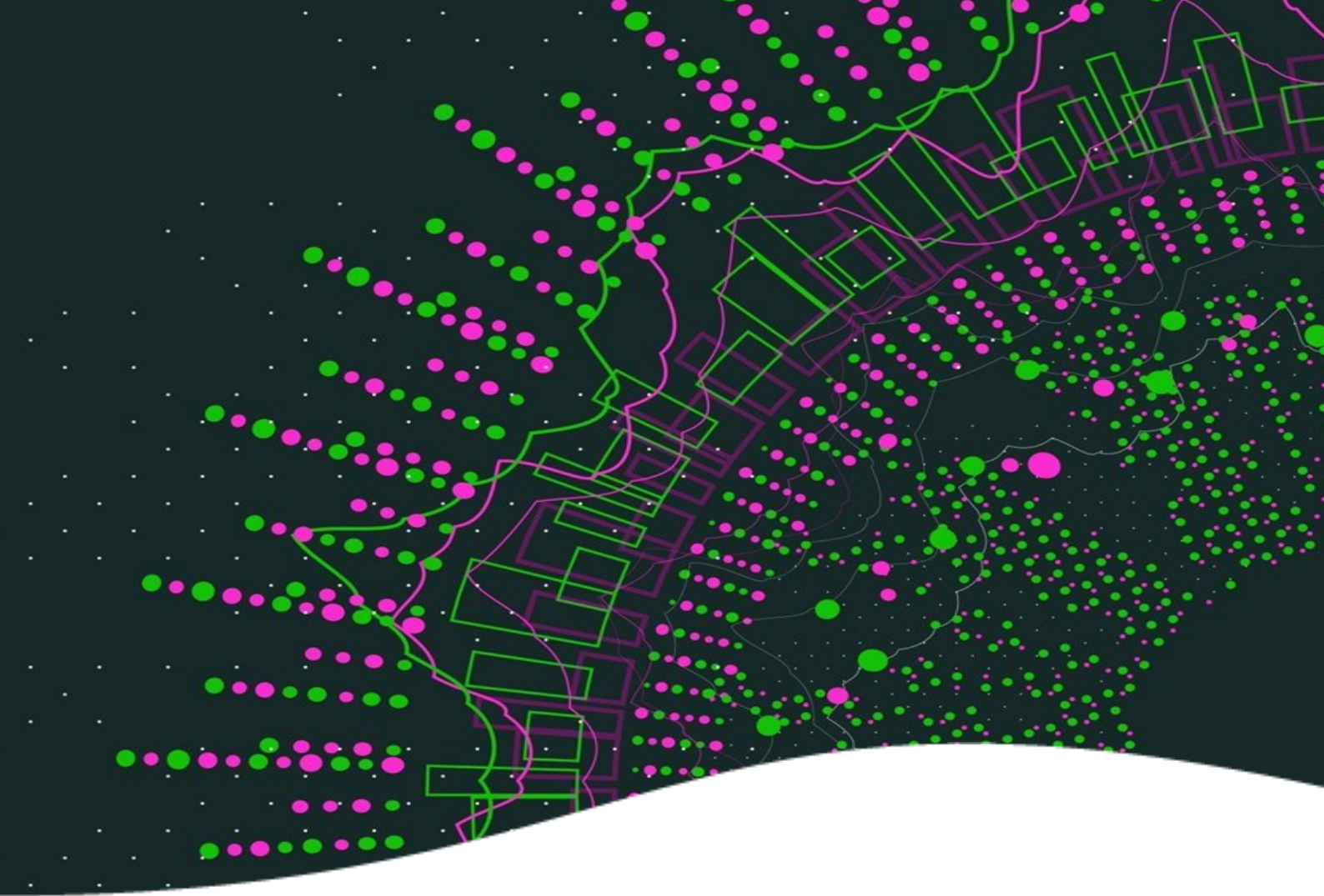




11th Colloquium of Genetics

Book of Abstracts

Ljubljana

25 September 2026

11th Colloquium of Genetics

Book of abstracts

Scientific committee:

Jernej Jakše
Nataša Štajner
Sabina Berne
Helena Volk
Maja Čemažar
Simon Horvat
Uroš Petrovič
Damjan Glavač

Organizing committee:

Jernej Jakše
Sabina Berne
Helena Volk
Nataša Štajner
Simona Kranjc Brezar
Boštjan Markelc

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WELCOME ADDRESS

Spoštovane mlade raziskovalke in mladi raziskovalci, drage kolegice in kolegi,

dobrodošli na 11. Kolokviju iz genetike, srečanju, ki ga Slovensko genetsko društvo namenja predvsem vam – mladim, ki s svojo radovednostjo, znanjem in svežimi idejami soustvarjate prihodnost genetike.

Letos smo se ponovno zbrali na Biotehniški fakulteti Univerze v Ljubljani, kjer je leta 2010 potekal prvi kolokvij Slovenskega genetskega društva, zato ima letošnje srečanje tudi poseben simbolni pomen – vračamo se tja, kjer se je začela tradicija dogodka, namenjenega predvsem mladim genetikom.

Genetika je danes eno najhitreje razvijajočih se in najbolj povezovalnih področij znanosti. Segaj od raziskovanja mikroorganizmov, rastlin in živali do razumevanja človekovega zdravja in boleznih ter pomembno prispeva k razvoju biotehnologije, medicine, kmetijstva in varovanja okolja. Kljub raznolikosti raziskovalnih tem nas združujeta želja po razumevanju življenja in odgovornost, da pridobljeno znanje uporabimo v dobro družbe. Srečanja, kot je današnje, nam omogočajo, da pogledamo preko meja lastnega raziskovalnega področja, spoznamo drugačne pristope in morda odkrijemo tudi nove ideje za raziskovanje na našem področju in nove priložnosti za sodelovanje.

Kolokvij mladim raziskovalkam in raziskovalcem ponuja priložnost, da predstavijo svoje delo, izmenjajo znanje in izkušnje ter navežejo nove strokovne in osebne stike. Predstavitev raziskovalnih rezultatov je pomemben korak na znanstveni poti, odprta razprava pa pogosto prinese nova vprašanja, zamisli in sodelovanja. Želimo si, da bi bil kolokvij prostor sproščenega, vključujočega in spodbudnega dialoga, v katerem je vsako dobro vprašanje enako dragoceno kot odgovor.

Naj vam letošnji 11. kolokvij prinese dragocene odzive, nova znanja in občutek, da ste pomemben del slovenske genetske skupnosti. Prihodnost genetike namreč nastaja tudi z vašim delom.

Vsem udeleženkam in udeležencem želimo uspešne predstavitve, ter veliko novih znanstvenih navdihov in poznanstev.

Maja Čemažar,
predsednica SGD



Dragi kolegi in mladi prijatelji genetike,

v imenu Slovenskega genetskega društva vas prisrčno pozdravljam na 11. kolokviju, ki ga skupaj pripravljata društvi SGD in SDHG.

V zadnjih nekaj letih je humana genetika doživela nesluten napredek, ki je genetiko premaknil iz osnovnih raziskav v neposredno zdravljenje bolnikov. Zdaj imamo 100-odstotno celoten genetski zapis človeka (Telomere-to-Telomere) in dešifriran preostali del genoma, ki je bil zaradi ponavljajočih se zaporedij prej nedostopen. To odpira vrata do razumevanja prej skritih genetskih vzrokov, predvsem za kompleksne bolezni. Uspešni so bili tudi pristopi k razvoju zdravil, ki temeljijo na tehnologiji CRISPR-Cas9 za zdravljenje npr. srpaste anemije in beta-talasemije. Združevanje genomskih podatkov milijonov ljudi v masovnih biobankah je raziskovalcem omogočilo razumevanje zapletenih povezav med geni in boleznimi. Umetna inteligenca (AI) zdaj lahko v kratkem času napove tridimenzionalne strukture proteinov in analizira mutacije. Orodja strojnega učenja so bistveno pospešila diagnosticiranje redkih bolezni in iskanje novih tarč za zdravila. Prihodnost genetike je res obetavna in sloni predvsem na vas, mladih.

Velika prednost vseh naših kolokvijev je interdisciplinarnost in povezanost vseh genetikov – od rastlinske, živalske in mikrobne do humane genetike. Ko smo skupaj, se drug od drugega učimo in tudi lažje rešujemo izzive.

Zato vam iskreno želim uspešne nastope in predstavitve na 11. kolokviju ter vas lepo pozdravljam.

Se vidimo!

Damjan Glavač,
predsednik SDHG



TABLE OF CONTENTS

<u>Program</u>	6
<u>List of Lectures</u>	8
<u>List of Posters</u>	9
<u>Animal Genetics Abstracts</u>	12
<u>Human Genetics Abstracts</u>	18
<u>Microbial Genetics Abstracts</u>	29
<u>Plant Genetics Abstracts</u>	34
<u>Author Index</u>	41
<u>Sponsors</u>	46

PROGRAM

Date: 25th September 2026

Venue: University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Lecture Hall A4

8:30 - 9:00 REGISTRATION

9:00 - 9:10 OPENING OF THE 11th COLLOQUIUM OF GENETICS

Jernej Jakše

9:10 - 9:40 PLENARY LECTURE

(Chair: Sabina Berne)

Sprayable RNAs: From gene silencing to gene expression in plants

Aline Koch (*Plant RNA Transport Group, Institute of Plant Sciences, University of Regensburg, Germany*)

9:45 - 11:30 ANIMAL GENETICS SHORT LECTURES

(Chairs: Simon Horvat, Tanja Kunej)

9:45 - 10:00 Relationship and inbreeding analysis based on pedigree in the population of Arabian Purebred horse in Slovenia

Anja Baš

10:00 - 10:15 The estimation of variance components for body weight in performance test of Jezersko-Solčava and Improved Jezersko-Solčava rams on the test station

Marko Bizjak

10:15 - 10:30 Fibre- and nucleus-resolved pipeline for spatial transcriptomics in skeletal muscle

Irma Zeljković

10:30 - 11:00 COFFEE BREAK AND POSTER SESSION (Lecture Hall A10)

11:00 - 12:00 HUMAN GENETICS SHORT LECTURES

(Chairs: Simona Kranjc Brezar, Damjan Glavač)

11:00 - 11:15 Development and *in vitro* functional evaluation of bacterial toxin-expressing plasmids for *in situ* cancer vaccination

Maja Cvetanoska

11:15 - 11:30 Pharmacokinetic optimization of electrochemotherapy for osteosarcoma mouse models

Saša Kupčič

11:30 - 11:45 Characterization of cell death pathways activated by electrochemotherapy

Lara Snoj

11:45 - 12:00 Advancing cancer gene therapy with electrotransfer of trans-amplifying mRNAs

Jaka Vrevc Žlajpah

12:00 - 13:00 MICROBIAL AND PLANT GENETICS SHORT LECTURES

(Chairs: Uroš Petrovič, Helena Volk)

- | | |
|---------------|--|
| 12:00 - 12:15 | Phased assemblies reveal hybrid genomes and wine-associated <i>Hanseniaspora</i> yeasts population structure
Katja Doberšek |
| 12:15 - 12:30 | Prevalence of siderophore synthesis genes among extended-spectrum β-lactam-resistant and susceptible <i>Escherichia coli</i> isolates from the Meža river and humans
Klemen Tršinar |
| 12:30 - 12:45 | Genome-wide association mapping of <i>Verticillium</i> wilt response in hop to support resistance breeding
Lucija Luskar |
| 12:45 - 13:00 | Genomic insights into composite populations of common bean (<i>Phaseolus vulgaris</i>) using whole genome sequencing
Eva Plestenjak |

13:00 - 14:15 LUNCH BREAK AND POSTER SESSION (Lecture Hall A10)

14:15 - 14:45 CLOSING LECTURE

(Chair: Jernej Jakše)

Electrochemical genosensor development

Samo B. Hočevár (*National Institute of Chemistry, Slovenia*)

14:45 - 15:30 CLOSING CEREMONY

Best lectures and posters awards, Closing of the 11th Colloquium of genetics and SGD assembly

LIST OF LECTURES

CODE	TITLE	PAGE
PLE	SPRAYABLE RNAS: FROM GENE SILENCING TO GENE EXPRESSION IN PLANTS	10
CLO	ELECTROCHEMICAL GENOSENSOR DEVELOPMENT	11
AL1	RELATIONSHIP AND INBREEDING ANALYSIS BASED ON PEDIGREE IN THE POPULATION OF ARABIAN PUREBRED HORSE IN SLOVENIA	12
AL2	THE ESTIMATION OF VARIANCE COMPONENTS FOR BODY WEIGHT IN PERFORMANCE TEST OF JEZERSKO-SOLČAVA AND IMPROVED JEZERSKO-SOLČAVA RAMS ON THE TEST STATION	13
AL3	FIBRE- AND NUCLEUS-RESOLVED PIPELINE FOR SPATIAL TRANSCRIPTOMICS IN SKELETAL MUSCLE	14
HL1	DEVELOPMENT AND <i>IN VITRO</i> FUNCTIONAL EVALUATION OF BACTERIAL TOXIN-EXPRESSING PLASMIDS FOR <i>IN SITU</i> CANCER VACCINATION	18
HL2	PHARMACOKINETIC OPTIMIZATION OF ELECTROCHEMOTHERAPY FOR OSTEOSARCOMA MOUSE MODELS	19
HL3	CHARACTERIZATION OF CELL DEATH PATHWAYS ACTIVATED BY ELECTROCHEMOTHERAPY	20
HL4	ADVANCING CANCER GENE THERAPY WITH ELECTROTRANSFER OF TRANS-AMPLIFYING mRNAs	21
ML1	PHASED ASSEMBLIES REVEAL HYBRID GENOMES AND WINE-ASSOCIATED HANSENIASPORA YEASTS POPULATION STRUCTURE	29
ML2	PREVALENCE OF SIDEROPHORE SYNTHESIS GENES AMONG EXTENDED-SPECTRUM β -LACTAM-RESISTANT AND SUSCEPTIBLE <i>ESCHERICHIA COLI</i> ISOLATES FROM THE MEŽA RIVER AND HUMANS	30
PL1	GENOME-WIDE ASSOCIATION MAPPING OF <i>VERTICILLIUM</i> WILT RESPONSE IN HOP TO SUPPORT RESISTANCE BREEDING	34
PL2	GENOMIC INSIGHTS INTO COMPOSITE POPULATIONS OF COMMON BEAN (<i>PHASEOLUS VULGARIS</i>) USING WHOLE GENOME SEQUENCING	35

