selection, purifying selection, adaptive evolution

Secondary metabolite (SM) biosynthetic gene clusters contribute to the ecological adaptation and pathogenicity of filamentous fungi, yet the evolutionary processes shaping their diversity within species remain insufficiently understood. We investigated the diversity and evolution of SM biosynthetic genes in European populations of *Fusarium graminearum* and across the *F. graminearum* species complex. Using a combination of pangenome analysis, population genomics, phylogenetics, and codon-based evolutionary models, we examined patterns of genetic variation and selective pressures acting on SM biosynthetic genes. The composition of SM biosynthetic gene repertoires reflected the established population structure of *F. graminearum*. Genetic variation was strongly population-dependent, with 105 of 122 population-specific SNPs identified in the East European population, while no population-specific SNPs were detected in the West European population. Although most SM backbone genes were subject to purifying selection, codon-based analyses detected positively selected sites in 19 of 57 SM gene clusters. Our findings demonstrate that secondary metabolism in *F. graminearum* is shaped by the interplay between strong purifying selection maintaining essential biosynthetic functions and localized adaptive evolution acting on individual amino acid residues. These patterns suggest that population-specific selective pressures contribute to the ongoing diversification of fungal secondary metabolism.

M16.P

Molecular Basis of DivIVA-Driven Polar Growth in *Streptomyces venezuelae*

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Słowa kluczowe: *Streptomyces venezuelae*; DivIVA; polar growth; bacterial cell polarity; polarisome; membrane curvature; lipid–protein interactions; X-ray crystallography.

Streptomyces venezuelae is a filamentous Gram-positive bacterium with a complex life cycle spanning vegetative growth, aerial mycelium formation, and sporulation. Beyond serving as a model for bacterial development, *Streptomyces* are prolific producers of antibiotics, antifungals, and antitumor compounds. Yet, despite their growth depending on polarized hyphal extension, how this polarity is established and maintained remains poorly understood. Polar growth in *Streptomyces* is coordinated by the polarisome, protein assemblies localized at hyphal tips and newly established branch sites. The essential coiled-coil scaffold DivIVA is a central component of these complexes and is proposed to accumulate at sites of negative membrane curvature. While DivIVA localization has been linked to membrane curvature, the molecular basis of this interaction remains unknown — whether curvature sensing alone, or specific lipid–protein interactions, drives its recruitment and activity at growth zones. To address this, we solved a high-resolution X-ray structure of the DivIVA N-terminus and, with Prof. Claessen, performed an alanine screen identifying key residues required for membrane binding. This work provides a first step toward decoding how DivIVA's membrane interactions underpin the spatial organization of *Streptomyces* growth.



B1.0

From gut to brain: The impact of bacterial functional amyloids on neurodegeneration

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Słowa kluczowe: neurodegenerative diseases, microbiome, amyloid, protein, Parkinson's disease

Amyloids are insoluble protein aggregates with a cross- β structure traditionally associated with neurodegenerative diseases. Functional amyloids, expressed predominantly by microorganisms, play important physiological roles, including bacterial biofilm stabilization. Using a bioinformatics approach, we identified functional amyloids in the human gut microbiome and investigated their potential impact on human health through the gut-brain axis. We detected a highly diverse set of putative bacterial functional amyloids across seven bacterial phyla and examined their possible involvement in gut-brain axis signaling. We further showed that the gut microbiomes of patients with Parkinson's disease (PD) contained a higher abundance of bacterial functional amyloids than those of healthy controls and patients with cerebral meningitis (CM) in two of the three analyzed datasets. Our findings reveal taxonomically diverse sources of functional amyloids and indicate that these proteins are frequently localized in the extracellular space. Predicted interactions between gut microbiome functional amyloids and human proteins suggest their potential to trigger inflammatory responses and modulate transport and signaling pathways. Furthermore, the structural similarity between bacterial functional amyloids and pathological human amyloids may facilitate molecular mimicry, thereby influencing the aggregation kinetics of pathological amyloids or activating common molecular pathways. For example, curli interacts with α -synuclein and A β both in vivo and in vitro, while inhibition of curli aggregation reduces neuronal death. Moreover, the curli precursor is recognized by the human immune system similarly to A β 42 because of shared structural features. The amyloid proteins identified in our

study are approximately 20-fold more likely to be extracellular than other bacterial proteins, increasing their potential to interact with human proteins. Consequently, interactions between bacterial functional amyloids and human proteins may occur on a much broader scale than previously appreciated. Overall, our results identify bacterial functional amyloids as an important, previously underrecognized component of the gut–brain axis.

B2.O

Evaluating Dimensionality Reduction Techniques and Neural Network Architectures for High-Dimensional, Small-Sample Microbiome Classification

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Słowa kluczowe: Deep Learning, Dimensionality Reduction, Microbiome

Deep learning (DL) serves as a dominant framework for pattern recognition, yet reliable pattern detection in biological data remains inherently difficult due to noise, sparsity, and complex feature dependencies. Microbiome datasets frequently present zero-inflated, compositional structures characterized by $p \gg n$ problem. Traditional statistical methods often struggle to capture higher-order patterns. Dimensionality reduction techniques (DRTs) and data-driven DL embeddings allow for projection of high-dimensional profiles into lower-dimensional latent spaces. This study evaluates and compares the classification performance of various combinations of traditional and DL-based DRTs paired with shallow (SNN) and deep (DNN) neural network architectures on sparse, high-dimensional, small-sample microbiome data. Utilizing a 16S rRNA sequencing dataset consisting of 124 intestinal samples from common carp (*Cyprinus carpio*) distributed across five effective microorganism supplementation schemes, the data comprised 126 bacterial families, representing a near one-to-one feature-to-sample ratio. Centered Log-Ratio (CLR) transformation was applied to handle the compositional nature of the data. Five distinct DRTs; Principal Component Analysis (PCA), Singular Value Decomposition (SVD), Non-negative Matrix Factorization (NMF), 1-D convolutional layers (Conv), and Autoencoders (AE), were implemented and paired with SNN and DNN classifiers. Performance was benchmarked against a random assignment null hypothesis and an XGBoost baseline. For the 5-class classification task, the highest test accuracy of 0.76 was achieved by both DNN and SNN architectures when combined with SVD, as well as by the SNN trained on unreduced features. NMF performed poorest (0.40 DNN, 0.44 SNN) due to its incompatibility with the value shifts required post-CLR transformation. Cross-validation highlighted that models omitting DRTs delivered the most stable and consistent performance (mean accuracy ~ 0.60), implying that small sample sizes can limit DRT generalizability. Deeper architectures showed no consistent performance advantage over shallow networks under these small-sample constraints. Our study shows that DL architectures can outperform more traditional machine learning approaches and that Dimensionality reduction can help extract meaningful patterns from microbiome data.

B3.O

Comparative evaluation of AVITI, MGI and Illumina NGS Platforms for Molecular Oncology Diagnostics

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Słowa kluczowe: NGS, bioinformatics, sequencing, somatic, germline

Background: Next-generation sequencing (NGS) has become an essential component of molecular oncology diagnostics, supporting cancer prevention strategies, precise diagnosis, and personalized therapeutic decision-making. Although Illumina systems are the major sequencing platform the current standard in clinical diagnostics, novel technologies such as AVITI and MGI are increasingly being evaluated as alternative approaches for molecular oncology applications.

Aim: In this study, we evaluated and compared the analytical performance of different NGS platforms, including Illumina MiniSeq (2016) and MiSeq i100 plus (2025), MGI DNBSEQ-G99 (2022), and AVITI (2022), for the detection of germline and somatic variants in targeted molecular oncology diagnostics.

Materials and Methods: We sequenced 11 somatic and 16 germline samples using four different NGS platforms (Illumina, MGI, AVITI) according to the manufacturers' protocols. Germline NGS libraries were performed using the Lynch Syndrome Detection Kit (SpaceGene) and somatic libraries using 3DMed Oncocore Tissue Kit (3DMed Diagnostics). Subsequently, single nucleotide variants (SNVs) and insertion-deletion (indels) were identified for all samples in order to compare the analytical performance across the sequencing platforms.

Results: All clinically relevant variants were consistently detected across all sequencing platforms. The highest stability of read quality across sequencing cycles was observed for MGI and MiSeq i100 Plus platforms, while AVITI generated the highest overall read quality (~Q40). Significant inter-platform differences were observed in sequencing workflow parameters, including turnaround time, throughput, compatibility with bioinformatic analysis software, and raw data conversion pipelines. These differences may substantially impact routine implementation in molecular diagnostic laboratories and should be considered during platform selection.

Conculsions: Our results demonstrate that regardless of whether an older-generation system such as MiniSeq or newer platforms are used, all evaluated NGS platforms provide high-quality sequencing data and sufficient amounts of coverage that are suitable accurate and reliable diagnostic results. The oldest system MiniSeq, showed slightly lower quality metrics - without impact on diagnostic validity.

B4.O

Comparison of Hyperparameter Optimization Tools in Deep Learning Using Autoencoders in the context of Classification

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Słowa kluczowe: AutoEncoder, classification, Deep Learning, hyperparameter tuning, Multilayer Perceptron

This study presents a comparison of selected hyperparameter optimization (HPO) tools in Deep Learning, exemplified by classifying aquaculture ponds. The analysis was carried out for two models: a classical Multilayer Perceptron (MLP) trained directly on a feature vector, and an AutoEncoder based model, in which the network simultaneously reconstructs the input data and performs classification based on the representation learned in the bottleneck layer. The comparison was performed for a baseline approach with manually selected hyperparameters as well as for automatic tuning using: KerasTuner (Random Search and Hyperband), Optuna (with and without pruning), and Ray Tune (with and without ASHA scheduler). The evaluation of the approaches considered three practical criteria: classification performance, total tuning time, and maximum memory usage. For the MLP model, the best validation accuracy was used as the main quality metric, while for the AutoEncoder additional analyses included the individual components of the loss function (classification and reconstruction) as well as the distributions of reconstruction errors. All experiments were executed in the same computational environment using a CPU, which enabled a fair comparison of computational costs under unified conditions. The results indicate that automatic hyperparameter optimization improves the performance of both models; albeit the magnitude of the improvement is moderate. At the same time, the HPO methods differ significantly in terms of computational cost. Hyperband proved to be the most time-consuming approach, Optuna achieved the best classification results but, in the AutoEncoder case, was associated with very high memory consumption. Ray Tune, especially when combined with ASHA (Asynchronous Successive Halving Algorithm), provided a favorable trade-off between computational time and model quality. Interestingly, the AutoEncoder model revealed that excellent reconstruction quality doesn't guarantee optimal classification performance, highlighting the importance of evaluating both aspects concurrently.

B5.O

Application of molecular dynamics for the analysis of selected peptide groups

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Słowa kluczowe: chloroplast transit peptides, mitochondrial transit peptides, signal peptides, molecular dynamics, three-dimensional structure

Understanding peptide function is essential for developing target-directed therapeutics and elucidating the underlying mechanisms of various diseases. Accordingly, this study employs molecular dynamics simulations to characterize the structural and dynamic profiles of selected peptide families. Three distinct groups with known amino acid sequences were investigated: chloroplast transit peptides, mitochondrial transit peptides, and signal peptides. Initial three-dimensional structural predictions were generated using AlphaFold 3, which subsequently served as starting conformations for 25-nanosecond molecular dynamics simulations performed in GROMACS. The resulting trajectories were evaluated based on five structural metrics: potential energy, root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), and intramolecular hydrogen bonding patterns. Our findings reveal that these distinct peptide groups exhibit divergent dynamic and conformational behaviors, directly correlating with their specific biological roles. This study highlights the power of integrating predictive bioinformatics with structural data, establishing a promising computational framework for classifying and differentiating diverse peptide families in future functional studies.

B6.O

Changes in codon usage during evolution of SARS-CoV-2 genomes

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Słowa kluczowe: SARS-CoV-2, codon usage bias, genetic code

Since the emergence of SARS-CoV-2 in late 2019, the adaptation of its codon usage to the human host has been a critical area of study. Because viral fitness depends on rapid replication and efficient protein synthesis, evolutionary pressures naturally favour mechanisms that optimize translational efficiency and fidelity. This study aimed to identify temporal and gene-specific shifts in synonymous codon usage to determine whether viral evolution increasingly mirrors human translational machinery. We analysed 84,324 SARS-CoV-2 genomes collected between January 2020 and October 2024, categorizing all protein-coding sequences into six groups based on human synonymous codon frequencies. Our analysis revealed diverging evolutionary trajectories across functional gene categories: structural genes developed a bias toward less optimal (infrequent) human codons, whereas non-structural genes (ORF1a and ORF1ab) progressively shifted toward more optimal (frequent) codons. Accessory genes displayed variable usage patterns. These findings suggest that SARS-CoV-2 codon adaptation is a dynamic, gene-specific process driven by a critical evolutionary trade-off between maximizing replication efficiency and evading the host immune response.

B7.O

SNP Analyser: a website tool for rapid bioinformatic interpretation of mitochondrial DNA variants

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Słowa kluczowe: mtDNA, SNP, indel, bioinformatic tool, genome

Mitochondrial DNA (mtDNA) is widely used in forensic and biomedical research due to its compact circular structure, conserved gene content, high copy number, and central role in oxidative phosphorylation. Although several resources support mitochondrial variant annotation, many available tools are restricted to a single organism, require specific input formats, or do not provide an integrated workflow for mutation effect prediction on protein secondary structure. To address these limitations, we developed SNP Analyser, an application for rapid analysis of mtDNA. The tool currently supports reference mtDNA of selected species, including *H. sapiens*, *C. lupus familiaris*, *S. scrofa*, and *B. taurus*. Reference records were retrieved from GenBank/RefSeq and stored locally for reuse, while gene annotations are parsed directly from GenBank feature tables. Users can select an organism and mitochondrial gene, inspect reference and editable nucleotide sequences in parallel, and introduce variants either manually or through batch input using simplified HGVS notation. The current parser supports single nucleotide substitutions, deletions, and insertions. Variants are represented internally by mutation type, position, reference nucleotide, and alternative sequence. Reference and edited sequences are translated using the vertebrate mitochondrial genetic code. For substitutions and indels located within complete codons, SNP Analyser determines codon context, nucleotide position within the codon, original and altered amino acids, and classifies the predicted effect. The generated report includes mutation count, nucleotide changes, coding interpretation, and comparative amino acid translations. SNP Analyser therefore integrates reference retrieval, gene annotation, effect prediction, and optional peptide characterisation in a single browser. The application is suitable for rapid exploratory analysis of mitochondrial variants and may be useful for screening of changes noted in the mtDNA. The tool is available for free on the website: <https://snpanalyser.com/>

B8.O

A Reproducible Bioinformatic Workflow for Nanopore-Based Bacterial Methylome Analysis

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Słowa kluczowe: Oxford Nanopore sequencing; bioinformatic workflow; methylation motif detection; genome annotation; *Streptomyces coelicolor* A3(2); 5mC; 6mA; 4mC; bacterial methylome

Oxford Nanopore Technologies (ONT) sequencing enables direct detection of modified DNA bases from sequencing signal, making it a powerful approach for bacterial methylome analysis. The ONT sequencing enables direct detection of DNA modifications from raw sequencing signal, making it a powerful approach for bacterial methylome analysis. However, reliable interpretation of methylation data requires a structured bioinformatic workflow, including read processing, alignment, modification calling, statistical filtering, motif identification, genomic annotation, and functional analysis.. The aim of this work was to establish a reproducible bioinformatic workflow for genome-wide analysis of bacterial DNA methylation using *Streptomyces coelicolor* A3(2) as a model organism. ONT sequencing data from three developmental time points, 18 h, 27 h, and 50 h, were processed to analyse 5mC, 6mA, and 4mC methylation. The workflow included sequencing quality control, read alignment to the reference genome, modified base processing, position-level methylation frequency extraction, statistical filtering of differentially methylated positions, motif detection, genomic annotation, and visualization. Methylated positions were assigned to promoter and ORF regions and further linked with biosynthetic gene clusters and KEGG pathway information. Custom scripts were used to organize outputs, compare methylation between time points, and generate summary tables and plots. The workflow enabled identification and annotation of 6646 differentially methylated positions across developmental time points. The pipeline further enabled extraction of methylation changes in selected biosynthetic gene clusters, including ACT, RED, CDA, and CPK, and supported functional interpretation through KEGG-based mapping. This workflow provides a practical framework for analysing bacterial methylomes from ONT sequencing data and can be adapted to other prokaryotic genomes.

B9.O

Exploratory DNA methylation profiling uncovers subgroup heterogeneity in BCOR-altered central nervous system tumors.