LIST OF POSTERS

CODE	TITLE	PAGE
AP1	HYPOTHALAMIC GENE EXPRESSION PROFILING IN MOUSE LINES DIVERGENTLY SELECTED FOR OBESITY AND LEANNESS	15
AP2	SPATIAL EXPRESSION OF EPIGENETICALLY PRIORITIZED CANDIDATE GENES IN SKELETAL MUSCLE OF FAT AND LEAN MOUSE LINES	16
AP3	GENOME-WIDE ANALYSIS OF INTRONIC SPLICE-SITE VARIANTS IN MOUSE LINES SELECTED FOR FATNESS AND LEANNESS	17
HP1	EVALUATION OF RNA SEQUENCING AS A COMPLEMENTARY DIAGNOSTIC APPROACH IN PATIENTS WITH SUSPECTED HEREDITARY PREDISPOSITION TO BREAST, OVARIAN, PROSTATE AND PANCREATIC CANCER	22
HP2	LONG-READ SEQUENCING RESOLVES COMPLEX CYP2D6 STRUCTURAL VARIATION WITH CLINICALLY RELEVANT CONSEQUENCES	23
HP3	INTEGRATIVE MULTI-OMICS ANALYSIS IDENTIFIES CANDIDATE GENES ASSOCIATED WITH DUPILUMAB RESPONSE IN ATOPIC DERMATITIS	24
HP4	IDENTIFICATION OF POTENTIALLY SPLICEOGENIC VUS IN SLOVENIAN PATIENTS WITH SUSPECTED LYNCH SYNDROME	25
HP5	IMPACT OF TELOMERE LENGTH AND TERT POLYMORPHISMS ON CARDIOTOXICITY OF ADJUVANT RADIOTHERAPY IN DCIS PATIENTS	26
HP6	LONG-READ NANOPORE SEQUENCING ENABLES INTEGRATED MOLECULAR GRADING OF MENINGIOMAS	27
HP7	CIRCULATING TUMOR CELL AND CLUSTER DYNAMICS ACROSS PRECLINICAL 4T1 MOUSE MODELS OF METASTATIC BREAST CANCER	28
MP1	PRELIMINARY CHARACTERIZATION OF EXTRACELLULAR VESICLE-ASSOCIATED RNA IN PHYTOPATHOGENIC FUNGUS <i>VERTICILLIUM NONALFALFAE</i>	31
MP2	INDUCTION OF PROPHAGES AND BACTERIOCIN GENES BY THE MITOMYCIN C-TRIGGERED SOS RESPONSE IN <i>ESCHERICHIA COLI</i>	32
MP3	WHOLE GENOME CHARACTERISATION AND BIOSYNTHETIC GENE CLUSTER MINING OF RHIZOSPHERE BACTERIA	33
PP1	HOW DISRUPTING VIROID BIOGENESIS IMPACTS VIROID PROPAGATION	36
PP2	FLOW CYTOMETRIC ANALYSIS OF A CHIMERIC COMMON BEAN	37
PP3	PARENTAL GLUCOSINOLATE PROFILES AS PREDICTORS OF HYBRID PERFORMANCE IN CABBAGE	38
PP4	VIRUS-DERIVED SMALL INTERFERING RNAs AND THEIR POTENTIAL HOST TARGETS DURING MIXED VIRAL INFECTION OF GRAPEVINE	39
PP5	EXPRESSION PROFILING OF PHYTOCANNABINOID BIOSYNTHESIS GENES ACROSS <i>CANNABIS SATIVA</i> GENOTYPES AND CHEMOTYPES	40

SPRAYABLE RNAS: FROM GENE SILENCING TO GENE EXPRESSION IN PLANTS

Aline Koch

Plant RNA Transport Group, Institute of Plant Sciences, University of Regensburg, Germany

The demonstration that plants are capable to take up spray-applied RNA will pave the way for various applications and innovations in both basic and applied research. For example, the discovery that foliar spraying of double-stranded RNA induces intra- and interspecies RNA interference (RNAi; post-transcriptional gene silencing) has led to the development of dsRNA-based pesticides. In addition, recent evidence suggests that even exogenously applied mRNA can enter plant cells for protein translation (spray-induced gene expression). These groundbreaking findings will allow the implementation of other RNA species (e.g., circRNA, CRISPR/Cas-guided sgRNA) as "sprayable RNAs" with distinct gene editing and regulation properties. However, the establishment of RNA sprays to mediate gene silencing, gene knockout, epigenetic gene editing and protein synthesis (avoiding the generation of negatively connotated GMOs) requires mechanistic knowledge of RNA uptake and transport.

Understanding the routes, cell fate and how exogenously applied RNAs can overcome various external and internal barriers is a prerequisite for improving RNA uptake, stability and transport to ensure the efficacy of extracellular RNA applications in plant science.

PLE

Plenary Lecture

ELECTROCHEMICAL GENOSENSOR DEVELOPMENT

Samo B. Hočevár¹, Nikola Tasić¹, Alnilan Lobato^{1,2}, Ivan Konjević^{1,3}, Sebastijan Radišek⁴, Jernej Jakše⁵, Helena Volk⁵

¹ Department of Analytical Chemistry, National Institute of Chemistry, Ljubljana, Slovenia

² International Postgraduate School Jožef Stefan, Ljubljana, Slovenia

³ Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

⁴ Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia

⁵ Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Emerging pathogens create an urgent demand for rapid, sensitive, selective, and point-of-need detection tools. For example, early identification of plant disease is key to halting transmission, lowering management costs, and protecting yields. When a pathogen lacks typical symptoms or exhibits only atypical ones in the early stages of infection, the importance of developing fast and accessible on-site detection methods is obvious. The identification of pathogens such as viruses and viroids relies on conventional or advanced molecular biology techniques [1]; however, these protocols are often costly, time-consuming, restricted to centralized laboratories, and require specialized personnel. Common examples include the gold standard polymerase chain reaction (PCR), reverse transcription quantitative PCR (RT-qPCR), loop-mediated isothermal amplification (LAMP), enzyme-linked immunosorbent assay (ELISA), and CRISPR-Cas-based diagnostics [2].

Electrochemistry offers a relatively simple and efficient framework for detecting various pathogens. Within this domain, electrochemical genosensing combines numerous approaches, primarily involving hybridization reactions and advanced technologies, such as catalytic hairpin assembly (CHA), CRISPR-Cas system(s), DNazymes, hybridization chain reaction (HCR), DNA nanowalker principles, etc., which are coupled with various electrochemical signal transduction strategies. Beyond offering unparalleled capabilities for miniaturization, electrochemical platforms can beneficially exploit the ongoing emergence of high-performing electrocatalysts to achieve very low limits of detection.

This lecture presents the development of a sensitive and selective genosensor for *Citrus bark cracking viroid* (CBCVd, *Cocadviroid rimocitri*) [3]. The genosensing platform utilizes streptavidin-agarose beads as a biocompatible immobilization matrix for a single-stranded DNA capture probe and leverages the superior performance of a 2D-layered electrocatalyst. Furthermore, some new detection principles and current limitations in electrochemical genosensor design will be addressed.

References:

1. F. Martinelli, R. Scalenghe, S. Davino, S. Panno, G. Scuderi, P. Ruisi, P. Villa, D. Stroppiana, M. Boschetti, L.R. Goulart, C.E. Davis, A.M. Dandekar, Advanced methods of plant disease detection. A review, *Agron Sustain Dev* 35 (2015) 1–25.
2. Z. Zhang, S. Li, Detection of viroids, in: *Fundamentals of Viroid Biology*, Elsevier, 2024: pp. 297–321.
3. J. Jakše, S. Radišek, T. Pokorn, J. Matousek, B. Javornik, Deep-sequencing revealed Citrus bark cracking viroid (CBCVd) as a highly aggressive pathogen on hop, *Plant Pathol* 64 (2015) 831–842.

Funding: Slovenian Research Agency: P1-0034, P4-0077, J1-3017, the Slovenian Research Agency's Young Researchers Program - grant agreements No. 56119 and 58179, and ViroiDoc - Horizon Europe MSCA Doctoral Networks, Reference Number HORIZON-MSCA-2023-DN-01-01, Marie Curie Grant Agreement Number: 101169421.

CLO

Closing Lecture

RELATIONSHIP AND INBREEDING ANALYSIS BASED ON PEDIGREE IN THE POPULATION OF ARABIAN PUREBRED HORSE IN SLOVENIA

Anja Baš¹, Barbara Luštrek¹, Nataša Gorišek², Mojca Simčič¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia

² University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia

THE ARABIAN PUREBRED (AP) horse is one of the oldest horse breeds, known for its endurance, agility, intelligence, and contribute substantially on other horse breeds. Breeding of AP horses in Slovenia began in the late 1980s with organized breeding established during the 1990s; Slovenia joined the World Arabian Horse Organisation in 2004.^{1,2} Its relatively small population makes monitoring relationship and inbreeding important for maintaining genetic diversity. The aim of this study was to describe the AP horse population in Slovenia and assess its population structure, relationship, and inbreeding.

We analysed pedigree and country-of-origin data from studbook records in the Central register of Equidae. After verification and harmonization, 3.378 animals were included in the analyses. Data were analysed using R.³ The pedigree analysis was carried out using the optiSel package.⁴ The AP horse population in Slovenia has increased over the last two decades, accompanied by more breeding animals and Slovenian-born foals. Analysis of animal origin revealed heterogeneous ancestry, with the greatest influence from horses born in the United States of America and Poland between 1970 and 1990. More than half of the foals born in Slovenia during the last ten years had parents also born and bred in Slovenia. The average maximum number of traced generations was 4.18, equivalent complete generations averaged 2.57, and the average pedigree completeness index was 49.3%. Almost 30% of animals were founders, while the deepest pedigree reached 15 generations. The average inbreeding coefficient (F) was 1.28%, with 32.8% of animals inbred. Among inbred animals, the highest individual F was 32.81%.

Both F and the proportion of inbred animals increased with pedigree completeness, suggesting that pedigree-based inbreeding may be underestimated due to incomplete pedigree information.

References:

1. Gorišek N. The Slovenian Stud Book of Arab Horses. Volume 1: 1990-2003. Ljubljana: Veterinary Faculty, University of Ljubljana. 2004.
2. Gorišek N. The Slovenian Stud Book of Arab Horses. Volume 3: 2009-2019. Ljubljana: Veterinary Faculty, University of Ljubljana. 2020 .
3. R Core Team. 2026. R: A Language in Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

AL1

Animal genetics

THE ESTIMATION OF VARIANCE COMPONENTS FOR BODY WEIGHT IN PERFORMANCE TEST OF JEZERSKO-SOLČAVA AND IMPROVED JEZERSKO-SOLČAVA RAMS ON THE TEST STATION

Marko Bizjak¹, Mojca Simčič¹, Anita Ule¹, Milena Kovač¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia

The two **SLOVENIAN SHEEP BREEDS** Jezersko-Solčava (JS) and Improved Jezersko-Solčava (IJS) are genetically related, as the IJS was developed by crossing JS with the Romanov breed. The aim of this study was to estimate variance components for body weight in IJS and JS rams during performance testing at the Logatec test station.

Young rams originating from different flocks in breeding programs were moved to the test station at an average age of 114.0 ± 19.9 days. Before the performance test period, they were housed in a quarantine barn to acclimatize to the new environment, penmates, and diet. Rams were weighed five times: at purchase (WTT), at the start of the performance test (WT0), and subsequently once per month at 30 ± 5 -day intervals (WT1, WT2, and WT3). Records from 2,263 rams tested between 2014 and 2024 were analysed, and a pedigree comprising 6,531 animals was constructed. The fixed part of the model included season, breed, litter size, and dam parity as class effects; ram age at weighing and the preceding lambing interval of the dam as linear regressions; and dam age as a quadratic regression nested within parity. For WTT, season was defined according to the ram's birth date, whereas for WT0–WT3 it was based on the month in which the performance test started. The random effects included the additive genetic effect and flock-year interaction. In addition, a repeatability animal model was fitted to the four body weights recorded during the performance test (WT0–WT3), treating repeated measurements as the same trait. Weighing order was included as a fixed effect, while the permanent environmental effect of the animal and flock-year interaction were fitted as random effects. Variance components and their ratios were estimated using REML as implemented in VCE-6.¹ Heritability estimates for WTT, WT0, WT1, WT2, and WT3 were 0.33, 0.40, 0.41, 0.42, and 0.43, respectively. The proportion of phenotypic variance explained by the flock-year interaction decreased from 0.25 at WTT to 0.16, 0.12, 0.10, and 0.09 at WT0, WT1, WT2, and WT3, respectively. In the repeatability model, heritability was estimated at 0.40, while the permanent environmental and flock-year effects accounted for 29% and 10% of the phenotypic variance, respectively.

The similar heritability estimates obtained from the single-trait and repeatability models indicate that a repeatability animal model is suitable for genetic evaluation of body weight in rams undergoing performance test at the test station.

References:

1. Groeneveld E, Kovač M, Mielenz N. VCE User's Guide and Reference Manual, version 6.0. 2010.

AL2

Animal genetics

FIBRE- AND NUCLEUS-RESOLVED PIPELINE FOR SPATIAL TRANSCRIPTOMICS IN SKELETAL MUSCLE

Irma Zeljković¹, Špela Simoneta¹, Denis Korady², Tanja Kunej¹, Uršula Prosenc Zmrzljak³, Simon Horvat¹, Miha Moškon⁴, Nejc Umek²

¹ University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia

² University of Ljubljana, Faculty of Medicine, Institute of Anatomy, Ljubljana, Slovenia

³ BIA Separations CRO, Molecular Biology Laboratory, Labena Ltd., Ljubljana, Slovenia

⁴ University of Ljubljana, Faculty of Computer and Information Science, Ljubljana, Slovenia

IMAGING-BASED SPATIAL TRANSCRIPTOMICS enables high-resolution analysis of gene expression while preserving the spatial organisation of intact tissue. Skeletal muscle presents distinct challenges for imaging-based spatial transcriptomics because its principal functional units are large, elongated, multinucleated myofibres that differ from conventionally segmented mononuclear cells [1]. Consequently, standard cell-based analytical pipelines may not adequately represent its biological and structural organisation. We developed and evaluated a skeletal muscle-specific computational pipeline for automated fibre- and nucleus-resolved analysis of Xenium spatial transcriptomics data.