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Słowa kluczowe: BCOR alterations, DNA methylation profiling, CNS tumors, Pediatric brain tumors

The BCL6 corepressor (BCOR) gene, located on the X chromosome, acts as a tumor suppressor for regulating cell differentiation and body structure development. Alterations in BCOR give rise to a group of rare central nervous system (CNS) neoplasms, recognized as distinct molecular entities based on their genetic, histopathological and immunophenotypic features. Two main types of alterations have been described: internal tandem duplications (ITDs) within exon 15 and gene fusions, including EP300::BCOR and BCOR::BCORL1. These two types define distinct patient groups. BCOR ITD tumors predominantly affect pediatric patients (median age 3.2 years, male-to-female ratio 1.13:1) and are associated with frequent recurrence, variable treatment response, and poor overall survival, although long-term survivors have been documented. In contrast, BCOR fusion-positive tumors occur in older patients (median age 30 years), with a female predominance. DNA methylation arrays allow for a global assessment of the methylation profile and exploration of regions showing the greatest variability. Reflecting the growing relevance of this approach, the machine learning-based Heidelberg/DKFZ classifier (Epignostix) has expanded from 91 CNS tumor classes in 2018 to 184 subclasses by 2025. This study analyzes publicly available methylation profiles from the Gene Expression Omnibus repository (GSE211156), supplemented by 7 institutional cases with confirmed BCOR ITD and 2 cases harboring EP300 and BCORL1 fusions, yielding a cohort of 46 cases. Although clear differences exist between the methylation profiles of BCOR ITD and fusion-positive tumors, unsupervised hierarchical clustering revealed a distinct subgroup within each group. To characterize it at the molecular level, differential methylated region (DMR) and pathway enrichment analyses were performed. This study is particularly relevant because both BCOR fusion-positive and BCOR-ITD tumors show variability in clinical course and outcome, suggesting that the current molecular classification may not capture all prognostically relevant subgroups. This research was funded by the Medical Research Agency, project BraimTOR (2021/ABM/01/00027).

B10.O

Attention-Based Imputation of Structural Variants: Genotype Prediction in Low-and High-LD Genomes

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Słowa kluczowe: Attention, Deep Learning, Genotype Imputation, Linkage Disequilibrium, Structural Variant, Transformer

Structural variants (SVs) affect more base pairs than single-nucleotide variants and are disproportionately represented among quantitative trait loci, yet comprehensive genotyping typically requires high-coverage long-read sequencing that is costly. Imputation is a practical alternative, but its accuracy may depend on the Linkage Disequilibrium (LD) regime of the target population. We examined this dependence by benchmarking imputation on two contrasting panels: the human 1000 Genomes Phase 3 SV callset (2,504 individuals; weak inter-SV LD) and a cattle HiFi long-read cohort (117 bulls of Braunvieh/Brown Swiss origin, 29 autosomes; strong LD). A compact windowed Transformer (146,755 parameters), trained by masked imputation with a class-weighted focal loss, was compared with a frequency baseline, a k-nearest-neighbour predictor, the Hidden Markov Model imputer Beagle 5.5, and the livestock imputer AlphaImpute2. All methods were evaluated on the same masked genotypes, using a relatedness-aware split in cattle. In the low-LD human panel, the Transformer reached the highest balanced accuracy (0.665, versus 0.602 for Beagle and 0.559 for the frequency baseline) and recovered more heterozygous carriers (0.52 versus 0.21). In the strong-LD cattle panel, the methods converged, with the Transformer nominally highest (0.670) and Beagle essentially tied (0.666). Quality control influenced performance more strongly than algorithm choice. Restricting the evaluation to read-supported genotypes lowered the apparent balanced accuracy of Beagle from 0.819 to 0.666, suggesting that part of its advantage reflected homozygous calls rather than imputation accuracy. The highest accuracy in cattle was obtained not with an SV-only imputer but by imputing SVs from flanking SNP haplotypes (balanced accuracy 0.803), requiring only SNP genotyping. These results suggest that attention-based models may be particularly advantageous in weak LD, while under strong LD, quality control and SNP information influence accuracy more than the choice of imputer.

B11.O

PLegio: app for comparative visualisation of Legionella genus plasmidome

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Słowa kluczowe: legionella, plasmid, nextflow, bioinformatics, microbiology

Plasmids – accessory genetic elements carrying virulence and resistance genes – are important vectors of horizontal gene flow in microbial communities. Despite this function, their coverage in user-friendly visual databases is often limited or poorly structured. Existing tools generally aim to cover all available bacterial data, which can hinder analyses focused on specific taxa and complicate systematic updates. To address this issue within the Legionella research community, we developed PLegio, a web-based application for interactive visualisation of the Legionella plasmidome. The app is designed to analyse available genomic data in a single, automated pipeline, followed by presentation through a clear, responsive interface. This architecture enables efficient, systematic updates using covered genomic databases and our own data. The app allows users to investigate plasmid-encoded genes, their functions, their genus prevalence and their localisation on a plasmid. Additionally, the application performs effector prediction and highlights candidate effector genes potentially involved in host infection. At present, the application covers all 170 publicly available valid Legionella plasmid sequences. PLegio aims to support Legionella focused research by facilitating presentation of plasmids diversity and improving understanding of plasmidome genes role in infection. In addition, its effector prediction functionality may provide additional insights into potential therapeutic targets.

B12.O

Alternative splicing in triple-negative breast cancer: from patient biopsies to patient-derived xenograft models

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Słowa kluczowe: triple-negative breast cancer, alternative splicing, transcriptomics, patient-derived xenografts, biomarker discovery

Triple-negative breast cancer (TNBC) is a clinically aggressive and molecularly heterogeneous subtype of breast cancer characterized by variable response to neoadjuvant chemotherapy and a high risk of disease recurrence. Although numerous studies have focused on genomic alterations associated with treatment outcome, the contribution of alternative splicing and transcript isoform usage to chemotherapy response remains incompletely understood. In our previous analyses of pre-treatment TNBC biopsies, we identified extensive isoform switching events associated with response to neoadjuvant chemotherapy. Differential transcript usage was observed in genes involved in DNA damage repair, immune signaling, and other pathways relevant to cancer progression and therapeutic resistance, suggesting that transcript-level alterations may represent an important regulatory layer that underlies treatment outcomes. To further investigate the biological significance of these observations, we are extending our analyses to a recently published, clinically annotated biobank of TNBC patient-derived xenograft (PDX) models generated in the ARTEMIS clinical trial (Rinkenbaugh et al., Cancer Research, 2025). This unique resource comprises 92 PDX samples derived from 75 patients, including longitudinal samples collected during treatment and disease progression, together with comprehensive clinical and molecular annotations. The availability of transcriptomic data from diverse TNBC subtypes and disease stages provides an opportunity to investigate alternative splicing patterns in the context of chemotherapy response, tumor evolution, and metastatic progression. Current analyses focus on transcript-level characterization, differential transcript usage, and isoform switching across TNBC models. By integrating patient-derived and preclinical transcriptomic datasets, we aim to identify robust splicing-associated molecular features linked to treatment outcome and to assess their relevance across different stages of disease evolution. Candidate genes and isoform-switching events identified through these analyses will serve as a basis for subsequent experimental validation in cellular models, enabling functional assessment of their role in chemotherapy response and resistance mechanisms. These findings may support future precision oncology strategies in TNBC.

B13.O

Omentin-induced transcriptomic remodeling of porcine granulosa cells: protein-coding and long non-coding RNA signatures of follicular microenvironment regulation

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Słowa kluczowe: omentin-1, granulosa cells, transcriptomics, RNA sequencing, long non-coding RNAs, gene expression regulation

Omentin-1 (ITLN1) is an adipokine produced mainly by visceral adipose tissue that enhances insulin sensitivity and exerts anti-inflammatory effects. While our previous studies demonstrated that ITLN1 regulates the proliferation, apoptosis, and steroidogenesis of granulosa cells (GCs), the molecular mechanisms underlying its actions remain unclear. Therefore, this study aimed to characterize the transcriptomic response of porcine GCs to ITLN1. GCs were isolated from medium-sized ovarian follicles (4-6 mm) collected from Large White pigs during the mid-luteal phase of the estrous cycle (days 10-12). Cells were cultured in the absence (control) or presence of ITLN1 (50 ng/mL) for 24 h. Total RNA was isolated and subjected to RNA sequencing. Differential expression analysis identified protein-coding genes and long non-coding RNAs (lncRNAs), followed by gene ontology, pathway enrichment, and cis/trans target prediction analyses. Selected transcriptomic changes were validated by technical and functional approaches. Analysis identified 4,160 differentially expressed protein-coding genes and 301 differentially expressed lncRNAs. The protein-coding signature revealed extensive remodeling of granulosa cell functions, including cytoskeletal organization and cell adhesion (↑MYLK, ↑TNS1, ↑ITGA6, ↑ITGB1, ↑PREX1), extracellular matrix remodeling (↑FN1, ↑VCAN, ↑COL11A1, ↑MMP16, ↑COL18A1), angiogenesis (↑VEGFC, ↑ANGPT1, ↑HIF1A, ↑TGFB2, ↑THBS1), cell junction organization and intercellular communication (↑GJA1, ↑ICAM1, ↓CDH1, ↓CLDN1), granulosa-oocyte metabolic coupling (↑GLS, ↑SLC6A9, ↑SLC16A7, ↓ITPR3), and follicular microenvironment regulation (↑GPER1, ↑CYP11B1, ↓CHI3L1, ↓C1QTNF1, ↓WNT7B). Furthermore, cis/trans target prediction linked lncRNAs to genes involved in cytoskeletal remodeling (↑CFL2, ↑MYO9A, ↑SNTB2), angiogenesis and hypoxia-related signaling (↑VEGFC, ↑THBS1, ↑HIF1AN), cell communication (↑GJA1), granulosa-oocyte signaling (↓ITPR3), and follicular microenvironment regulation (↑CYP11B1, ↓WNT7B, ↓C1QTNF1). Key transcriptomic changes were confirmed by RT-qPCR, and functional validation assays supported the transcriptomic findings. These findings identify ITLN1 as a regulator of follicular microenvironment remodeling through coordinated modulation of protein-coding and long non-coding RNAs affecting granulosa cell architecture, extracellular matrix dynamics and intercellular communication. Funding: National Science Centre, Poland (2020/37/B/NZ9/01154).

B14.O

Variant-aware prediction and experimental validation of CRISPR-Cas9 nucleases and base editor off-target sites in HEK293T cells

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Słowa kluczowe: CRISPR-Cas9; base editors; off-target prediction; variant-aware analysis; CRISPRitz; HEK293T cells; experimental validation

Accurate identification of CRISPR off-target sites is essential for both functional studies and therapeutic applications. Standard off-target prediction typically relies on searching the reference genome for sequences similar to the guide RNA, allowing a limited number of mismatches and, in some tools, bulges. However, reference-based prediction may overlook the effect of genetic variants present in a given individual or cell line. By contrast, variant-aware prediction incorporates sequence variation directly into the search space, enabling the identification of candidate off-target sites absent from the reference genome and the exclusion of reference-derived sites disrupted by local variation. This distinction is particularly relevant in HEK293T cells, whose genomic background may differ substantially from the canonical reference assembly. Several computational tools have been developed for off-target prediction, including Cas-OFFinder, FlashFry, OFF-Spotter, CRISPOR, CHOPCHOP, and CRISPRitz. Among these, CRISPRitz is of particular interest because it combines genome-wide search with support for mismatches, DNA/RNA bulges, and genetic variants, while also enabling functional annotation of predicted loci. Nevertheless, the reliability of CRISPRitz-like predictions requires experimental validation. Such tools rely primarily on sequence similarity between the guide and candidate genomic sites, most often represented through edit distance, mismatch position, and bulge structure. Although these factors are important, they are unlikely to fully explain off-target activity, which may also depend on chromatin accessibility, local sequence context, and editor-specific biochemical constraints. This need is especially evident for base editors, whose activity depends on editing windows and sequence preferences not fully captured by nuclease-oriented prediction frameworks. In this work, we evaluate variant-aware off-target predictions in HEK293T cells and discuss their experimental validation for both CRISPR-Cas9 nucleases and base editors.

B15.O

Bioinformatic Identification of HMB-Responsive Genes and Pathways in a DMDmdx Rat Model

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Keywords: DMD, HMB, DMD^{mdx} rat model, RNA-Seq, Muscle inflammation and fibrosis

Duchenne Muscular Dystrophy (DMD) is a severe X-linked neuromuscular disorder caused by variants in the dystrophin gene, leading to dystrophin deficiency, recurrent muscle injury, inflammation, and progressive functional decline. Although genetic and pharmacological therapies are advancing, dystrophin-independent approaches targeting secondary pathology are still needed. β -hydroxy- β -methylbutyrate (HMB), a leucine-derived metabolite, has been associated with improved muscle regeneration through IGF/AKT signaling and reduced fibrosis via lower TGF β 1 levels. It may also support anabolic balance by increasing mTOR phosphorylation and reducing E3 ubiquitin ligases such as MAFbx and MuRF1, while influencing MAPK/ERK and PI3K/Akt pathways involved in myogenic proliferation, differentiation, and survival.

This study investigated HMB-associated transcriptomic responses in dystrophic skeletal muscle using the DMD^{mdx} rat model. Male DMD^{mdx} rats aged 13–14 weeks were allocated to a dystrophic control group receiving calcium carbonate (CaCO₃) or an HMB-treated group receiving calcium HMB (Ca-HMB). After 42 days of intervention, biceps femoris samples were analyzed by RNA-Seq using the Illumina NovaSeq X Plus platform. Differential expression analysis was performed with DESeq2, followed by KEGG pathway, Gene Ontology (GO), STRING/Cytoscape, MCODE, and CytoNCA analyses. HMB treatment was associated with 830 differentially expressed genes, including 100 upregulated and 730 downregulated genes compared with dystrophic controls. Integrated MCODE and CytoNCA analyses identified hub genes including *Il6*, *Ccl2*, *Mmp9*, *Fos*, *Klf4*, *Agt*, *Ncam1*, *Cxcr4*, *Kdr*, *Slc17a7*, *Selp*, *Grin1*, *Lif*, *Gria4*, *Serpib5*, *Slc6a4*, *Nppb*, *Fosb*, *Drd1*, *Serpib6a*, and *Crhr1*. Enriched pathways included calcium signaling, cAMP signaling, IL-17 signaling, and TNF signaling. GO biological processes further highlighted responses to activity, mechanical stimulus, calcium-mediated signaling, cellular response to tumor necrosis factor, and positive regulation of cytosolic calcium ion concentration. Overall, HMB induced broad transcriptional remodeling related to muscle activity, mechanical stress, calcium homeostasis, inflammatory signaling, and tissue adaptation. These findings support further functional validation of HMB as a candidate supportive intervention in DMD.

Research funded by the Polish National Science Center, grant no. 2020/37/B/NZ5/01744.

B16.O

Comparative Hepatic microRNA Profiling in Pigs Identifies Novel DE Transcripts Associated with *Andrographis paniculata* Hepatoprotection and Aflatoxin B1 Hepatotoxicity

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Słowa kluczowe: microRNA, Transcriptomics, microRNA-seq, pig, Aflatoxin B1, *Andrographis paniculata*, hepatoprotection, hepatotoxic.

Background: Aflatoxin B1 (AFB1) causes severe hepatotoxicity in livestock, hypercatabolism, and cellular stress. *Andrographis paniculata* (AP) possesses potent anti-inflammatory and hepatoprotective properties. This study used small RNA sequencing to map the porcine hepatic microRNA (miRNA) landscape and to decipher the post-transcriptional regulatory networks that drive AP-mediated protection against AFB1.

Methods: A total of 16 commercial crossbred TN70 pigs (Norsvin Landrace x Large White) were investigated. Porcine liver small RNA libraries (n = 6 AFB1; n = 10 AP) were sequenced to identify differentially expressed (DE) miRNAs. Downstream transcriptomic impacts were predicted using RNAhybrid to construct a target interaction map. Modulated networks were identified via GO and KEGG pathway enrichment analyses utilizing Bonferroni step-down correction.

Results: Comparative profiling between the AP and AFB1 cohorts revealed 26 significant DE miRNAs (17 upregulated, 9 downregulated) that clearly partitioned the treatment groups. The 17 upregulated transcripts featured high-magnitude induction of ssc-miR-100 ($\log_2FC = +3.16$), ssc-miR-1271-5p, and ssc-miR-221-5p. Targeting 7,978 unique genes, these miRNAs demonstrated highly significant suppression ($p_{adj} < 0.002$) of "protein ubiquitination," "macromolecule catabolic processes," and hyperactive signaling cascades via the "mTOR," "MAPK," and "Sphingolipid" pathways, indicating a reset of metabolic checkpoints. Conversely, 9 miRNAs were significantly downregulated, including ssc-miR-708-5p ($\log_2FC = -1.30$), ssc-miR-10386, and ssc-miR-33b-5p. The de-repression of their 7,611 predicted target genes revealed a robust activation ($p_{adj} < 0.003$) of cellular repair and structural integrity networks, dominated by "establishment of protein localization," "protein transport," "Rap1 signalling," and "IgSF CAM signalling." **Conclusion** *Andrographis paniculata* counteracts AFB1 hepatotoxicity through a dual-action miRNA regulatory network. AP restores liver homeostasis by epigenetically suppressing toxin-induced hypercatabolism and hyperactive signaling (mTOR/MAPK), while simultaneously activating essential intracellular protein transport and cellular adhesion pathways (Rap1/IgSF CAM) to repair hepatic damage.

Acknowledgements: This research was funded by Narodowe Centrum Nauki: UMO-2021/43/I/NZ9/02612, and Deutsche Forschungsgemeinschaft: 504983453 (WI 1754/18).