Our pipeline comprises custom myofibre and nuclear segmentation, spatial transcript assignment, transcript density-based quality control adapted to variation in myofibre size, clustering, cell- and fibre-type annotation, and spatially variable gene (SVG) detection. We evaluated the pipeline using 19 Xenium datasets generated from *tibialis anterior* muscles of 16 mice. We assessed segmentation performance against manual reference annotations. We evaluated fibre clustering and annotation using immunohistochemical fibre-type annotations and benchmarked SVG detection methods using experimental datasets, simulated spatial expression patterns, and adjacent serial sections.

Custom model training substantially improved nuclear segmentation compared with the default Cellpose model, whereas myofibre segmentation was already robust with the default model and showed no clear additional improvement after custom training. Transcript density-based quality control reduced the influence of myofibre size on downstream analyses. Fibre-level analysis recovered the expected organisation of canonical muscle fibre types, whereas integrated analysis of 160,048 nuclei identified biologically plausible myonuclear, vascular, stromal, immune, and myogenic populations.

Benchmarking revealed substantial differences among clustering approaches and, consistent with previous benchmarking studies, among SVG detection methods [2]. Clustering methods differed in their agreement with immunohistochemical fibre-type annotations and in their ability to generate internally coherent clusters. Similarly, SVG detection methods varied markedly in the number of identified SVGs, agreement between methods, performance on synthetic datasets, and reproducibility across adjacent tissue sections. More conservative methods generally identified smaller SVG sets, whereas more permissive approaches returned larger sets that often included these genes. Our findings demonstrate that analytical choices at multiple stages of the pipeline can substantially affect the biological interpretation of skeletal muscle spatial transcriptomics data.

By treating myofibres and nuclei as distinct analytical units and systematically evaluating alternative approaches, the proposed pipeline provides practical guidance for reproducible analysis of skeletal muscle Xenium data and may inform similar spatial transcriptomics approaches in other structurally complex tissues.

References:

1. Umek N, et al. *In situ* spatial transcriptomic analysis of human skeletal muscle using the Xenium platform. *Cell Tissue Res.* 2025;399(3):291–302.
2. Li Z, et al. Systematic benchmarking of computational methods to identify spatially variable genes. *Genome Biol.* 2025;26(1):285.

AL3

Animal genetics

HYPOTHALAMIC GENE EXPRESSION PROFILING IN MOUSE LINES DIVERGENTLY SELECTED FOR OBESITY AND LEANNESS

Luka Puhar¹, Jernej Jakše², Martin Šimon¹, Simon Horvat¹, Nicholas M. Morton³, Špela Mikec^{1,4}, Kalyani Pandya⁵, James Prendergast⁵, Tanja Kunej¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia

² University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia

³ Nottingham Trent University, Centre for Systems Health and Integrated Metabolic Research, Clifton Campus, NG11, 8NG, United Kingdom

⁴ Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Slovenia

⁵ University of Edinburgh, The Queen's Medical Research Institute, Centre for Cardiovascular Science, Edinburgh, United Kingdom

OBESITY is a complex metabolic condition in which the central nervous system, particularly the hypothalamus, plays a key role in regulating feeding behavior, metabolism, and hormonal balance¹. The association between variation in hypothalamic gene expression and body mass has been demonstrated repeatedly, yet many underlying molecular mechanisms remain unclear. Previous studies in the FLI (Fat) and FHI (Lean) mouse lines have focused on SNP analysis² and alternative polyadenylation³, but the hypothalamic transcriptome in polygenic obesity models remains insufficiently explored. In this study, we performed RNA-seq on hypothalamic samples from these two mouse lines, divergently selected for obesity and leanness. Building on our previous identification of 19 differentially expressed protein-coding genes mapping within *Fob* (Fat-line obesity) QTL regions, we extended the set of differentially expressed protein-coding genes from 171 to 195 based on an updated annotation assessment (using the same statistical cut-off: Bonferroni-adjusted $p \leq 0.01$; $|\text{fold change}| > 1.3$). The extended set was then used to identify additional candidate genes beyond previously mapped obesity QTL regions. We subsequently obtained functional annotations from publicly available databases and performed a literature review to investigate their biological roles. Of the 19 *Fob* QTL candidates, seven genes (*Pla2g4e*, *Phf6*, *Ifrd1*, *Bub1b*, *Spint1*, *Scg5*, *Ly6a*) have well-established associations with adipocyte differentiation, regulation of food intake, insulin signaling, and metabolic homeostasis. Among the genes outside the QTL regions, *Ahcy* and *Mill2* emerged as potential candidate genes with *Ahcy* located at the edge of the mapped *Fob* region and *Mill2* previously associated with obesity. The remaining differentially expressed genes represent additional candidates warranting further functional investigation.

Collectively, these findings expand the identification of potential obesity-associated genes beyond previously mapped *Fob* QTL regions and highlight candidates that may contribute to the genetic basis of obesity.

References:

1. Bell CG, Walley AJ, Froguel P. The genetics of human obesity. *Nat Rev Genet.* 2005;6(3):221–34.
2. Šimon M, Mikec Š, Atanur SS, Konc J, Morton NM, Horvat S, et al. Whole genome sequencing of mouse lines divergently selected for fatness (FLI) and leanness (FHI) revealed several genetic variants as candidates for novel obesity genes. *Genes Genom.* 2024;46(5):557.
3. Mikec Š, Horvat S, Wang H, Michal J, Kunej T, Jiang Z. Differential alternative polyadenylation response to high-fat diet between polygenic obese and healthy lean mice. *Biochem Biophys Res Commun.* 2023;666:83–91.

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AP1

Animal genetics

SPATIAL EXPRESSION OF EPIGENETICALLY PRIORITIZED CANDIDATE GENES IN SKELETAL MUSCLE OF FAT AND LEAN MOUSE LINES

Špela Simoneta¹, Irma Zeljković¹, Nejc Umek², Uršula Prosenc Zmrzljak³, Simon Horvat¹, Tanja Kunej¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia

² University of Ljubljana, Faculty of Medicine, Institute of Anatomy, Ljubljana, Slovenia

³ BIA Separations CRO, Molecular Biology Laboratory, Labena Ltd., Ljubljana, Slovenia

OBESITY is a complex multifactorial disease resulting from interactions between genetic and environmental factors. Its genetic basis is predominantly polygenic, involving numerous genetic variants with cumulative effects, and can be efficiently studied with mouse models generated through long-term selective breeding for obesity-associated traits. Epigenetic mechanisms, particularly DNA methylation, may contribute to obesity-associated variations in gene expression. Spatial transcriptomics enables gene expression to be examined within skeletal muscle tissue [1]. We examined the spatial expression of candidate genes associated with DNA methylation, fat deposition and *Fob* quantitative trait loci (QTLs) in skeletal muscle of divergently selected fat (FLI) and lean (FHI) mouse lines [2].

We selected 48 candidate genes based on their location within *Fob* QTL regions, the presence of CpG islands, published evidence on DNA methylation and obesity, and information from relevant biological databases. Spatial gene expression was analysed using the Xenium Prime 5k Mouse Pan Tissue & Pathways Panel (10x Genomics) on fresh-frozen tibialis anterior (TA) muscle tissues from 14-week-old male FLI and FHI mice. Muscle fibers were segmented using Cellpose, a deep-learning model trained on manually annotated image patches, to analyse gene expression and its spatial distribution within individual fibers. Four candidate genes were differentially expressed between fat and lean mouse lines ($p_{\text{adj}} < 0.05$; $|\log_2\text{FC}| > 1$). Among these, *Baiap2l1* (BAI1-associated protein 2-like 1) showed statistically significant differential expression across muscle fiber types 2a, 2b and 2x.

These findings identify candidates for targeted investigation of DNA methylation and its relationship with gene expression and muscle fiber phenotype in polygenic obesity.

References:

1. Umek N, et al. In situ spatial transcriptomic analysis of human skeletal muscle using the Xenium platform. *Cell Tissue Res.* 2025;399(3):291–302.
2. Horvat S, et al. Mapping of obesity QTLs in a cross between mouse lines divergently selected on fat content. *Mamm Genome.* 2000;11(1):2–7.

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AP2

Animal genetics

GENOME-WIDE ANALYSIS OF INTRONIC SPLICE-SITE VARIANTS IN MOUSE LINES SELECTED FOR FATNESS AND LEANNESS

Matevž Žula¹, Martin Šimon¹, Simon Horvat¹, Tanja Kunej¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia

OBESEITY is a complex polygenic phenotype shaped by genetic, environmental, and regulatory mechanisms. Alternative RNA splicing contributes to transcript and protein diversity, and single nucleotide polymorphisms (SNPs) located at 5' splice donor and 3' splice acceptor sites may modify splice-site recognition and transcript processing. However, the contribution of splice-site variants to fatness and leanness has not yet been systematically investigated. Therefore, this study established a catalogue of intronic splice-site SNPs in mouse lines divergently selected for fatness (FLI) and leanness (FHI). The catalogue was constructed using whole-genome sequencing data from FLI and FHI mouse lines [1]. The dataset comprised 1,320 unique splice-site SNPs associated with 1,209 genes. Variants were subsequently prioritized according to mouse line specificity, splice donor/acceptor position, protein-coding transcript annotation, and overlap with fatness-related quantitative trait loci (QTL). Only variants fulfilling all prioritization criteria were retained. This annotation-based approach highlighted five candidate genes: *2310069G16Rik*, *Snappc1* (small nuclear RNA activating complex polypeptide 1), and three olfactory receptor genes; *Or4f57*, *Or4f56*, and *Or4c10b*, which were interpreted cautiously given the complex organization of the olfactory receptor gene family. To complement this annotation-based selection, variants were further evaluated using AlphaGenome, a sequence-based deep learning model for regulatory variant effect prediction [2]. This *in silico* analysis estimated allele-dependent changes in splice-site, splice-site-usage, and splice-junction signals, which were interpreted as separate model outputs. In the broader analysis, rs29216035 in *Snappc1* ranked among the top ten predicted splice-affecting variants. The most informative output for this variant was the splice-junction score, suggesting a potential change in donor-acceptor connectivity rather than only a local change in splice-site strength. Because SNAPC1 contributes to the transcription of small nuclear RNAs (snRNA), several of which are essential components of the spliceosome, rs29216035 was highlighted for potential further RNA-level validation of possible *Snappc1* transcript changes.

Overall, this catalogue links genome-wide splice-site discovery with targeted RNA-level validation in these selected mouse lines.

References:

1. Šimon M, Mikec Š, Atanur SS, Konc J, Morton NM, Horvat S, et al. Whole genome sequencing of mouse lines divergently selected for fatness (FLI) and leanness (FHI) revealed several genetic variants as candidates for novel obesity genes. *Genes Genom.* 2024;46(5):557.
2. Avsec Ž, Latysheva N, Cheng J, Novati G, Taylor KR, Ward T, et al. Advancing regulatory variant effect prediction with AlphaGenome. *Nature.* 2026;649(8099):1206–1218.

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AP3

Animal genetics

DEVELOPMENT AND IN VITRO FUNCTIONAL EVALUATION OF BACTERIAL TOXIN-EXPRESSING PLASMIDS FOR *IN SITU* CANCER VACCINATION

Maja Cvetanoska^{1,2}, Maja Čemažar^{1,2,3}, Gregor Serša^{1,2,4}, Urška Kamenšek^{1,2,5}

¹ Institute of Oncology Ljubljana, Department of Experimental Oncology, Ljubljana, Slovenia

² University of Ljubljana, Faculty of Medicine, 1000 Ljubljana, Slovenia

³ University of Primorska, Faculty of Health Sciences, Izola, Slovenia

⁴ University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia.

⁵ University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia.

***IN SITU* CANCER VACCINATION** represents a promising strategy to induce antitumor immunity directly within the tumor microenvironment by promoting tumor antigen release and local immune activation. In this study, we develop a novel plasmid-based approach for *in situ* cancer vaccination using secreted forms of bacterial toxins. The approach combines pore-forming toxins (PFTs), which induce tumor cell lysis and antigen release by forming pores in the cell membrane, with superantigens (SAGs), which enhance local immune activation through potent T-cell stimulation. This study aims to identify candidate PFTs and SAGs for the development of plasmids encoding secreted toxin forms, construct the plasmids, and evaluate their expression and biological activity *in vitro*.

The most promising PFT- and SAG-encoding plasmids were selected for *in vivo* evaluation in murine tumor models, where the plasmids were delivered using intratumoral gene electrotransfer (GET). Candidate toxins were identified through literature analysis, and their coding sequences were codon-optimized for mammalian expression. Secretion signals derived from perforin-1 (PRF1) and interleukin-2 (IL2) gene were initially evaluated using green fluorescent protein (GFP) reporter plasmids. Secretion efficiency was assessed in B16-F10 murine melanoma cells following GET by comparing intracellular and extracellular GFP fluorescence and quantifying GFP in culture supernatants by ELISA. Based on these experiments, the PRF1 secretion signal was selected and fused upstream of toxin genes. Constructs were synthesized, cloned into a mammalian expression vector, and verified by restriction analysis and whole-plasmid sequencing. Toxin transcription following GET was evaluated by qRT-PCR. SAG activity was evaluated in immune-cell co-cultures by measuring secretion of selected immunostimulatory cytokines (IFN- γ , IL-2, and TNF- α) by ELISA and T-cell proliferation using Carboxyfluorescein succinimidyl ester (CFSE) dilution assays. Plasmids encoding six PFT candidates: cytolysin A, Shiga toxin 2, streptolysin O, aerolysin, alpha-hemolysin, and a synthetic nanopore and three SAG candidates: staphylococcal enterotoxins A and B and toxic shock syndrome toxin were successfully generated. Reporter experiments demonstrated extracellular GFP following transfection, supporting the functionality of the selected PRF-1 secretion signal. Transcription of the toxin genes was confirmed by qRT-PCR.

These results establish the initial feasibility of a plasmid-based platform for local expression and secretion of bacterial toxins as mediators of *in situ* cancer vaccination. Further functional studies are required to confirm the biological activity of toxins produced by transfected tumor cells.