B1.P

Bioinformatic assessment of tumour mutational burden and clinical heterogeneity in colon cancer: insights from TCGA-COAD and the perspective of isomiR profiling

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Słowa kluczowe: colorectal cancer; tumor mutational burden; molecular biomarkers, miRNAs; isomiRs

Colorectal cancer (CRC) is among the most common malignancies and a leading cause of cancer-related mortality worldwide. Its clinical course and treatment response are shaped by molecular heterogeneity and genomic instability. Tumour mutational burden (TMB), derived from somatic mutation counts, is a potential biomarker of immunotherapy response; however, its clinicopathological relevance and relationship with post-transcriptional regulation in CRC remain unclear. Publicly available data from The Cancer Genome Atlas Colon Adenocarcinoma cohort (TCGA-COAD) were analysed. TMB was calculated from somatic mutation profiles, and samples were stratified into high- and low-TMB groups. Associations with clinicopathological variables and patient survival were assessed. miRNA and isomiR expression profiles will be compared between TMB groups to identify discriminatory variants. Candidate miRNAs/isomiRs will be used to develop and validate a TMB associated expression signature, evaluated for discrimination, calibration, and clinical utility. Functional analysis of target genes and associations with immune-response markers will also be assessed. Preliminary analyses established an integrated TCGA-COAD cohort and stratified patients by TMB. Associations between TMB and selected clinicopathological characteristics were observed, while miRNA and isomiR analyses are ongoing. This study provides a framework for assessing TMB in colon cancer stratification. Integrating mutation data with miRNA and isomiR profiles may identify post-transcriptional mechanisms linked to TMB and immune response. Seed-region isomiRs may be particularly informative because they can alter predicted target-gene repertoires.

B2.P

Optimization of the base editor BE4max and evaluation of its activity in cell culture

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Słowa kluczowe: Cytosine Base Editor (CBE), Genome Editing, BE4max, Gibson Assembly, HEK293T cells

Cytosine base editors are precise genome editing tools capable of converting C to T bases without inducing double-strand breaks. Although BE4max is designed to be superior to the BE3 editor, initial tests in HEK293T cells revealed that our BE4max mRNA exhibited significantly reduced activity of 5% compared to 30% for the BE3 generation. Sequencing analysis of the BE4max plasmid identified two point mutations, specifically G2788A in the Cas9 domain and G5773A in the UGI subunit, which were identified as the primary cause of this suboptimal performance. The objective of this research is to correct these mutations individually and in combination to restore the expected superior functionality of the BE4max editor. The project incorporates multidimensional in silico analyses to evaluate the impact of these mutations on the protein structure and dynamics, drawing on insights from our previous research involving the BE3 variant that contained the EGFP reporter. The research process includes: (1) primer design and Gibson assembly to generate repair variants; (2) construct propagation in *E. coli* and sequence validation; and (3) mRNA synthesis via in vitro transcription. In the final stage, editing efficacy in HEK293T cells is assessed via mRNA transfection. On-target editing efficiency is verified through Sanger sequencing, and the results are correlated with findings from our earlier studies conducted with the BE3 construct that was tagged with EGFP. Integrating computational predictions with experimental data allows for a comprehensive characterization of the enzymatic specificity of the editor. These findings elucidate how the identified mutations affected the stability and activity of BE4max, providing an optimized and highly efficient tool for advanced genetic research.

B3.P

Evaluating maximum parsimony and maximum likelihood methods for estimating mutational pressures

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Słowa kluczowe: molecular evolution, maximum likelihood, maximum parsimony, substitution models, mutational pressure

Quantifying mutational pressures via nucleotide substitution matrices is fundamental to molecular phylogenetics. Standard frameworks like the General Time Reversible (GTR) model are computationally efficient but biologically unrealistic due to their assumptions of reversibility and stationarity. Even the complex, non-reversible UNREST model remains constrained by stationarity. Because real-world mutational processes often violate these assumptions, standard maximum likelihood (ML) methods can misrepresent biological reality. Conversely, maximum parsimony (MP) does not assume stable mutation rates over time, potentially offering more reliable estimates. To evaluate MP against ML (under GTR and UNREST), we conducted 5 million computational simulations across diverse nucleotide compositions, testing both constant and time-variable mutational pressures. Across all frameworks, estimation errors were high in highly similar sequences due to limited data, reached a minimum at intermediate divergence, and rose again in highly divergent sequences due to multiple substitutions. Notably, ML required higher sequence variability to yield accurate predictions, whereas MP achieved accuracy at lower divergence levels. However, MP error rates surpassed ML in highly divergent datasets. Under constant mutational pressure simulated via GTR, ML-GTR provided the most accurate predictions, though differences between MP and ML-UNREST were minor. When sequences evolved under constant UNREST dynamics, ML-UNREST typically performed best. Crucially, MP demonstrated a distinct advantage under time-variable mutational pressures—scenarios that best mirror real-world biological evolution. Consequently, our results indicate that the maximum parsimony method is the most suitable approach for studying mutational pressures across closely related genomes, whereas the widely used GTR method proves to be the least accurate.

B4.P

PhyloPlast: An Integrated Platform for Plastid Protein Evolution and Subplastid Localization Prediction

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Słowa kluczowe: Database, Plastid, Plastid localization

Understanding plastid evolution and protein targeting requires comprehensive protein-centered resources combined with accurate predictive tools. Here, we present PhyloPlast, a newly developed plastid-focused database that integrates curated protein, phylogenetic, and subplastid localization data with advanced machine-learning algorithms. PhyloPlast provides: (i) comprehensive information on plastid types and their evolutionary history; (ii) interactive browsing and downloading of protein records by gene/protein name, plastid type, localization, and taxonomy; (iii) visualization and retrieval of plastid genome maps; (iv) homology searches for nucleotide and protein sequences; and (v) subplastid localization prediction using dedicated computational models. A core component of PhyloPlast is PlastoGram, an ensemble machine-learning framework designed to predict protein localization within plastids and determine whether proteins are plastid- or nuclear-encoded. PlastoGram classifies proteins into envelope, stroma, thylakoid membrane, or thylakoid lumen compartments and additionally predicts Sec or Tat import pathways for luminal proteins. The model was trained on a manually curated dataset and explicitly addresses challenges associated with limited and highly imbalanced classes. Importantly, analysis of model decision rules revealed that informative n-grams (short amino acid motifs) recover experimentally validated targeting signals, demonstrating that the learned features capture biologically meaningful determinants of plastid protein import. To complement PlastoGram approach, we developed ChloroGuide, a transformer-based predictor leveraging protein language models to enhance subplastid localization inference. Together, PlastoGram and ChloroGuide offer complementary predictive approaches that improve the analysis of plastid protein localization within a single integrated platform PhyloPlast.

B5.P

Prada versus Versace – Deep-Learning-based SNP imputation on the genomewide scale

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Słowa kluczowe: AutoEncoder, Deep Learning, Imputation, Genotyping Microarray, Single Nucleotide Polymorphism, Whole Genome Sequence

The imputation of SNP genotypes from the genotypic microarray level to the level of whole genome variation has important implication for Genome-Wide Association Studies enabling the creation of large cohorts with phenotyped and genotyped individuals, such as the UK-Biobank data. Moreover, imputation has also clinical value through facilitating the estimation of polygenic risk scores and aiding in diagnosis to inform clinical decision-making. Most of the current SNP imputation software packages prioritize overall imputation accuracy, rather than prediction of individual variants. However, variants with low Minor Allele Frequencies or located in regions of low Linkage Disequilibrium, are often the ones that are the most important for predicting phenotypes and disease risks. Our study uses the subset of 742 training and 186 test bulls from the 1000 Bull Genome cohort to predict SNP genotypes across the entire genome (here exemplified by the BTA28 containing 297 605 SNPs) using the Illumina 50K Bead Chip as a reference. The focus of this study is on investigating the performance of Deep Learning-based architectures to accurately predict genotypes of SNPs with low Minor Allele Frequencies and to identify patterns underlying poorly predicted variants. For the purpose four DL architectures were applied: a vanilla AutoEncoder based only on the set of Feed Forward Dense Neural Networks (FNN1), a modification of AE1 implementing SNP positional encoding via 1D Convolution (FNN2), a variational AutoEncoder (VAE) and the most architectonical heavy AutoEncoder implementing parallel attention mechanisms to process different sources of SNP-related information (TAE). Results demonstrate that two most basic approaches - FNN1 and VAE yielded accurate predictions of rare SNP genotypes with remarkably low computational costs. The results highlight the fact that very fashionable, extensive Deep Learning architectures are not always compatible with data sets that contain limited information.