Based on these analyses, one PFT and one SAG candidate will be selected for *in vivo* validation following intratumoral GET in murine tumor models. Functional characterization of the secreted toxins is ongoing and will determine their cytotoxic, membrane-permeabilizing, and immunostimulatory activities.

HL1

Human genetics

PHARMACOKINETIC OPTIMIZATION OF ELECTROCHEMOTHERAPY FOR OSTEOSARCOMA MOUSE MODELS

Saša Kupčič^{1,2}, Urška Kamenšek^{1,3}, Maja Čemažar^{1,4}, Urša Lampreht Tratar^{1,5}

¹ Department of Experimental Oncology, Institute of Oncology, Ljubljana, Slovenia

² Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

³ Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

⁴ Faculty of Health Sciences, University of Primorska, Izola, Slovenia

ELECTROCHEMOTHERAPY (ECT) is a local ablative treatment that combines the administration of cytostatic drugs with electric pulses to enhance intracellular drug delivery. ECT has already shown promising efficacy and a positive safety profile in the treatment of bone metastases (1–3). However, previously investigated protocols have not been specifically adapted to the unique characteristics of bone tissue. The cytostatics currently used in clinical ECT are bleomycin (BLM) and cisplatin (CDDP), while oxaliplatin (OXA), a third-generation platinum compound, has recently emerged as a promising alternative (4).

To further explore the feasibility of using ECT with these cytostatics for treatment of osteosarcoma (OS) tumors, we first characterized their pharmacokinetic profiles in subcutaneous and orthotopic OS tumors to identify the optimal time window for electric pulse delivery (Approval no. U34401-17/2023/1).

Upon tumor establishment, cytostatics were delivered intravenously. Blood and tumor samples were subsequently collected at predefined time points, and cytostatic concentrations were determined. Based on these pharmacokinetic profiles, the optimal timing of pulse delivery was selected according to the time point associated with the highest intratumoral drug concentration. We subsequently tested the antitumor effectiveness of ECT in subcutaneous and orthotopic OS tumors. Based on the pharmacokinetic findings, electric pulses were delivered 3 min after rapid intravenous bolus administration of the cytostatics in the subcutaneous model and 10 min after administration in the orthotopic model.

Analysis of tumor growth delay demonstrated a clear antitumor effect of ECT with CDDP in both tumor models. In subcutaneous tumors, ECT with BLM also resulted in a pronounced tumor growth delay. Survival analysis revealed no differences between treatment groups in the subcutaneous model, whereas in the orthotopic model, treatment with ECT combined with either CDDP or BLM was associated with improved survival.

In this study we characterized crucial pharmacokinetic parameters for three cytostatics. The optimized protocols were subsequently evaluated for their antitumor activity. These findings provide a pharmacokinetic rationale for adapting ECT protocols to bone tumors and support further investigation of ECT as a local treatment strategy for osteosarcoma.

References

- 1 Mir LM, Orlowski S, Belehradek J, et al. Electrochemotherapy Potentiation of Antitumour Effect of Bleomycin by Local Electric Pulses. *Eur J Cancer* 1991;27(1):6–72.
- 2 Campanacci L, Cevolani L, De Terlizzi F, et al. Electrochemotherapy Is Effective in the Treatment of Bone Metastases. *Current Oncology* 2022;29(3):1672–1682; doi: 10.3390/CURRONCOL29030139.
- 3 Deschamps F, Tselikas L, Yevich S, et al. Electrochemotherapy in radiotherapy-resistant epidural spinal cord compression in metastatic cancer patients. *Eur J Cancer* 2023;186:62–68; doi: 10.1016/j.ejca.2023.03.012.
- 4 Ursic K, Kos S, Kamenšek U, et al. Comparable effectiveness and immunomodulatory actions of oxaliplatin and cisplatin in electrochemotherapy of murine melanoma. *Bioelectrochemistry* 2018;119:161–171; doi: 10.1016/J.BIOELECTCHEM.2017.09.009.

HL2

Human genetics

CHARACTERIZATION OF CELL DEATH PATHWAYS ACTIVATED BY ELECTROCHEMOTHERAPY

Lara Snoj^{1,2}, Michael Dengler³, Amar Balihodžić³, Ziva Pisljar^{1,2}, Masa Omerzel^{1,2}, Tanja Jesenko^{1,2}, Gregor Sersa^{1,2,4}, Maja Cemazar^{1,2,5}, Bostjan Markelc^{1,2,6}

¹ University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

² Institute of Oncology Ljubljana, Department of Experimental Oncology, Ljubljana, Slovenia

³ Medical University of Graz, Department of Oncology, Graz, Austria

⁴ University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia

⁵ University of Primorska, Faculty of Health Sciences, Izola, Slovenia

⁶ University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

ELECTROCHEMOTHERAPY (ECT) is a local ablative treatment in which electric pulses transiently permeabilize the cell membrane and thereby enhance intracellular drug delivery. Bleomycin (BLM) is particularly suited to this approach, as electroporation markedly increases its cytotoxic activity at the treatment site. DNA damage is regarded as the principal cause of cell death after ECT with BLM, primarily leading to mitotic catastrophe and apoptosis. The cellular response may also engage several distinct forms of regulated cell death, including necroptosis, pyroptosis, ferroptosis and immunogenic cell death (ICD), which is characterized by the release of danger-associated molecular patterns (DAMPs) and stimulation of antitumor immunity. Although ECT with BLM has been shown to induce ICD, the temporal dynamics of cell death and DAMP release have not been defined. This information is essential for understanding both the cytotoxic and the immunogenic potential of the treatment.

We therefore set out to characterize the kinetics of cell death following ECT with BLM and to determine the contribution of individual cell-death pathways to treatment response. The sensitivity of murine melanoma (B16F10) and colon carcinoma (CT26) cells was assessed by cell viability (PrestoBlue) and long-term proliferative capacity (clonogenic assay). This defined two BLM concentrations that killed 70 % of the cells: IC70-H (PrestoBlue) and IC70-L (clonogenic assay). Lactate dehydrogenase (LDH) release did not differ substantially between experimental groups, indicating limited and relatively stable membrane lysis after ECT, whereas PrestoBlue revealed a pronounced, time-dependent loss of cell viability. Consistently, time-lapse microscopy showed that most of the treated cells remained viable while adopting an enlarged, flattened, senescent-like morphology. Cell death and membrane integrity were further assessed by propidium iodide staining and flow cytometry. To dissect the pathways involved, we used CRISPR-Cas9 gene editing to generate tumor cell lines deficient in key regulators of apoptosis (Bax/Bak), pyroptosis (GSDMD/E) and necroptosis (MLKL). After ECT with BLM, Bax/Bak- and GSDMD/E-knockout (KO) cells differed only marginally from wild-type (WT) cells, showing slightly reduced sensitivity to treatment. To corroborate these in vitro observations RNA sequencing was performed. The in vivo relevance of individual pathways was then tested in subcutaneously induced CT26 WT and MLKL KO tumor models in Balb/c mice. Notably, MLKL KO tumors responded worse to ECT with BLM than WT tumors.

Together, our results indicate that ECT with BLM does not result in rapid cell death, especially at the IC70-L; however, there is a pronounced effect on the proliferative capacity of the cells. Further, in the CT26 tumor model in vivo the results showed that necroptosis importantly contributes to antitumor efficacy of ECT with BLM. Ongoing characterization of the KO models will further clarify the cellular responses underlying ECT and their potential impact on treatment efficacy and immunogenicity.

HL3

Human genetics

ADVANCING CANCER GENE THERAPY WITH ELECTROTRANSFER OF TRANS-AMPLIFYING mRNAs

Jaka Vrevc Žlajpah ^{1,2}, Maja Čemažar ^{1,2,3}, Gregor Serša ^{1,2,4}, Urša Lampreht Tratar ^{1,2,5}, Urška Kamenšek ^{1,2,6}, Boštjan Markelc ^{1,2,6}, Katja Uršič Valentinuzzi ^{1,2}

¹ Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia

² University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

³ University of Primorska, Izola, Faculty of Health Sciences, Slovenia

⁴ University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia

⁵ University of Ljubljana, Veterinary Faculty, Ljubljana Slovenia

⁶ University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

The clinical success of mRNA vaccines, high transfection efficiency and absence of insertional mutagenesis have accelerated interest in **mRNA-THERAPEUTICS** for cancer therapy. However, its short-lived protein expression requires repeated administrations, limiting efficient clinical translation. To address this limitation, we investigated gene electrotransfer (GET) of naked, adapted bipartite trans-amplifying RNA (taRNA) system as a new platform for cancer gene therapy.

The system consisted of unmodified non-replicating mRNA encoding the replicase of Semliki Forest virus (nrRNA-REPL), which amplifies a co-delivered unmodified reporter mRNA encoding green fluorescent protein (trRNA-EGFP, *in vitro*) or luciferase (trRNA-LUC2, *in vivo*). Transfection efficiency (flow-cytometry, live-cell imaging, ddPCR) and viability (PrestoBlue®) were monitored on B16-F10 cells and taRNA *in vivo* performance on B16-F10-tumor-bearing C57BL/6 mice (IVIS® imaging platform, U34401-3/2022/17). To optimize delivery and amplification, five electroporation protocols (EP1: 1300 V/cm, 1 Hz, 100 µs, 8 pulses; EP2: 1300 V/cm, 5 kHz, 100 µs, 8 pulses; EP3: 600 V/cm, 1 Hz, 5 ms, 8 pulses; EP4: 120 V/cm, 1 Hz, 20 ms, 2 pulses; EP5: HV (4+4 pulses): 1300 V/cm, 100 µs, 1 Hz; LV: 8 pulses, 140 V/cm, 50 ms, 2Hz), hyaluronidase pre-treatment and a wide range of taRNA concentrations were tested.

In vitro, we confirmed trans-amplification by describing a population of highly positive GFP cells (hGFP), which were non-existent after GET of trRNA-eGFP alone, and is responsible for elevated and prolonged GFP expression in taRNA groups. EP3 was the most efficient protocol for GET of taRNA-eGFP, and, when compared to lipofection (standard for mRNA delivery), resulted in higher fraction of hGFP cells. Trans-amplification was nrRNA-REPL dose dependent and was most prominent when 4 µg or 8 µg of nrRNA-REPL were used. Increasing nrRNA-REPL dose resulted in reduced cell viability. At mRNA level, amplification was detected as early as 6 h post GET, reaching a remarkable 384-median-fold change between copies in taRNA-eGFP and trRNA-eGFP groups at 24 h post GET. GET of taRNA enables concomitant amplification of at least two transgenes (trRNA-eGFP and trRNA-LUC2) *in vitro*.

At *in vivo* level, we efficiently delivered naked mRNAs intratumorally and showed successful trans-amplification, as co-delivery of nrRNA-REPL resulted in median 108.6-fold increase in area under the bioluminescence curve (compared to trRNA-LUC2 alone). EP5 alone and EP3 with hyaluronidase pre-treatment resulted in the most efficient delivery of taRNA components. Strong amplification of bioluminescence intensity was observed at low trRNA-LUC2 doses (0.078 µg), whereas increasing the dose reduced the amplification effect. 10 µg and 20 µg of nrRNA-REPL yielded comparable amplification levels.

Our work establishes an mRNA-based platform for cancer therapy with enhanced, prolonged, but transient transgene expression and represents a first-time description of naked taRNA use in oncology setting. Ongoing studies aim to further evaluate the safety and therapeutic applications of this approach.

HL4

Human genetics

EVALUATION OF RNA SEQUENCING AS A COMPLEMENTARY DIAGNOSTIC APPROACH IN PATIENTS WITH SUSPECTED HEREDITARY PREDISPOSITION TO BREAST, OVARIAN, PROSTATE AND PANCREATIC CANCER

Gregor Hertl¹, Vita Šetrajčič Dragoš^{1,2}, Srdjan Novaković², Lorena Kunc¹, Vida Stegel^{1,3}

¹ Department of Molecular Diagnostics, Institute of Oncology Ljubljana, Slovenia

² Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

³ Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

HEREDITARY PREDISPOSITION accounts for a clinically important proportion of breast, ovarian, prostate and pancreatic cancers and is most commonly associated with pathogenic or likely pathogenic variants (P/LPV) in genes linked to hereditary breast and ovarian cancer syndrome (HBOC). Identification of P/LPV carriers is essential for targeted surveillance, risk-reducing management and cascade testing of relatives. Standard genetic testing relies primarily on short-read next-generation sequencing (NGS) of exonic and flanking intronic regions and copy-number analysis. However, deep intronic and complex structural variants may remain undetected, while the functional effect of variants of uncertain significance (VUS) on splicing often cannot be resolved by DNA analysis alone. RNA transcript analysis can reveal aberrant splicing and may therefore complement DNA-based testing. Our aim was to optimize a blood-based RNA-sequencing workflow and evaluate whether RNA transcript analysis as a complement to DNA sequencing can increase diagnostic yield and improve the clinical interpretation of VUS in patients with suspected HBOC after negative or inconclusive standard short-read DNA testing.

The RNA-sequencing workflow was initially optimized using 16 peripheral-blood RNA samples. Because transcript abundance of some target genes in peripheral blood is low, three approaches were compared: whole-transcriptome sequencing using TruSeq Stranded mRNA, target-enriched RNA sequencing, and target-enriched RNA sequencing after an additional DNase treatment. Target-enriched libraries were prepared using Illumina RNA Prep with Enrichment and sequenced on a NextSeq 550 platform. The optimized workflow will subsequently be evaluated in at least 100 adult patients with suspected HBOC, including patients with negative standard short-read DNA testing and a subgroup carrying VUS predicted in silico to affect RNA splicing. RNA-seq data will be analyzed to identify aberrant splicing and transcript alterations that may indicate underlying genetic variants and guide targeted DNA analysis. Preliminary results from 16 samples showed that target enrichment substantially improved coverage of HBOC-associated genes and transcripts compared with whole-transcriptome sequencing alone, with coverage increases exceeding 100-fold across the majority of targeted regions. Residual genomic DNA contamination was observed in target-enriched libraries, and additional DNase treatment partially reduced, but did not completely eliminate, this contamination. Further optimization, including incorporation of poly(A) selection into the target-enrichment workflow, may increase the proportion of informative RNA reads and improve transcript coverage. RNA transcript analysis is also expected to provide functional evidence for the interpretation and reclassification of splicing-relevant VUS.