B6.P

Bioinformatic investigation of starch metabolism in ciliates

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Słowa kluczowe: Ciliates, Starch, Glucan water dikinase, Phosphoglucan water dikinase, Phylogenetics

Ciliates are a diverse group of unicellular eukaryotes. Although they are heterotrophic organisms and there is no evidence that they originated from photosynthetic ancestors, several observations suggest that they accumulate starch – a storage polysaccharide characteristic of plants and algae. In my research, I set out to investigate the presence of starch in this lineage. Using bioinformatic approaches, I identified genes encoding α -glucan, water dikinase (GWD) and phosphoglucan, water dikinase (PWD) – key enzymes involved in starch metabolism – in the genomes of numerous ciliate species. To accurately translate the nucleotide sequences of these genes into the corresponding protein sequences, I developed a Python script that uses BLAST hits to identify coding regions and reconstruct complete coding sequences, even in the presence of frameshifts. My phylogenetic analyses indicate that both GWD and PWD genes were already present in the last common ancestor of ciliates. Furthermore, the results suggest a shared evolutionary origin of starch metabolism in ciliates and their close relatives, dinoflagellates and apicomplexans. Additionally, I validated the bioinformatic findings using biochemical and microscopic approaches. Together, these results substantially expand our understanding of the distribution of starch across the tree of life while opening new avenues for the potential practical applications of ciliate storage polysaccharides. This work was supported by the National Science Centre grant no. 2024/53/N/NZ8/02479.

S.O

Investigative Genetic Genealogy: From Genome-wide SNP Data to Investigative Leads

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Słowa kluczowe: investigative genetic genealogy; forensic genetics; forensic genealogy; SNP analysis; kinship analysis; cold cases

Background: Investigative genetic genealogy (IGG) is an interdisciplinary methodology integrating high-density autosomal SNP analysis with genealogical research to generate investigative leads in cases involving unidentified human remains and unresolved criminal investigations. Unlike conventional forensic DNA analysis, which relies on direct comparison of STR profiles, IGG uses genome-wide SNP data to identify biological relatives of an unknown individual through consumer genealogical databases. Following its landmark application in criminal investigations in 2018, IGG has transformed cold-case investigations internationally, yet important methodological and legal distinctions remain insufficiently recognised.

Aim: This presentation defines IGG as a method for generating investigative hypotheses rather than forensic identification. IGG narrows the pool of potential candidates and guides investigative work toward a testable hypothesis, which must subsequently be confirmed using validated forensic DNA methods.

Methodology: The IGG workflow begins with uploading high-density SNP data to genealogical databases, followed by analysis of shared DNA segments, clustering of genetic matches, and reconstruction of family trees. Successful implementation requires close collaboration among forensic geneticists, genetic genealogists, and law enforcement agencies, integrating genetic evidence with documentary records, administrative registers, publicly available information, and other investigative data. Tools such as DNA Painter for segment visualisation and BanyanDNA for network-based kinship modelling support interpretation of complex or endogamous populations. Polish context. Implementation in Poland faces several challenges, including data protection requirements and the absence of dedicated legislation. Additional limitations include the underrepresentation of Central and Eastern European populations in consumer genealogical databases and the need to ensure compliance with database terms of service, user consent, and privacy requirements.

Conclusions: IGG offers substantial investigative value when its methodological scope and hypothesis-generating role are clearly understood. As the method emerges in Poland, shared methodological standards, greater legal clarity, and interdisciplinary collaboration will be essential for its responsible implementation.



S1.O

Validation of the FORCE Panel for Extended Kinship Analysis Using Massively Parallel Sequencing

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Słowa kluczowe: human identification, kinship, SNP panel

Extended kinship analysis represents a significant challenge in forensic genetics, particularly in cases involving distant biological relationships, degraded biological material, or limited availability of reference samples. While short tandem repeat (STR)-based methods remain the standard approach for close relationship testing, their discriminatory power decreases substantially when evaluating more distant relatives such as cousins, avuncular pairs, or half-siblings. Recent advances in massively parallel sequencing (MPS) and high-density single nucleotide polymorphism (SNP) panels offer new opportunities to improve the accuracy and reliability of such investigations. Here we present validation and implementation of the QIAseq FORCE Panel in a forensic genetics laboratory for extended kinship analysis. The panel comprises more than 5,000 autosomal SNP markers selected for high heterozygosity, low linkage disequilibrium, broad genomic distribution, and suitability for identity-by-descent (IBD) analyses. Its design is optimized for forensic applications, including the examination of degraded and low-template DNA samples. The validation study included the development and optimization of laboratory and bioinformatic workflows using the Ion S5 sequencing platform. Performance characteristics were assessed in accordance with international forensic validation recommendations, including sensitivity, repeatability, reproducibility, and data quality metrics. The study consisted of Certified Reference Material (CRM) and samples from individuals with known family relationships representing different degrees of relatedness. Bioinformatic analysis were performed using both commercial and open-source software solutions, while kinship inference was conducted using likelihood-based statistical approaches implemented in FamLink2. The project is expected to establish a validated methodology for routine forensic application of SNP-based extended kinship analysis. Its implementation will expand the laboratory's capabilities in human identification, missing persons investigations, disaster victim identification, and complex relationship testing, while aligning forensic practice with current international standards and developments in forensic genomics.

S2.O

From DNA to the Most Recent Common Ancestor: The Scientific Foundations of Forensic Genetic Genealogy

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Słowa kluczowe: sądowa genealogia genetyczna, FGG, SNP, WGS, Identity by Descent (IBD), Most Recent Common Ancestor (MRCA), genetyka sądowa, genealogia genetyczna

Background: Forensic Genetic Genealogy (FGG) is an emerging forensic discipline that integrates genome-wide SNP analysis with genealogical and archival research to generate investigative leads in violent crime investigations and the identification of unidentified human remains. The rapid expansion of genome-wide technologies and genetic genealogy databases has transformed the ability to identify distant relatives and reconstruct complex family relationships.

Methods: FGG extends conventional forensic DNA analysis beyond STR profiling by employing dense single nucleotide polymorphism (SNP) datasets generated by microarrays or whole-genome sequencing (WGS), particularly for highly degraded DNA. Kinship inference is based on the detection of Identity-by-Descent (IBD) segments, quantified in centimorgans (cM), shared with individuals in genealogical databases such as GEDmatch and FamilyTreeDNA. These genetic matches are integrated with documentary evidence from genealogical and archival records to reconstruct pedigrees and identify the Most Recent Common Ancestor (MRCA). Recent studies have further introduced stochastic dynamic programming approaches to optimize genealogical search strategies.

Results: The combination of genome-wide SNP profiling, IBD-based relationship inference, genealogical reconstruction and computational optimization substantially improves the efficiency of forensic investigations by reducing the search space and accelerating pedigree reconstruction. FGG has demonstrated considerable value in resolving cold cases and identifying previously unidentified human remains while maintaining confirmation through conventional forensic DNA profiling.

Conclusions: FGG represents a paradigm shift in forensic human identification by combining molecular genetics, genealogy and computational science. Its broader implementation will require standardized analytical workflows, validated quality assurance procedures, specialized training, and harmonized legal and ethical frameworks governing the forensic use of investigative genetic genealogy.

S3.O

FacePainter – a comprehensive tool for forensic DNA phenotyping

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Słowa kluczowe: forensic DNA genotyping; bioinformatics pipeline; MPS sequencing; validation studies

Forensic DNA phenotyping (FDP) has emerged as a powerful approach for generating investigative leads in cases where conventional STR-based identification fails. By enabling the prediction of externally visible characteristics (EVCs) and biogeographic ancestry (BGA) from trace DNA, FDP can support criminal investigations, disaster victim identification (DVI), and the analysis of human skeletal remains. Recent advances in massively parallel sequencing (MPS) technologies have further expanded the scope and accuracy of such predictions, while simultaneously increasing the demand for robust and automated bioinformatic solutions. The aim of this study was to develop a comprehensive bioinformatic pipeline for the analysis and interpretation of MPS data generated using the Ion Torrent technology, and to apply this pipeline in the developmental validation of a custom SNP panel designed for simultaneous prediction of selected appearance traits and BGA. The panel integrates 161 genetic markers for sex determination and associated with pigmentation, hair morphology, and other facial features, enabling extended phenotype inference. The tool was designed to utilise previously described and novel predictive algorithms. The performance of the assay and the accompanying analytical workflow was evaluated according to forensic validation guidelines, including sensitivity, reproducibility, repeatability, inhibitor tolerance, species specificity, and the ability to analyse mixed, degraded, and low-template DNA samples, including bone-derived

material. The results demonstrated the method's high robustness and sensitivity, with reliable genotyping achieved from low DNA input quantities. Predictions for 16 known reference samples were consistent with the available phenotype data. In conclusion, the FacePainter, together with the bioinformatic pipeline offer a sensitive, robust, and straightforward solution for forensic applications. This streamlined approach facilitates efficient analysis, supporting both facial reconstruction in forensic anthropology and comprehensive DNA phenotyping.