Overall, the combined DNA/RNA approach is expected to increase diagnostic yield, help define patient subgroups that may benefit most from complementary RNA testing, and provide a basis for its future implementation in routine clinical diagnostics of HBOC.

HP1

Human genetics

LONG-READ SEQUENCING RESOLVES COMPLEX CYP2D6 STRUCTURAL VARIATION WITH CLINICALLY RELEVANT CONSEQUENCES

Urška Janžič¹, Špela Mikec², Barbara Jenko Bizjan^{2,3}, Jernej Kovač^{2,3}, Vita Dolžan¹

¹Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia

²Clinical Institute of Special Laboratory Diagnostics, Division of Paediatrics, University Medical Centre Ljubljana, Ljubljana, Slovenia

³Faculty of Medicine, University of Ljubljana, Slovenia

CYP2D6 pharmacogenomic genotyping remains challenging because of extensive homology with CYP2D7, frequent copy-number variation, and hybrid alleles (1). We first benchmarked CYP2D6 genotyping tools on three complex reference samples with established diplotypes, HG001, HG01190 and NA19758. FASTQ data was obtained from European nucleotide archive and The International Genome Sample Resource. For long-read sequencing (LRS), pb-StarPhase, Aldy4, and ONT Chinook tools were evaluated using Oxford Nanopore Technologies data, while PyPGx and Aldy4 were evaluated using Illumina short-read sequencing (SRS) data. pb-StarPhase and PyPGx showed 100% concordance with the reference genotypes and were therefore selected for downstream comparison. Thirteen randomly selected samples were sequenced using the Illumina NovaSeq 6000 and ONT PromethION platforms. Pharmacogenomic analysis of SRS data was performed using PyPGx, whereas LRS data were analyzed using pb-StarPhase. Discordant cases were further investigated using OpenArray genotyping on the QuantStudio 12K Flex platform, copy-number assays targeting the 5'UTR and exon 9, phased-read inspection in IGV, and pb-StarPhase structural reconstruction. LRS provided additional phasing and structural information in 3/13 samples.

In case 1, SRS using PyPGx assigned CYP2D6 *1/*36+*10, corresponding to a normal metabolizer phenotype, whereas LRS using pb-StarPhase assigned *1/*4x3, corresponding to an intermediate metabolizer. OpenArray reported an undetermined genotype with two possible configurations, *1/*68+*4 or *10/*68+*4, both including the decreased-function *4 allele, consistent with the LRS result. Also copy-number analysis showed 5'UTR CN=4 and exon 9 CN=2, supporting a complex hybrid configuration. In case 2, SRS identified candidate *1, *2, and *65 alleles but could not reconstruct a definitive diplotype and therefore could not assign a phenotype. In contrast, LRS resolved *1/*68+*2. Orthogonal copy-number testing showed 5'UTR CN=3 and exon 9 CN=2, consistent with an additional CYP2D6/CYP2D7 hybrid copy and supporting the long-read structural interpretation. In case 3, SRS using PyPGx assigned *68+*4/*68+*4, whereas LRS using pb-StarPhase reconstructed the more complex *4x2/*68x2+*4 configuration. OpenArray analysis showed 5'UTR CN=5 and exon 9 CN approximately 2–2.5, supporting copy-number complexity. Long-read inspection additionally provided haplotype-specific structural evidence. However, the rare *68x2+*4 configuration did not have an established function assignment in the current phenotype-translation framework, resulting in unassigned function despite improved structural resolution.

These findings demonstrate three clinically relevant contributions of LRS: possible phenotype reclassification, resolution of an otherwise unreportable diplotype, and identification of a rare structural haplotype beyond current clinical interpretation frameworks.

References:

1 Turner AJ, Nofziger C, Ramey BE, Ly RC, Bousman CA, Agúndez JAG, et al. PharmVar tutorial on CYP2D6 structural variation testing and recommendations on reporting. Clin Pharmacol Ther. 2023;114(6):1220-1237. doi:10.1002/cpt.3044.

HP2

Human genetics

INTEGRATIVE MULTI-OMICS ANALYSIS IDENTIFIES CANDIDATE GENES ASSOCIATED WITH DUPILUMAB RESPONSE IN ATOPIC DERMATITIS

Martina Krušič¹, Mario Gorenjak¹, Uroš Potočnik^{1,2,3}, Maruška Marovt^{4,5}

¹ University of Maribor, Faculty of Medicine, Centre for Human Molecular Genetics and Pharmacogenomics, Maribor, Slovenia

² University of Maribor, Faculty of Chemistry and Chemical Engineering, Maribor, Slovenia

³ University Clinical Centre Maribor, Department for Science and Research, Maribor, Slovenia

⁴ University of Maribor, Faculty of Medicine, Maribor, Slovenia

⁵ University Clinical Centre Maribor, Dermatovenereology, Maribor, Slovenia

ATOPIC DERMATITIS (AD) is a heterogeneous chronic inflammatory disease, and clinical response to dupilumab varies considerably among patients (1-3). Reliable biomarkers predicting treatment response could facilitate personalized therapeutic strategies. We aimed to identify peripheral blood molecular signatures associated with clinical response to dupilumab using an integrative transcriptomic and genomic approach.

Peripheral blood samples were collected from 18 dupilumab-naïve patients with moderate-to-severe AD at baseline and after 16–18 weeks of treatment. Five patients were classified as non-responders based on failure to achieve EASI75. RNA sequencing was performed using a multi-stage analytical strategy designed to reduce treatment- and inflammation-related transcriptional signals before comparing baseline responders and non-responders. Differentially expressed genes were further investigated using gene ontology enrichment and weighted gene co-expression network analysis. Genomic profiling was performed in 34 patients, including the initial cohort and 16 additional long-term responders. Candidate genes were prioritized through integration of transcriptomic and genomic data. Selected candidates were additionally assessed by RT-qPCR.

After systematic removal of therapy- and inflammation-associated signals, 817 genes were differentially expressed between baseline responders and non-responders. Functional analyses identified response-associated pathways related to ribosomal structure, cell-cycle regulation, protein kinase activity, chromatin organization, and DNA-related processes. Integration of transcriptomic and genomic findings prioritized eight candidate genes potentially associated with dupilumab response: RPL18A, RPS28, FAU, MASTL, AURKA, TAF2, BUB1B, and RNF135. RT-qPCR demonstrated concordant expression direction for BUB1B, AURKA, and RPS28, although no candidate reached statistical significance after regression analysis.

This integrative multi-stage approach identified candidate peripheral blood molecular signatures potentially associated with clinical response to dupilumab in AD. Given the limited sample size, these results should be considered exploratory and hypothesis-generating and require validation in larger independent cohorts before clinical application.

References

1 Weidinger S, Novak N. Atopic dermatitis. The Lancet. 2016;387(10023):1109-22.

2 Mastrafatti S, Vrioni G, Bakakis M, Nicolaidou E, Rigopoulos D, Stratigos AJ, et al. Atopic Dermatitis: Striving for Reliable Biomarkers. J Clin Med. 2022;11(16).

3 Bakker D, de Bruin-Weller M, Drylewicz J, van Wijk F, Thijs J. Biomarkers in atopic dermatitis. J Allergy Clin Immunol. 2023;151

HP3

Human genetics

IDENTIFICATION OF POTENTIALLY SPLICEOGENIC VUS IN SLOVENIAN PATIENTS WITH SUSPECTED LYNCH SYNDROME

Lorena Kunc^{1,2}, Vita Šetrajčič Dragoš^{1,2}, Vida Stegel^{1,3}, Srdjan Novaković^{1,2}

¹ Institute of Oncology Ljubljana, Department of Molecular Diagnostics, Ljubljana, Slovenia

² University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

³ University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

LYNCH SYNDROME (LS) is the most common hereditary colorectal cancer syndrome and is caused by germline pathogenic/likely pathogenic (P/LP) variants in the mismatch repair (MMR) genes. A subset of variants of uncertain significance (VUS) are identified during routine genetic testing, some of which may alter pre-mRNA splicing. Spliceogenic variants located beyond canonical GT-AG splice sites are particularly difficult to interpret and are therefore frequently classified as VUS. In silico splicing tools are valuable for identifying such variants, but computational predictions alone do not establish their actual effect on mature mRNA. We therefore assessed how many VUS identified during routine genetic testing were predicted to affect pre-mRNA splicing and could benefit from further RNA analysis.

Between 2020 and 2025, 690 patients referred for germline genetic testing due to suspected LS underwent multigene panel testing of hereditary colorectal cancer predisposition genes: APC, BMPR1A, EPCAM, MLH1, MSH2, MSH3, MSH6, MUTYH, NTHL1, PMS2, POLD1, POLE, PTEN, SMAD4, STK11 and TP53. Targeted NGS was performed using hybrid capture library preparation with the TruSight Hereditary Cancer Panel (Illumina). Splicing effects were assessed using SpliceAI, with a delta score ≥ 0.20 considered predictive of a potential spliceogenic effect. Variants were classified according to ACMG/AMP guidelines.

P/LP variants were identified in 19% of the 690 patients. Among patients without a P/LP variant, 186 (33.2%) harbored at least one VUS. In total, 127 distinct VUS were identified in these patients, five (3.9%) of which were predicted to affect pre-mRNA splicing.

VUS remain a major challenge in genetic diagnostics because their clinical significance is uncertain and they cannot be used to guide clinical management. In our cohort, five VUS were predicted to affect pre-mRNA splicing and were therefore identified as potential candidates for further RNA analysis. Functional assessment of their effects on mature mRNA could provide additional evidence for variant reclassification, improve molecular diagnosis, and support appropriate genetic counselling and surveillance of patients and their at-risk relatives.

HP4

Human genetics

IMPACT OF TELOMERE LENGTH AND TERT POLYMORPHISMS ON CARDIOTOXICITY OF ADJUVANT RADIOTHERAPY IN DCIS PATIENTS

Maja Marinčič¹, Tanja Marinko^{2,3}, Katarina Trebušak Podkrajšek¹, Vita Dolžan¹, Katja Goričar¹

¹ Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

² Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

³ Institute of Oncology Ljubljana, Ljubljana, Slovenia

DUCTAL CARCINOMA *in situ* (DCIS) is a non-invasive form of breast cancer commonly treated with surgery and adjuvant radiotherapy (RT). RT reduces the risk of local recurrence but is associated with potential cardiotoxicity. Because radiation can impair genomic stability maintained by telomeres, this study aimed to determine whether leukocyte telomere length (LTL) or TERT polymorphisms affect early cardiotoxicity of adjuvant RT.

Our study included 183 DCIS patients treated with adjuvant RT. Cardiotoxicity was assessed by measuring serum NT-proBNP levels after RT and 6 months after RT. Genomic DNA was isolated from peripheral blood samples, and all patients were genotyped for TERT rs2736098, rs2736100 and rs10069690 using competitive allele-specific PCR. Relative LTL was determined using monochrome multiplex quantitative PCR and expressed as the ratio between the telomere and housekeeping gene amplicons. Nonparametric statistical tests and logistic regression were used for data analysis. Median LTL increased from 0.86 (0.75-0.97) before RT 0.92 (0.79-1.03) to after RT ($p < 0.001$) and remained elevated 6 months after RT at 0.91 (0.82-1.08, ($p < 0.001$)). LTL after RT and 6 months after RT were not associated with NT-proBNP levels (all $p > 0.050$). Carriers of the TERT rs2736098 CT genotype showed a higher risk of elevated NT-proBNP levels after RT (OR = 2.17 (1.04–4.50), $p = 0.038$), even when adjusted for cardiac disease and age (OR = 2.66 (1.19–5.98), $p = 0.020$). In the dominant model, the association was only observed in multivariable analysis (OR = 2.33 (1.06–5.12), $p = 0.035$). No significant association was observed between TERT rs2736100 or rs10069690 and NT-proBNP levels after RT or at 6-month follow-up (all $p > 0.050$).

In conclusion, LTL changes significantly after RT but is not associated with early cardiotoxicity of RT, whereas the TERT polymorphism might affect NT-proBNP levels, thereby potentially serving as a biomarker of early RT-induced cardiotoxicity.

Acknowledgements

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HP5

Human genetics

LONG-READ NANOPORE SEQUENCING ENABLES INTEGRATED MOLECULAR GRADING OF MENINGIOMAS

Sara Petrin¹, Špela Kert¹, Miha Jerala¹, Matic Bošnjak¹, Andrej Zupan¹, Alenka Matjašič¹

¹Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

MENINGIOMAS are the most common primary central nervous system tumours. Despite their generally favourable prognosis, they are biologically and clinically heterogeneous, with variable risks of progression and recurrence. Molecular grading of meningiomas can improve risk stratification beyond conventional histopathological grading based on the World Health Organization Classification of Central Nervous System (CNS WHO) tumours. DNA methylation profiling and copy number alterations (CNAs) provide important information for molecular grading of meningiomas and can both be assessed from a single long-read nanopore sequencing assay. We therefore evaluated the potential of long-read nanopore sequencing to provide molecular information relevant for integrated risk stratification of meningiomas.

We performed long-read nanopore sequencing of DNA extracted from 20 fresh-frozen meningioma tissue samples, comprising 10 CNS WHO grade 1 and 10 CNS WHO grade 2 tumours. Rapid methylation-based classification using the bioinformatics tool nanoDx assigned all 20 tumour samples to the meningioma methylation family. Data generated after 72 hours of sequencing were further analysed using the bioinformatics tool MNP-Flex, which classified 19/20 tumours into either the benign or intermediate meningioma methylation class, while one sample showed high-confidence scores for both methylation classes and could not be unambiguously classified. Genome-wide CNA profiles were generated from long-read nanopore data using QDNAseq, enabling the identification of chromosome-arm losses. The derived molecular data were subsequently integrated with histopathological features to determine the Integrated Score (IntS) and Integrated Grade (IntG), which stratify meningioma tumours based on the risk of tumour progression and recurrence. Among CNS WHO grade 1 meningiomas two were reclassified from low risk to intermediate risk, while among CNS WHO grade 2 meningiomas, one was reclassified as low risk and three samples were reclassified as high risk for tumour progression and recurrence. Such risk stratification could support more individualised clinical management and follow-up of patients with meningiomas.

Our results demonstrate that a single long-read nanopore sequencing assay can provide both methylation and genome-wide copy number information relevant for molecular grading of meningiomas. Integration of these data with conventional histopathological features enables molecularly informed risk stratification and shows the potential of long-read nanopore sequencing as a multimodal approach for meningioma characterization and grading.

HP6

Human genetics

CIRCULATING TUMOR CELL AND CLUSTER DYNAMICS ACROSS PRECLINICAL 4T1 MOUSE MODELS OF METASTATIC BREAST CANCER

Veronika Škrjanc^{1,2}, Simona Kranjc Brezar^{1,2}, Maja Čemažar^{1,2,3}, Bostjan Markelc^{1,2,4}, Živa Pišljarić^{1,2}, Cvetka Grašič Kuhar^{1,2}, Simona Miceska^{1,2}, Veronika Kloboves-Prevodnik^{1,2,5}, Tanja Jesenko^{1,2}

¹ Institute of Oncology Ljubljana, Ljubljana, Slovenia

² University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

³ University of Primorska, Faculty of Health Sciences, Izola, Slovenia

⁴ University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

⁵ University of Maribor, Faculty of Medicine, Maribor, Slovenia

CIRCULATING TUMOR CELLS (CTCs), both as individual cells and as clusters, are key mediators of metastatic dissemination. However, their dynamics in preclinical models remain insufficiently understood. This study compared three 4T1 triple-negative breast cancer mouse models: i) a subcutaneous (SC) model and ii) an orthotopic mammary fat pad (MFP) model in immunocompetent BALB/c mice, and iii) an orthotopic MFP model in immunodeficient nude mice. The aim was to establish an *in vivo* mouse platform for the investigation of CTCs, enabling their isolation, characterization, and *ex vivo* expansion. CTCs and CTC clusters were isolated from blood collected at predefined tumor volumes using the Parsortix® system. They were then characterized by morphological and immunofluorescence (IF) analyses, which involved staining for Hoechst, pan-cytokeratin, CD45, vimentin, and N-cadherin. CTCs were detected in all models from the earliest tumor stages, and their numbers increased with tumor volume, showing a positive correlation with primary tumor burden. Their phenotypic characterization by IF showed a predominance of hybrid (epithelial-mesenchymal) CTCs across all models. In contrast, CTC clusters were present throughout tumor progression at stable levels, showed no correlation with tumor volume or metastatic burden, and consisted of both homotypic and heterotypic clusters. Neither CTC nor CTC cluster size changed with increasing tumor burden, indicating stable physical characteristics during disease progression. In conclusion, all three 4T1 models are suitable for studying CTCs with CTC numbers increasing with primary tumor burden. However, higher CTC yields make the orthotopic nude mouse model particularly suitable for downstream applications such as *ex vivo* culture and expansion, where maximal CTC isolation is required.

Overall, the findings indicate that while tumor burden influences the quantity of CTCs, key qualitative features of CTCs including clustering behavior, size, and phenotypic diversity remain consistent across different 4T1 models and host immune conditions.

HP7

Human genetics

PHASED ASSEMBLIES REVEAL HYBRID GENOMES AND WINE-ASSOCIATED *HANSENIASPORA* YEASTS POPULATION STRUCTURE

Katja Doberšek¹, Mia Žganjar², Eva Praprotnik³, Jernej Jakše⁴, Jaka Razinger³, Uroš Petrovič², Neža Čadež¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Ljubljana, Slovenia

² Jožef Stefan Institute, Department of Molecular and Biomedical Sciences, Ljubljana, Slovenia

³ Agricultural Institute of Slovenia, Plant Protection Department, Ljubljana, Slovenia

⁴ University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia

HANSENIASPORA YEASTS are characterised by accelerated evolution, reduced genomes with low GC content, and extensive gene loss compared to other Saccharomycotina yeasts. Their genomes are highly dynamic, displaying strong variation in heterozygosity, structure, and ploidy both between species and among isolates, representing a rare example of long-term hypermutator lineages in fungi. We investigated two closely related species with different ecological histories: *H. uvarum*, long associated with wine environments in Europe, and *H. opuntiae*, originally isolated from cacti in Hawaii but now increasingly found in European vineyards. Many of the European *H. opuntiae* isolates show hybrid ancestry with *H. pseudoguilliermondii*, suggesting hybridization facilitated their spread into wine-associated habitats.

To explore the genomic basis of adaptation to vineyard environments, we generated haplotype resolved genome assemblies for representative *Hanseniaspora* isolates and sequenced and phenotyped a collection of 200 isolates comprising *H. uvarum*, *H. opuntiae*, and putative hybrids originating from diverse environments. Population structure analyses revealed distinct lineages within each species that largely correlate with geographic origin, indicating ongoing local adaptation and region-specific evolutionary histories. Phenotypic assays uncovered predominantly species-specific profiles, where hybrid isolates consistently outperformed both, *H. uvarum* and *H. opuntiae* isolates under vineyard related stress conditions. Ploidy estimation revealed that most hybrids are triploid, supporting a 2:1 genomic contribution from *H. opuntiae* and *H. pseudoguilliermondii*.

Overall, our results indicate that hybridization and changes in genomic architecture are likely important contributors to fitness variation and adaptation in *Hanseniaspora*.

ML1

Microbial genetics

PREVALENCE OF SIDEROPHORE SYNTHESIS GENES AMONG EXTENDED-SPECTRUM β -LACTAM-RESISTANT AND SUSCEPTIBLE *ESCHERICHIA COLI* ISOLATES FROM THE MEŽA RIVER AND HUMANS

Klemen Tršinar¹, Vanesa Pivar¹, Kristina Arko¹, Valerija Tkalec^{2,3}, Tadeja Vajdič¹, Marjanca Starčič Erjavec^{1,2}

¹University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, Maribor, Slovenia

²National Laboratory of Health, Environment and Food, Department of Medical Microbiology, Maribor, Slovenia

³University of Maribor, Faculty of Medicine, Department of Microbiology, Maribor, Slovenia

⁴University of Ljubljana, Biotechnical Faculty, Department of Microbiology, Ljubljana, Slovenia

ESCHERICHIA COLI requires iron for growth and acquires it through high-affinity chelating molecules called siderophores [1]. The aim of the study was to determine the prevalence of synthesis genes for three different siderophores, associated with the pathogenicity of *E. coli* in humans: *iroB* (salmochelins), *iucD* (aerobactin) and *irp2* (yersiniabactin), in four different groups of *E. coli*: A) River Meža isolates resistant to extended spectrum β -lactam antibiotics (ESBL Meža) [2], B) River Meža isolates sensitive to extended spectrum β -lactam antibiotics (non-ESBL Meža) [2], C) humans clinical isolates resistant to extended spectrum β -lactam antibiotics (ESBL human) and D) human non-clinical isolates sensitive to extended spectrum β -lactam antibiotics (non-ESBL human) [3].

A total of 80 non-clonal isolates (20 per group) showing the largest halos on CAS medium, indicating siderophore synthesis, were subjected to amplification of the targeted siderophore genes *iroB*, *iucD*, and *irp2* by PCR. The presence of amplicons (expected sizes 663bp for *iroB*, 602bp for *iucD*, 287bp for *irp2*) was confirmed by agarose gel electrophoresis. Results were statistically analyzed. The most prevalent gene was *irp2* (61%), followed by *iucD* (50%), while *iroB* was the least prevalent (26%). Among the tested isolates, 3 from the ESBL Meža group, 10 from the non-ESBL Meža group, 0 from the ESBL human group, and 4 from the non-ESBL human group lacked all targeted siderophore genes, despite exhibiting halos on CAS medium. Significant difference in gene prevalence were observed between the four groups for *irp2* and *iucD* (χ^2 , $p < 0.01$), while no significant difference was observed for *iroB* gene (χ^2 , $p = 0.28$). Post-hoc Fisher's exact tests with Bonferroni correction showed significantly higher prevalence of *irp2* in the ESBL human group compared with both non-ESBL groups. For *iucD*, a significantly higher prevalence was observed in the ESBL human group compared with the non-ESBL Meža.

These patterns reflect ecological difference between the groups. In the continuation of the research, quantitative real-time PCR analysis of siderophore gene expression will be performed to evaluate their transcriptional activity in selected isolates.

References:

1. Mey AR, Gómez-Garzón C, Payne SM. Iron Transport and Metabolism in *Escherichia*, *Shigella*, and *Salmonella*. EcoSal Plus 2021;9:eESP-0034-2020.
2. Starčič Erjavec M, Vrecl E, Zajec T, et al. Occurrence of multi-drug resistant *Escherichia coli* in a river without hospital effluents. ASM Microbe. June 4-7, 2026, Washington, DC, 2026.
3. Starčič Erjavec M, Jesenko B, Petkovšek Ž, Žgur-Bertok D. Prevalence and Associations of *tcpC*, a gene encoding a Toll/Interleukin-1 receptor domain-containing protein, among *Escherichia coli* urinary tract infection, skin and soft tissue infection, and commensal isolates. J Clin Microbiol 2010;48:966-8.

PRELIMINARY CHARACTERIZATION OF EXTRACELLULAR VESICLE-ASSOCIATED RNA IN PHYTOPATHOGENIC FUNGUS *VERTICILLIUM NONALFALFAE*

Miona Kovachevikj¹, Tjaša Cesar¹, Taja Jeseničnik¹, Veronika Kralj-Iglič², Jernej Jakše¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia

² University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia

EXTRACELLULAR VESICLES (EVs) are increasingly studied as carriers of biologically active molecules and potential mediators of communication between fungal pathogens and their hosts. While EV-associated RNA has been described in several fungal species, its composition and potential functions in *Verticillium nonalfalfae* remain uncharacterized.

In this study, we performed a preliminary characterization of the RNA cargo associated with EVs produced by *V. nonalfalfae* under different *in vitro* growth conditions. Fungal cultures were grown on Czapek–Dox (CD) and xylem-simulating medium (XSM), followed by EV isolation and total RNA sequencing. Preliminary comparison of EV-associated transcriptomes identified 318 differentially expressed transcripts between the two conditions, including 283 downregulated and 35 upregulated transcripts in XSM-derived EVs. Gene Ontology enrichment analysis associated the differentially expressed transcripts primarily with translation, ribosome-related processes, RNA processing, metabolism, and transport. These preliminary findings provide an initial overview of the transcript composition of *V. nonalfalfae* EVs and provide a basis for further investigation of their RNA cargo.

Future work will include expanded total RNA sequencing, small RNA sequencing, and prediction of fungal microRNA-like RNAs (miRNAs) and their potential targets. EV-associated RNA will also be compared with intracellular fungal RNA and between *V. nonalfalfae* isolates with contrasting virulence to investigate selective RNA packaging and potential pathogenicity-related RNA signatures.

MP1

Microbial genetics

INDUCTION OF PROPHAGES AND BACTERIOCIN GENES BY THE MITOMYCIN C-TRIGGERED SOS RESPONSE IN *ESCHERICHIA COLI* – A PRELIMINARY STUDY

Pia Poglajen¹, Tadeja Vajdič¹, Marjanca Starčič Erjavec^{1,2}

¹ University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, Maribor, Slovenia

² University of Ljubljana, Biotechnical Faculty, Department of Microbiology, Ljubljana, Slovenia

THE BACTERIAL SOS RESPONSE is a global regulatory network that is activated by DNA damage, with mitomycin C being one of the most commonly used inducers due to its DNA-damaging activity. In addition to activating DNA repair pathways, the SOS response can trigger prophage induction into the lytic cycle and stimulate the synthesis and release of bacteriocins [1]. Many *Escherichia coli* strains harbour prophages and bacteriocin determinants that can be activated by the bacterial SOS response [2]. The aim of this study was to evaluate the prevalence of SOS response-inducible bacteriophages and bacteriocins in selected *E. coli* environmental bacterial isolates in comparison with faecal *E. coli* isolates from healthy humans.

Twenty-one bacterial isolates, 10 in the environmental group (ACC20-9, ACC20-10, AMC1-1, AZC1-13, FMC100-1, KMC100-6, KMC100-9, PMC1-1, PZC1-4, PZC1-19) and 11 in the human faecal group (BJ2, BJ3, BJ4, BJ9, BJ10, BJ14, BJ15, BJ16, BJ17, BJ18 and BJ26) were cultivated in LB broth and treated with mitomycin C at a final concentration of 1 µg/mL. Bacterial growth was monitored by measuring optical density at 600 nm (OD₆₀₀). Chloroform-treated lysates were prepared from cultures that exhibited a reduction in OD₆₀₀ following mitomycin C treatment and were subsequently tested for activity using spot and overlay assays with an indicator strain. After addition of mitomycin C the OD₆₀₀ value of 8 environmental isolates (ACC20-9, ACC20-10, AMC1-1, FMC100-1, KMC100-6, KMC100-9, PZC1-4, and PZC1-19) and 8 human faecal isolates BJ2, BJ4, BJ9, BJ10, BJ14, BJ15, BJ16 and BJ17) was reduced. Spot and overlay assays using an indicator strain revealed that two environmental isolates (ACC20-10 and AMC1-1) and four human faecal isolates (BJ2, BJ4, BJ15, and BJ16) harboured inducible prophages, whereas two environmental isolates (FMC100-1 and PZC1-4) and three human faecal isolates (BJ9, BJ10, and BJ17) produced inducible bacteriocins. Subsequent overlay assays with diluted lysates showed phage titres of 2.3×10^6 , 2.0×10^2 , 6.3×10^4 , 2.6×10^6 , 4.4×10^6 , and 1.0×10^2 PFU/mL for isolates ACC20-10, AMC1-1, BJ2, BJ4, BJ15, and BJ16, respectively. These results suggest differences in the prevalence of mitomycin C-inducible prophages and bacteriocins between environmental and human faecal isolates. However, further studies involving a larger number of isolates are required to confirm these observations.

Furthermore, this study highlights the importance of combining growth monitoring, spot assays, and dilution analyses to reliably distinguish bacteriophage activity from bacteriocin-mediated inhibition.

References:

1. Lima-Noronha MA, Fonseca DLH, Oliveira RS, Freitas RR, Park JH, Galhardo RS. Sending out an SOS - the bacterial DNA damage response. Genet Mol Biol. 2022;45:e20220107.
2. Podlessek Z, Žgur Bertok D. The *Escherichia coli* SOS Response: Much More than DNA Damage Repair. In: Starčič Erjavec M, editor. *Escherichia coli* - Old and New Insights. London: IntechOpen; 2021.