S4.O

Assessment of the Kinfinder kit suitability for analyzing bone material for identification purposes

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Słowa kluczowe: Kinfinder, GlobalFiler, Human Identification, STR comparison

The Kinfinder kit, enables the analysis of 50 STR loci in two multiplex PCR reactions. It has been validated for routine casework using reference material. Population studies confirmed a very high polymorphism of the analysed loci, with a mean heterozygosity of 88.07%. However, until now, there had been no assessment of its suitability for analyzing DNA from bone material. 25 genetic profiles obtained from bone samples were analysed to compare the performance of the Kinfinder and GlobalFiler™ kits. In 15 cases, at least 19 fully identified loci were obtained. Higher DNA concentrations were associated with more complete profiles, whereas in samples with low DNA content the number of detectable markers decreased significantly. The relationship with the degradation index was less straightforward, as some samples with high DI yielded better results than samples with moderate one. In 9 out of 12 cases of calculations performed on actual bone material, the Kinfinder kit produced higher statistical kinship analysis results. LR distributions for defined kinship scenarios were simulated in Familias, using 1,000 simulated profiles per calculation. Full profiles for the GlobalFiler™ and Kinfinder kits, as well as profiles limited to the 19 most frequently detected loci, were included. Kinfinder achieved higher LR values for first and second degree kinship and for part of third degree kinship analyses. Its usefulness in analyzing fourth-degree relationships, though limited, was still higher than that of traditional kits. When considering only the 19 most frequently typed loci, in paternity tests as well as in siblings, LR values two orders of magnitude higher were obtained. For more distant relationships, the results were comparable, with a slight advantage for Kinfinder. Despite Kinfinder was more prone to drop-out, even partial profiles provided LR values comparable to, or even higher than those obtained using GlobalFiler™.

S5.O

From Turing models to neural networks: the genetic architecture of fingerprints in DNA phenotyping and forensic systems

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Słowa kluczowe: dermatoglyphics; Turing model; EVI1 gene; artificial intelligence; DNA phenotyping; SMARCAD1

Advances in developmental biology and computational genetics have radically changed our understanding of the process by which fingerprints are formed. Recent research includes multicenter genome-wide association studies (GWAS), three-dimensional microscopic models of fetal development, and numerical mathematical simulations of reaction-diffusion systems. Furthermore, deep neural networks (DNNs) are being implemented in automated fingerprint identification systems (AFIS). The scientific literature unequivocally refutes the traditional paradigm, according to which fingerprints are treated as random products of mechanical pressure in the uterus. Instead, it has been demonstrated that macrostructures (vortices, loops, arcs) are a direct consequence of the expression of genes responsible for limb development (including the EVI1 gene), which modifies the anatomy of the palmar pad between the 9th and 15th weeks of pregnancy. At the cellular level, the formation of epidermal ridges follows Alan Turing's mathematical reaction-diffusion model, driven by the antagonism between activators (WNT, EDAR) and fast-acting inhibitors (BMP). The collision of biochemical waves within confined spatial boundaries generates unique minutiae. Recent studies estimate that developmental noise accounts for approximately 20% of the total variance, providing a definitive scientific explanation for why identical twins exhibit different microstructural features. In forensics, these discoveries constitute a new discipline: genomic dactyloscopy. Comprehensive marker identification enables the deployment of predictive systems (DNA phenotyping) capable of generating a probabilistic fingerprint template directly from a suspect's genetic profile. Concurrently, Deep Neural Networks (DNN) redefine comparative analysis (AFIS-CODIS), allowing AI to perform unprecedented cross-domain database retrieval based on ridge orientation vector fields. Furthermore, precise mapping of the SMARCAD1 gene enables forensic medicine to instantly differentiate congenital adermatoglyphia from intentional destruction of fingertips by criminals. This paper provides an overview of the latest literature. The study was financed by statutory funding 506.534.04.00 from the Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Poland.

S6.O

Dilemmas in Predicting Biogeographical Ancestry from Mitochondrial DNA: The Case of the "Vampire" of Pień

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Słowa kluczowe: biogeographic origin, fossil DNA, mtDNA, phylogeography

Biogeographical ancestry prediction within forensic genetics, population genetics, and anthropology provides the most reliable outcomes when utilizing extensive panels of autosomal single nucleotide polymorphisms. Conversely, this modality of prediction primarily offers indications regarding affiliation with major continental groups—namely Africa, Europe, and Central and East Asia—whereas it performs suboptimally for specimens of admixed ancestry, such as those from the Middle East or Latin America. Furthermore, the analysis of thousands of SNP markers via massively parallel sequencing (MPS) is frequently unfeasible for ancient and degraded skeletal specimens. Under such circumstances, mitochondrial DNA analysis often represents the sole alternative; at its maximum attainable resolution—specifically, whole-genome sequencing coupled with phylogeographic analysis—it can provide the definitive insight into ancestry. A pertinent illustration of this scenario is evidenced by data obtained through MPS sequencing of the complete mtDNA from skeletal material excavated from a grave at a 17th-century cemetery in Pień (Dąbrowa Chełmińska commune, Bydgoszcz district). This burial contained the remains of a young woman with a sickle placed across her neck and a padlock affixed to her toe, features indicative of protective (anti-vampiric) practices. The comprehensive analysis of the mitochondrial genome derived from the bone, integrated with the phylogenetic reconstruction of haplogroup J1c2c1 utilizing all published whole-genome data, substantiated the hypothesis that the individual's maternal lineage potentially originated from southern Scandinavia. In conjunction with non-genetic evidence, these findings establish a framework for exploring the underlying rationales of this atypical burial.

S7.0

Comparison of EuroForMix and DNASTatistiX in likelihood ratio analysis of DNA mixtures

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Słowa kluczowe: DNA mixtures, LR, EuroForMix, DNASTatistix, forensic genetics, validation

The interpretation of DNA mixtures is one of the key challenges in contemporary forensic genetics. In laboratory practice, probabilistic tools are increasingly used to calculate likelihood ratios (LRs) for alternative hypotheses regarding the origin of genetic material. However, the application of such software requires validation, including an assessment of the range of obtained results, their stability, and concordance between different analytical tools. The aim of this study was to compare two programs used for DNA mixture analysis: EuroForMix and DNASTatistiX. The study was based on a set of artificially generated genetic profiles and a specially prepared allele frequency database, in which identical frequencies were assigned to alleles within each locus. This approach made it possible to reduce the impact of population variability and to focus the analysis on the properties of the mixtures themselves and on how they are interpreted by both programs. For the purposes of the study, 100 artificial genetic profiles were constructed, simulating mixtures originating from two or three contributors. For each variant, LR calculations and selected auxiliary parameters were obtained, allowing assessment of the distribution of resulting values, repeatability of results, and the impact of the number of contributors on interpretation. The analysis also included the identification of situations in which the results generated by the two programs may show the greatest differences. This study forms part of the validation of probabilistic tools used in the interpretation of DNA mixtures. The obtained results may support the assessment of the practical applicability of the analyzed programs and the development of local interpretation procedures in forensic genetic testing.

S8.O

Comparison of Likelihood Ratios for Real DNA Casework Mixtures Using DNASTatist X and LRMix Studio.

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Słowa kluczowe: Forensic statistical analysis, Likelihood ratio; Mixture interpretation.

Interpretation of DNA profiles from evidence is often challenging due to the presence of complex mixtures, and various stochastic effects resulting from low DNA quantity/quality and/or a high degree of degradation. There are several statistical tools based on qualitative and quantitative models developed for probabilistic evaluation of DNA mixtures. The aim of this study was to compare LR calculations estimated for real casework DNA mixed profiles using the LRMix Studio software v.2.1.5, which is routinely used in many DNA laboratories, with the more advanced DNASTatist X system. The study included two categories of DNA profiles: good quality two- and three-donor mixtures and incomplete profiles exhibiting stochastic effects. The same DNA profiles were analyzed with two computer programs that implement different mathematical approaches. LRMix Studio is a qualitative model (semi-continuous) that focuses on allele presence or absence, while DNASTatist X software is a more advanced tool that uses a quantitative model (continuous model) that additionally integrates allele peak heights. Analysis performed with the DNASTatist X software yielded considerably higher and more robust LR values compared to LRMix Studio for both types of DNA mixtures examined. The difference reached at least 2–3 orders of magnitude, and in some cases reached up to ten orders of magnitude. In conclusion, quantitative analysis of DNA mixtures as implemented in DNASTatist X software is computationally more demanding than LRMix Studio but it should be considered as a significant step forward in statistical interpretation of DNA mixtures and the evaluation of the weight of evidence. Incorporation of peak height data in addition to allele presence enables a more informative and reliable analysis of DNA profiles. The study confirms that quantitative models provide a more comprehensive approach in probabilistic DNA mixture interpretation.