MP2

Microbial genetics

WHOLE GENOME CHARACTERISATION AND BIOSYNTHETIC GENE CLUSTER MINING OF RHIZOSPHERE BACTERIA FROM MARIBOR ISLAND INHIBITING ESBL- AND CARBAPENEMASE-PRODUCING ENTEROBACTERIALES

Tjaša Zajec¹, Tadeja Vajdič¹, Luka Jeršič¹, Valerija Tkalec², Urška Kramar², Jan Gojznikar³, Polonca Ferk³, Marjanca Starčič Erjavec^{1,4}

¹ University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, Koroška cesta 160, 2000 Maribor, Slovenia

² National Laboratory of Health, Environment and Food, Centre for Medical Microbiology, Department of Medical Microbiology, Maribor, Slovenia

³ University of Ljubljana, Faculty of Medicine, ELIXIR-SI Centre / IBMI, Karantanska ulica 37, 2000 Maribor, Slovenia

⁴ University of Ljubljana, Biotechnical Faculty, Department of Microbiology, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

The 2024 WHO Bacterial Priority Pathogens List ranks carbapenem-resistant and third-generation cephalosporin-resistant Enterobacterales among the critical-priority pathogens, highlighting the urgent need for research and development of novel **ANTIBACTERIAL AGENTS** against these organisms. [1]. Members of the genus *Streptomyces* are responsible for the production of more than two-thirds of naturally derived antibiotics used in human medicine and continue to be intensively explored for novel bioactive molecules, as *Streptomyces* genomes encode dozens of biosynthetic gene clusters (BGCs) [2,3]. We bioprospected the rhizosphere of Maribor Island, a protected alluvial river island. Crowded-plate method, agar overlay assays, ERIC-PCR, Sanger sequencing of 16S rDNA, short-read sequencing on the Illumina NextSeq 2000 platform and bioinformatical analysis, including antiSMASH 8.0, were used to reveal and characterise isolates with potential antimicrobial activity. Sixty-four isolates with potential antimicrobial activity were recovered by the crowded-plate method from the rhizosphere of five trees. Agar overlay assays using *Escherichia coli* DH5α and two uropathogenic *E. coli* strains as indicators identified nine isolates with potential antimicrobial activity. Based on 16S rDNA gene sequencing, seven isolates were identified as *Streptomyces*, one as *Bacillus*, and one as *Pseudomonas*. These isolates were subsequently sequenced on an Illumina NextSeq 2000 platform, and their genomes assembled *de novo*. Bioinformatic analysis showed that *Streptomyces* genomes spanned 8.0–11.8 Mb at 70.9–72.2% GC; orthologue clustering grouped 67,984 proteins into 10,275 clusters, 3,588 of them core, with 12.9% strain-specific singletons, and antiSMASH predicted 336 BGC regions (35–50 per genome), 178 of which matched no characterised cluster. All nine isolates were tested at 37 °C for their antimicrobial activity against a panel of twenty clinical carbapenem-resistant or third-generation cephalosporin-resistant *E. coli* and *Klebsiella pneumoniae* isolates. The five *S. xanthophaeus* isolates inhibited every clinical isolate, with inhibition radii of 16.8–27.2 mm, whereas the two unassigned *Streptomyces*, the *Bacillus wiedmannii* and the *Pseudomonas* isolate gave only marginal zones (up to 8.1 mm).

The performed mining uncovered a large reservoir of uncharacterised BGCs that can be in future exploited for novel antimicrobial therapy against carbapenem-resistant and third-generation cephalosporin-resistant Enterobacterales.

References:

1. World Health Organization. WHO Bacterial Priority Pathogens List, 2024. Geneva: World Health Organization; 2024.
2. Quinn GA, Banat AM, Abdelhameed AM, Banat IM. *Streptomyces* from traditional medicine: sources of new innovations in antibiotic discovery. J Med Microbiol. 2020;69(8):1040-1048.
3. Lee N, Hwang S, Kim W, Lee Y, Kim JH, Cho S, Kim HU, Yoon YJ, Oh MK, Palsson BO, Cho BK. Systems and synthetic biology to elucidate secondary metabolite biosynthetic gene clusters encoded in *Streptomyces* genomes. Nat Prod Rep. 2021;38(7):1330-

MP3

Microbial genetics

GENOME-WIDE ASSOCIATION MAPPING OF VERTICILLIUM WILT RESPONSE IN HOP TO SUPPORT RESISTANCE BREEDING

Lucija Luskar^{1,2}, Martin Waldinger³, Nicholi J. Pitra⁴, Alexander Feiner³, Sebastjan Radišek⁵, Jernej Jakše², Andreja Čerenak¹

¹ Department for Plants, Soil and the Environment, Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia

² University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia

³ Hopsteiner, Simon H. Steiner, Hopfen, Mainburg, Germany

⁴ Hopsteiner, S.S. Steiner, Inc., New York, USA

⁵ Plant Protection Diagnostics Laboratory, Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia

VERTICILLIUM WILT (VW), caused by the xylem-invading fungus *Verticillium nonalfalfae*, represents a major threat to European hop (*Humulus lupulus* L.) production. The development and progression of VW are determined by a complex interaction between the host plant and the pathogen. Susceptible cultivars may activate strong induced defence responses, however, these responses are often insufficient to prevent pathogen colonisation and vascular damage¹. In contrast, resistant genotypes appear to depend on more efficient vascular defence mechanisms, which may involve both rapid inducible responses and constitutive protective traits. Identifying the genomic regions that contribute to these contrasting disease outcomes is therefore essential for the development of reliable marker-assisted selection strategies.

In this study, we investigated a bi-parental F1 population derived from a cross between the resistant hop cultivar Wye Target and the susceptible hop BL2/1. Disease response was evaluated following controlled inoculation with *V. nonalfalfae* and quantified using a disease severity index (DSI) ranging from 0 to 5². The progeny was genotyped by sequencing and aligned to Apollo, a haplotype-resolved, reference genome. Because of the phased structure of the reference, the two genome phases were analysed independently. Association mapping was performed using five genome-wide association study frameworks: the mixed linear model implemented in TASSEL 5 and the MLM, MLMM, FarmCPU, and BLINK models implemented in GAPIT. To evaluate the practical value of the identified markers, the association signals were subsequently incorporated into a Random Forest model for genomic prediction.

Although the quantitative inheritance of resistance and the relatively limited population size affected the statistical power and stringency of genome-wide correction procedures, the analysis identified several reproducible association signals. Comparison of results across models and genome phases enabled the prioritisation of candidate loci with plausible roles in plant-microbe interactions. A prominent region on chromosome 1 was consistently detected and contained clusters of kinase-related genes, including wall-associated kinases (WAKs). These proteins are involved in the perception of cell-wall damage and pathogen-derived signals and may contribute to the activation of downstream defence pathways³.

Overall, the integration of haplotype-resolved association mapping with genomic prediction identified promising candidate loci and marker guidelines for VW resistance. In particular, the repeated detection of WAK- and kinase-rich regions provides new insights into the hop-*Verticillium* interaction and offers valuable resources for future hop resistance-breeding programmes.

References:

1 Luskar L, Jakše J, Čerenak A. Interaction of hop (*Humulus lupulus* L.) and *Verticillium nonalfalfae*. Hmeljarski Bilten. 2022;29:5–22.

2 Flajšman M, Radišek S, Javornik B. Pathogenicity assay of *Verticillium nonalfalfae* on hop plants. Bio Protoc. 2017;7(6):e2171. doi:10.21769/bioprotoc.2171.

3 Yang K, Qi L, Zhang Z. Isolation and characterization of a novel wall-associated kinase gene TaWAK5 in wheat (*Triticum aestivum*). Crop J. 2014;2(5):255–266. doi:10.1016/j.cj.2014.04.010.

PL1

Plant genetics

GENOMIC INSIGHTS INTO COMPOSITE POPULATIONS OF COMMON BEAN (*PHASEOLUS VULGARIS*) USING WHOLE GENOME SEQUENCING

Eva Plestenjak¹, Mohamed Neji¹, Barbara Pipan¹

¹Agricultural Institute of Slovenia, Crop Science Department, Ljubljana, Slovenia

The increasing demand for **RESILIENT CROP VARIETIES** has renewed interest in genetically diverse plant materials capable of adapting to variable environmental conditions. In common bean (*Phaseolus vulgaris*), seed coat colour is one of the most visible traits and is frequently used for variety identification. However, unlike conventional varieties that must fulfil the requirements of distinctness, uniformity and stability (DUS), the heterogeneity of composite populations limits their suitability for variety registration but increases their value for organic and low-input agriculture.

We investigated 50 composite populations of common bean containing between two and five distinct seed coat colour phenotypes. To determine whether seed coat colour variation reflects broader diversity, individual phenotypes were characterised at morphological, genetic and genomic levels over two years using 86 morphological descriptors and 47 molecular markers. Four representative composite population (17 phenotypes), together with two commercial varieties, were further selected for whole genome sequencing on the Illumina platform.

Sequencing generated high-quality short-read data with an average genome coverage of 30× and a mapping rate of 97.7% to the common bean reference genome. More than 8.6 million SNPs were identified, of which approximately 98% were located in non-coding regions, suggesting an important role of gene regulation in phenotypic diversity. Genomic analyses revealed marked differences among composite populations. The population exhibiting the highest number of seed coat phenotypes also displayed the greatest nucleotide diversity ($\pi = 0.22$) and allelic richness (1.38), whereas populations differing only in the proportion of background seed colour showed considerably lower nucleotide diversity ($\pi = 0.07$). Population structure analyses showed that phenotypes originating from the same composite population generally grouped together despite their visual differences, indicating a shared genetic background. Comparison of colour-stable and colour-segregating populations identified reduced genetic diversity in the latter group and revealed signatures of selection within specific genomic regions. Candidate regions detected by complementary XP-CLR and XP-EHH analyses were enriched for genes involved in phosphatidylinositol signalling pathways, highlighting their potential role in pigmentation processes and pigment transport regulation. In addition, molecular marker analyses revealed substantial within-population diversity, including variation in resistance-associated loci. This genetic variation was also reflected at the phenotypic level, where individuals differed in growth habit, pod characteristics, phenology and anthocyanin pigmentation.

Our results demonstrate that seed coat colour variation reflects much broader genetic and genomic differentiation than previously assumed. Seed coat colour should therefore be viewed not merely as a varietal descriptor but as a visible indicator of the diversity present within a population. Such heterogeneity offers considerable potential for future breeding strategies aimed at improving crop resilience and adaptation.

Acknowledgements

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PL2

Plant genetics

HOW DISRUPTING VIROID BIOGENESIS IMPACTS VIROID PROPAGATION

João Colaço¹, Helena Volk¹, José-Antonio Daròs², Jernej Jakše¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia

² Instituto de Biología Molecular y Celular de Plantas, CSIC, Universitat Politècnica de València, Spain

VIROIDS are small, non-coding RNA pathogens that infect and impair economically important plants, such as potatoes, tomatoes and citrus. Despite lacking protein-coding capacity, viroids completely rely on host cellular machinery for replication, movement, and pathogenicity. Host factors such as VirP1 and RNA polymerase II, have been implicated in viroid infection cycles, highlighting the importance of plant–viroid interactions at the transcriptomic level. Identifying conserved and species specific host genes can reveal novel factors involved in viroid infection, with potential for further gene silencing and decrease in virulence. This study aims to find host proteins and biological processes associated with infection across diverse plant species.

We uncovered differentially expressed genes and enriched pathways linked to viroid pathogenicity, by establishing a computational pipeline for large-scale plant transcriptomic analyses. Meta-analysis was used to integrate RNA-Seq transcriptional data from 21 different studies, 13 genomes, and varied experimental conditions. By aggregating datasets we improved statistical power for identifying DEGs. VIGS-targets is a scalable and modular bioinformatic workflow that automates the retrieval, processing, and analysis of publicly available RNA-seq plant-viroid experiment datasets. The pipeline features sample quality control, host plant and viroid genomes mapping, transcript quantification, and differential gene expression analysis. The workflow was designed to handle heterogeneous datasets originating from multiple plant species, experimental designs, and different sequencing strategies, while maintaining reproducibility and up-to-date plant-viroid transcriptomic studies.

Ongoing work is focus on within-study and cross-study normalization methods, clustering of DEGs by orthologous groups across species, gene reannotation, functional enrichment analysis, and to select up to 6 host genes with potential for Virus Induced Gene Silencing (VIGS) in hops or a model plant. The identification of conserved host factors potentially involved in viroid replication, host susceptibility, or immune response is a primary goal of this study.

This pipeline is the groundwork for a systematic viroid infection transcriptomic analysis and may contribute to the discovery of candidate targets for improving plant resistance to viroid diseases.

PP1

Plant genetics

FLOW CYTOMETRIC ANALYSIS OF A CHIMERIC COMMON BEAN (*PHASEOLUS VULGARIS*) EXHIBITING DIMORPHIC POD CROSS-SECTIONS

Gaja Ernecl¹, Barbara Pipan¹

¹Agricultural Institute of Slovenia, Crop Science Department, Ljubljana, Slovenia

CHIMERISM is characterized by the presence of genetically distinct cell lineages within a single organism and represents a form of intraorganismal genetic heterogeneity that may contribute to phenotypic diversity. In common bean (*Phaseolus vulgaris* L.), a diploid species ($2n = 2x = 22$) with a genome size of approximately 587 Mbp¹, chimerism is a rare phenomenon. Early reports described plants producing flat and round pods on different branches, indicating naturally arising chimeras². Despite their significance for breeding and evolutionary studies, mechanisms underlying such chimeras remain unclear. The aim of this study was to characterize ploidy level and nuclear DNA content in a chimeric common bean population exhibiting a dimorphic pod cross-section phenotype.