S9.O

Epigenetic age prediction in sperm cells for forensic applications using ForSpEC model

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Słowa kluczowe: Forensic epigenetics, Age estimation, Sperm DNA methylation, Epigenetic clock, Semen analysis

Semen is one of the most frequently encountered biological materials in forensic investigations, particularly in sexual assault cases. While standard DNA profiling of sperm cells enables donor identification when reference profiles are available, additional investigative intelligence is essential when database matches are lacking. Age estimation based on sperm-specific DNA methylation patterns has therefore emerged as a promising complementary approach. ForSpEC (Forensic Sperm Epigenetic Clock) is a compact model for chronological age prediction based on DNA methylation at seven CpG sites. Developed using Illumina EPIC array data, it achieved high predictive accuracy, with a strong correlation with chronological age ($R^2 = 0.7$) and a mean absolute error (MAE) of 3.13 years. Cross-platform validation performed on a subset of samples confirmed its robustness in targeted sequencing approaches, including Ion AmpliSeq technology, with only a minor reduction in performance (MAE = 3.24 vs. 3.76 years). An extended forensic validation framework was implemented, including comparisons between purified sperm cells and whole semen samples, evaluation across population groups, assessment of repeatability, and benchmarking against alternative models. The results

demonstrated that cellular composition significantly affects prediction accuracy, due to sensitivity of marker cg14728380 to somatic DNA contamination (MAE = 10.22 vs. 3.08 years). To address this limitation, an alternative mathematical model was introduced, improving accuracy in contaminated samples and enhancing model robustness (MAE = 10.22 vs. 4.21 years). Furthermore, despite using only seven CpG markers, ForSpEC achieved the second-best performance among various evaluated models. The effect of potential confounders, including lifestyle factors (smoking, alcohol consumption, BMI) and semen quality parameters, was also examined. These variables showed minimal influence on methylation levels and did not affect prediction accuracy. Overall, the extended validation demonstrates that ForSpEC is a robust and accurate tool for forensic age estimation from sperm DNA, with strong potential for practical implementation as an investigative method.

S10.O

Automation makes the difference – manual vs automated DNA preferential lysis in sexual assault cases

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Słowa kluczowe: sexual assault, DNA extraction, differential lysis, automated DNA extraction, EZ2 platform

In the analysis of biological evidence from sexual assault cases, the separation of mixed cellular material is a critical step in DNA extraction. Preferential lysis enables the selective isolation of epithelial cell DNA (of potential victim origin) from the sperm fraction (of potential perpetrator origin), based on differences in cellular resistance to chemical reagents. This process is particularly useful in samples with a high imbalance between sperm cells and other cellular material. However, the manual procedure has certain limitations, which have led to efforts to automate this step. One such solution is the Investigator Sep&Prep Kit used on the EZ2 platform. The aim of this study was to evaluate automated preferential lysis and DNA extraction using the EZ2 workstation and Investigator Sep&Prep Kit (Qiagen), in comparison with a workflow involving manual preferential lysis followed by automated extraction on the QIA Symphony SP/AS using the QIA Symphony DNA Investigator Kit (Qiagen). Samples with varying sperm-to-epithelial cell ratios, as well as different storage conditions and durations, were analyzed. DNA quantification (qPCR) and STR profile quality, assessed using the NGM Select kit (Life Technologies), served as evaluation criteria. The findings indicate that the EZ2 Investigator Sep&Prep Kit improves DNA extraction performance, particularly in the sperm fraction, and reduces processing time compared to the manual method. In addition, automated lysis enhanced the separation of epithelial and sperm fractions, often yielding single-source profiles or dominant profiles with minimal admixture resulting from inter-fraction carryover. This resulted in higher-quality STR profiles and an increased proportion of detected alleles, in some cases reaching complete (100%) allele detection. These results support the implementation of automated workflows to increase standardization and efficiency in forensic laboratories.

S11.O

Utilizing DNA Methylation Markers in Bone and Cartilage for Victim Identification

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Słowa kluczowe: methylation, epigenetics, disaster victim identification

Identification of severely compromised human remains poses a complex challenge that often exceeds the capabilities of standard STR profiling when no reference samples are available for comparison. To address these limitations, we explored the potential of DNA methylation (DNAm) markers for tissue differentiation and age estimation in skeletal remains. Conducted as part of the European project ForMAT (Forensic Methylation Analysis Toolsets), this study systematically evaluates the DNA methylation profiles in both bone and cartilage, proposing cartilage as highly practical yet less commonly used alternative to bone samples. We utilized a multi-tiered screening and validation strategy to isolate robust CpG sites. First, we performed a meta-analysis of publicly available methylome data from the Gene Expression Omnibus (GEO), aggregating bone (N=84, three datasets) and cartilage (N=68, three datasets) profiles. This bioinformatic screening isolated top-performing candidate markers located in genes such as ELOVL2, PDE4C, KLF14, ASPA, TRIM59, PI4KB, FAM59B, PRMT8, and SCGN. Subsequently, we generated novel genome-wide DNAm data using the Illumina Infinium MethylationEPIC v2.0 array on 136 forensic autopsy samples (96 femur and skull; 40 rib cartilage). Integrating our experimental cohort with public datasets facilitated the development of a highly predictive, compact epigenetic marker panel. Ultimately, these validated loci will be incorporated into a novel DNAm-based Disaster Victim Identification (DVI) kit, bridging the gap between molecular epigenetics and applied forensic science. The study received funding from the European Union's Horizon Europe Programme under Grant agreement no. 101225631.

Comparative Assessment of DNA Preservation in Human Skeletal Remains

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Słowa kluczowe: auditory ossicles, forensic genetics, DNA preservation, DNA degradation, DNA isolation, STR typing

Recent advances in genetic and analytical methods have greatly improved DNA research; however, obtaining high-quality genetic material from skeletal remains remains challenging due to DNA degradation and the presence of PCR inhibitors. Traditionally, long bones, the petrous part of the temporal bone, and teeth have been preferred for genetic analyses. Although these tissues often provide good results, DNA recovery is not always successful and usually requires destructive sampling of valuable skeletal elements. To reduce damage to human remains, researchers continue to seek alternative skeletal structures that can yield high-quality DNA while requiring only minimal intervention. In this study, we investigated the potential of auditory ossicles, small bones of the middle ear that are rarely analysed, as a source of genetic material for forensic and historical applications. Auditory ossicles were collected from above 100 individuals. Whenever possible, teeth from the same individuals were also sampled to serve as comparative material. All specimens underwent identical analyses, including DNA quantification and STR profiling. The results showed that ossicles consistently outperformed teeth. Mean concentrations of large DNA fragments were 0.110 ng/μL in ossicles and 0.030 ng/μL in teeth ($p = 6.4 \times 10^{-9}$). For small fragments, ossicles yielded 0.240 ng/μL compared with 0.126 ng/μL from teeth ($p = 2.7 \times 10^{-6}$). DNA preservation was also significantly better in ossicles, as indicated by a lower degradation index (mean DI = 3.02) than in teeth (mean DI = 22.4; $p = 1.4 \times 10^{-5}$). Higher DNA yields and lower degradation translated into superior downstream results, including more complete genetic profiles and improved allelic balance. Overall, auditory ossicles performed better than teeth across all evaluated parameters. These findings indicate that auditory ossicles are a valuable alternative source of high-quality DNA, particularly when preservation of skeletal integrity is a priority.

AMEL-Y dropout in forensic genetic testing

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Słowa kluczowe: amelogenin, deletion, multiplex-PCR, Y-chromosome, kinship analysis, forensic genetics

The amelogenin gene (AMEL) encodes a protein present in dental enamel and is located on the human sex chromosomes. The AMEL-X/AMEL-Y marker, included in many multiplex PCR kits, is routinely used for sex determination in forensic genetics. However, the absence of amplification of the AMEL-Y fragment in a male sample may result from a deletion within the short arm of the Y chromosome and may consequently lead to misclassification of the sample as female. This issue constitutes a significant interpretative challenge, particularly in kinship analyses, including paternity testing. The aim of this study was to analyze the occurrence of deletions in the AMEL-Y region and adjacent Y-STR markers in a population of men from central Poland, as well as to evaluate their detectability using various commercial multiplex PCR kits. The study material consisted of samples collected from men undergoing genetic testing at the Department of Forensic Medicine, Medical University of Lodz, between 2005 and 2025. Detected deletions within the AMEL-Y region were verified using selected multiplex kits containing the amelogenin marker, differing in primer localization and the length of amplified fragments, as well as using Y-STR markers. This approach allowed us to assess whether the observed lack of signal resulted from an actual deletion within a specific region of the Y chromosome or from limitations of a particular multiplex kit. The results confirm that AMEL-Y deletions may significantly affect the reliability of forensic conclusions in both kinship analyses and criminal casework. The study highlights the importance of applying additional Y chromosome markers, multiple PCR kits and real-time PCR assays to confirm male origin. Taking into account the possibility of AMEL-Y deletions is therefore crucial for the correct interpretation of results and for reducing the risk of drawing erroneous conclusions in forensic genetic testing.