A local heterogeneous Slovenian common bean population was investigated, comprising three phenotypic groups: plants with round pod cross-sections, plants with flat pod cross-sections, and chimeric plants expressing both pod types simultaneously. In 2025, 800 plants were grown and harvested individually according to phenotype to determine segregation patterns. In 2026, 83 plants per phenotypic group (3) were cultivated under greenhouse conditions and evaluated morphologically. Leaf tissue from selected individuals was sampled for flow cytometric analyses and subsequent DNA extraction for simple sequence repeat (SSR) marker analysis and whole-genome sequencing (WGS). Flow cytometric analyses were performed using a CyFlow Ploidy Analyser equipped with a UV-LED (365 nm) and an Nd: YAG green laser (532 nm, 30 mW). Ploidy level was assessed using DAPI (4',6-diamidino-2-phenylindole), while nuclear DNA content was determined using propidium iodide (PI) with black clover (*Trifolium pratense*) reference standard.

Flow cytometry revealed no variation in ploidy level among phenotypic groups, with all analysed individuals confirmed as diploid ($2n = 2x = 22$). The estimated 1C genome size ranged from 405.58 to 433.21 Mbp and averaged 420.0 ± 5.17 Mbp, with no statistically significant differences observed among round, flat and chimeric plants (ANOVA, $p = 0.097$). These results indicate that the dimorphic pod phenotype is not associated with changes in ploidy level or nuclear DNA content. The absence of cytological variation points to a genetic or developmental basis of the observed chimerism, which will be further elucidated through ongoing SSR genotyping and WGS.

Characterization of this unique common bean population will improve understanding of naturally occurring chimerism and its potential use as a source of phenotypic variation in breeding.

References

- 1 Schmutz J, McClean PE, Mamidi S, Wu GA, Cannon SB, Grimwood J, et al. A reference genome for common bean and genome-wide analysis of dual domestications. *Nat Genet.* 2014;46:707–13.
- 2 Pearson OH. Unstable gene systems in vegetable crops and implications for selection. *HortScience.* 1968;3(4).

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PP2

Plant genetics

PARENTAL GLUCOSINOLATE PROFILES AS PREDICTORS OF HYBRID PERFORMANCE IN CABBAGE

Primož Fabjan¹, Maja Mikulič Petkovšek¹, Damijana Kastelec¹, Adriana Podržaj¹, Katarina Rudolf Piliš¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia

GLUCOSINOLATES (GSLs) are sulfur-containing secondary metabolites with important roles in plant defense and human health [1, 2]. Developing cabbage (*Brassica oleracea* var. *capitata*) hybrids with increased GSL content represents a promising approach to improve both resistance and health-related traits in cabbage (*Brassica oleracea* var. *capitata*). Because GSLs are moderately to highly heritable in related *Brassica* crops [3] parental phenotypes may help predict hybrid performance and support breeding high-GSL cabbage hybrids.

Here, we evaluated GSL composition, variation, heterosis, and parent–hybrid correlations in 14 cabbage doubled haploid lines and 11 derived hybrids. Thirteen GSLs were detected and quantified using ultra-high-16 performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS). Total GSL contents ranged from 19.7 to 67.8 $\mu\text{mol g}^{-1}$ dw, with aliphatic GSLs dominating the profile (73.0%), followed by indolyl (26.9%) and aromatic (0.1%). Hierarchical clustering revealed two major genotype groups differentiated primarily by 3C and 4C aliphatic GSLs, with correlation analysis reflecting coordinated regulation and trade-offs in their accumulation. Mid-parent heterosis (MPH) for GSLs varied by compound and parental combination.

In parallel, agronomic traits showed uniformly positive MPH, with head weight exhibiting the highest values. Linear regression models showed that mid-parent values explained a high proportion of hybrid variability for major aliphatic glucosinolates and indolyl GBS. Building on these results, we screened 35 genetically diverse breeding lines for GSL content and composition to identify elite parental genotypes for breeding high-GSL cabbage hybrids.

Overall, these results support integrating parental GSL profiling into cabbage breeding to improve nutritional and defense-related traits alongside yield, thereby reducing the need for extensive testcrossing and field trials.

References

- 1 Variyar PS, Banerjee A, Akkarakaran JJ, Suprasanna P. Role of Glucosinolates in Plant Stress Tolerance. In: Ahmad P, Rasool S, editors. Emerging Technologies and Management of Crop Stress Tolerance. Cambridge: Academic Press; 2014. p. 271–291.
- 2 Dinkova-Kostova AT, Kostov RV. Glucosinolates and isothiocyanates in health and disease. Trends Mol Med. 2012; 18:337–347.
- 3 Ze-su, H, Paisan L, Thitiporn M, Ze-hui C, Wen-dong D, Rong. T. Cabbage: Breeding and Genomics. Vegetable Science. 2023; 50:231–43.

PP3

Plant genetics

VIRUS-DERIVED SMALL INTERFERING RNAs AND THEIR POTENTIAL HOST TARGETS DURING MIXED VIRAL INFECTION OF GRAPEVINE (*VITIS VINIFERA* L.)

Katja Jamnik¹, Vanja Miljanić¹, Klara Erzar¹, Jernej Jakše¹, Nataša Štajner¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia

VIRUS-DERIVED SMALL INTERFERING RNAs (vsiRNAs) are small RNA molecules generated from viral RNAs during plant infection and play an important role in the antiviral RNA-silencing response¹. vsiRNAs can originate from viral double-stranded RNA replication intermediates or highly structured regions of single-stranded viral RNA and are processed by Dicer-like enzymes (DCLs) into small RNAs, predominantly 21–22 nt in length. These vsiRNAs can be loaded into Argonaute (AGO) proteins and guide RNA-induced silencing complexes (RISCs) to complementary viral RNAs, promoting their cleavage and contributing to antiviral defense². In addition to targeting viral RNAs, vsiRNAs may also target host transcripts and potentially affect host gene expression³.

In this study, vsiRNAs were characterized in two grapevine cultivars, Refošk (“Terrano”) and Zeleni Sauvignon (“Sauvignon Vert”), co-infected with Grapevine Pinot gris virus (GPGV), Grapevine rupestris stem pitting-associated virus (GRSPaV), and Grapevine rupestris vein feathering virus (GRVfV). Small RNA sequencing data were used to characterize vsiRNA size distribution, 5' terminal nucleotide composition, and genomic distribution. Degradome sequencing was performed to identify potential vsiRNA-mediated cleavage sites in grapevine transcripts, and selected targets were subsequently analyzed by RT-qPCR.

Across all three viruses and both cultivars, 21-nt vsiRNAs were the most abundant size class, followed by 22-nt vsiRNAs. In GPGV- and GRSPaV-derived vsiRNAs, uridine (U) was the most frequent 5' terminal nucleotide, while G and C were most frequent among GRVfV-derived vsiRNAs. Several genomic regions showed increased vsiRNA accumulation, with most hotspots generally located near the 3' ends of the viral genomes. Degradome analysis identified multiple potential vsiRNA targets in grapevine transcripts associated with diverse biological processes, including stress responses, defense, and photosynthesis. RT-qPCR analysis showed reduced abundance of glutathione S-transferase TCHQD transcripts at the predicted cleavage site in virus-infected plants, supporting its potential regulation by vsiRNA-mediated cleavage.

These findings provide insights into vsiRNA-mediated interactions between grapevine and viruses during mixed infection and identify candidate host targets for further functional studies.

References

- 1 Llave C. Virus-derived small interfering RNAs at the core of plant-virus interactions. *Trends Plant Sci.* 2010;15(12):701-707. doi:10.1016/j.tplants.2010.09.001
- 2 Prasad A, Sharma N, Muthamilarasan M, Rana S, Prasad M. Recent advances in small RNA mediated plant-virus interactions. *Crit Rev Biotechnol.* Taylor and Francis Ltd. 2019;39(4):587-601. doi:10.1080/07388551.2019.1597830
- 3 Huang X, Li F, Zhang X, et al. A virus-derived small RNA targets the rice transcription factor ROC1 to induce disease-like symptom. *Phytopathology Research.* 2022;4(1). doi:10.1186/s42483-022-00112-6

PP4

Plant genetics

EXPRESSION PROFILING OF PHYTOCANNABINOID BIOSYNTHESIS GENES ACROSS CANNABIS SATIVA GENOTYPES AND CHEMOTYPES

Polona Jamnik¹, Sinja Svetik¹, Jana Murovec¹

¹ University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia

CANNABIS SATIVA L. is a genetically diverse plant with different chemotypes characterized by differences in phytocannabinoid composition. The production of phytocannabinoids can also change during plant development. Previous studies have shown differences in the expression of cannabinoid synthase genes between chemotypes and during different developmental stages (1,2). Therefore, studying gene expression in different genotypes, chemotypes and developmental stages can help us better understand the regulation of phytocannabinoid biosynthesis.

In this study, the expression of selected genes involved in phytocannabinoid biosynthesis was investigated in flowers of *C. sativa* plants representing four different chemotypes and different genotypes. Flowers were collected at several time points to follow changes in gene expression during flower development. Three biological replicates were analyzed for each sampling point. Total RNA was isolated from flower tissue using the Monarch Spin RNA Isolation Kit (New England Biolabs). Subsequently, cDNA was synthesized from the isolated RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific). Gene expression was analyzed by reverse transcription quantitative PCR (RT-qPCR) using gene-specific primers, with EF-1 α used as the reference gene for normalization. Relative expression was calculated using the comparative 2^{- $\Delta\Delta$ Ct} method.

The aim of the study is to determine how the expression of phytocannabinoid biosynthesis genes differ between genotypes and chemotypes and how it changes during flower development. The study focuses on genes involved in the biosynthesis of major phytocannabinoids, including CBD, THC, CBC and CBG. The expression data will help identify differences in gene activity among the studied plants and across the different sampling time points. Results will be used to select candidate genes for further functional studies and CRISPR/Cas9-mediated genome editing. Establishing the expression patterns of these genes will provide a baseline for evaluating changes following targeted genome modification.

This study is therefore an important step for better understanding the regulation of phytocannabinoid biosynthesis and the potential use of genome editing to study this pathway in *C. sativa*.

References

- 1 Fulvio F, Paris R, Montanari M, Citti C, Cilento V, Bassolino L, et al. Analysis of sequence variability and transcriptional profile of cannabinoid synthase genes in *Cannabis sativa* L. Chemotypes with a focus on cannabichromenic acid synthase. *Plants*. 2021 Sep 1;10(9). doi:10.3390/plants10091857
- 2 Fulvio F, Mandolino G, Citti C, Pecchioni N, Cannazza G, Paris R. Phytocannabinoids biosynthesis during early stages of development of young *Cannabis sativa* L. seedlings: Integrating biochemical and transcription data. *Phytochemistry*. 2023 Oct 1;214. doi:10.1016/j.phytochem.2023.113793 PubMed PMID: 37479208.

PP5

Plant genetics

AUTHOR INDEX

Author	Pages
Alenka Matjašič	27
Alexander Feiner	34
Aline Koch	10
Alnilan Lobato	11
Amar Balihodzic	20
Andrej Zupan	27
Andreja Čerenak	34
Anita Ule	13
Anja Baš	12
Barbara Jenko Bizjan	23
Barbara Luštrek	12
Barbara Pipan	35,37
Boštjan Markelc	20,21,28
Cvetka Grašič Kuhar	28
Damijana Kastelec	38
Denis Korady	14
Eva Plestenjak	35
Eva Praprotnik	29
Gaja Ernecl	37
Gregor Hertl	22
Gregor Serša	18,20,21
Helena Volk	11,36
Irma Zeljković	14,16
Ivan Konjević	11
Jaka Razinger	29
Jaka Vrevc Žlajpah	21
Jan Gojznikar	33
Jana Murovec	40
Jernej Jakše	11,15,29,31,34,36,39
Jernej Kovač	23
José-Antonio Daròs	36
João Colaço	36
Kalyani Pandya	15
Katarina Rudolf Piliš	38
Katarina Trebušak Podkrajšek	26

AUTHOR INDEX

Author	Pages
Katja Doberšek	7,29
Katja Goričar	26
Katja Jamnik	39
Katja Uršič Valentinuzzi	21
Klara Erzar	39
Klemen Tršinar	30
Kristina Arko	30
Lara Snoj	20
Lorena Kunc	22,25
Lucija Luskar	34
Luka Jeršič	33
Luka Puhar	15
Maja Cvetanoska	18
Maja Marinčič	26
Maja Mikulič Petkovšek	38
Maja Čemažar	18,19,20,21,28
Mario Gorenjak	24
Marjanca Starčič Erjavec	30,32,33
Martin Waldinger	34
Martin Šimon	15,17
Martina Krušič	24
Matevž Žula	17
Matic Bošnjak	27
Mia Žganjar	29
Miha Jerala	27
Miha Moškon	14
Milena Kovač	13
Miona Kovachevikj	31
Mohamed Neji	35
Mojca Simčič	12,13
Nataša Gorišek	12
Nataša Štajner	39
Nejc Umek	14,16
Neža Čadež	29
Nicholas M. Morton	15

AUTHOR INDEX

Author	Pages
Nikola Tasić	11
Pia Poglajen	32
Polona Jamnik	40
Polonca Ferk	33
Primož Fabjan	38
Sara Petrin	27
Saša Kupčič	19
Sebastjan Radišek	11,34
Simon Horvat	14,15,16,17
Simona Kranjc Brezar	28
Simona Miceska	28
Sinja Svetik	40
Spela Mikec	15,23
Srdjan Novaković	22,25
Tadeja Vajdič	30,32,33
Taja Jeseničnik	31
Tanja Jesenko	20,28
Tanja Kunej	14,15,16,17
Tanja Marinko	26
Tjaša Cesar	31
Tjaša Zajec	33
Uroš Petrovič	29
Uroš Potočnik	24
Urša Lampreht Tratar	19,21
Urška Janžič	23
Urška Kamenšek	18,19,21
Uršula Prosenc Zmrzljak	14,16
Valerija Tkalec	30,33
Vanja Miljanić	39
Veronika Kloboves-Prevodnik	28
Veronika Kralj-Iglič	31
Veronika Škrjanc	28
Vida Stegel	22,25
Vita Dolžan	23,26
Vita Šetrajčič Dragoš	22,25
Špela Kert	27
Špela Simoneta	14,16
Živa Pišljarič	28

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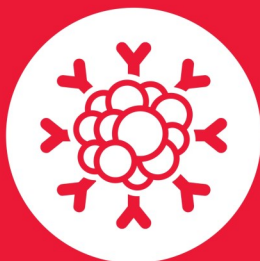
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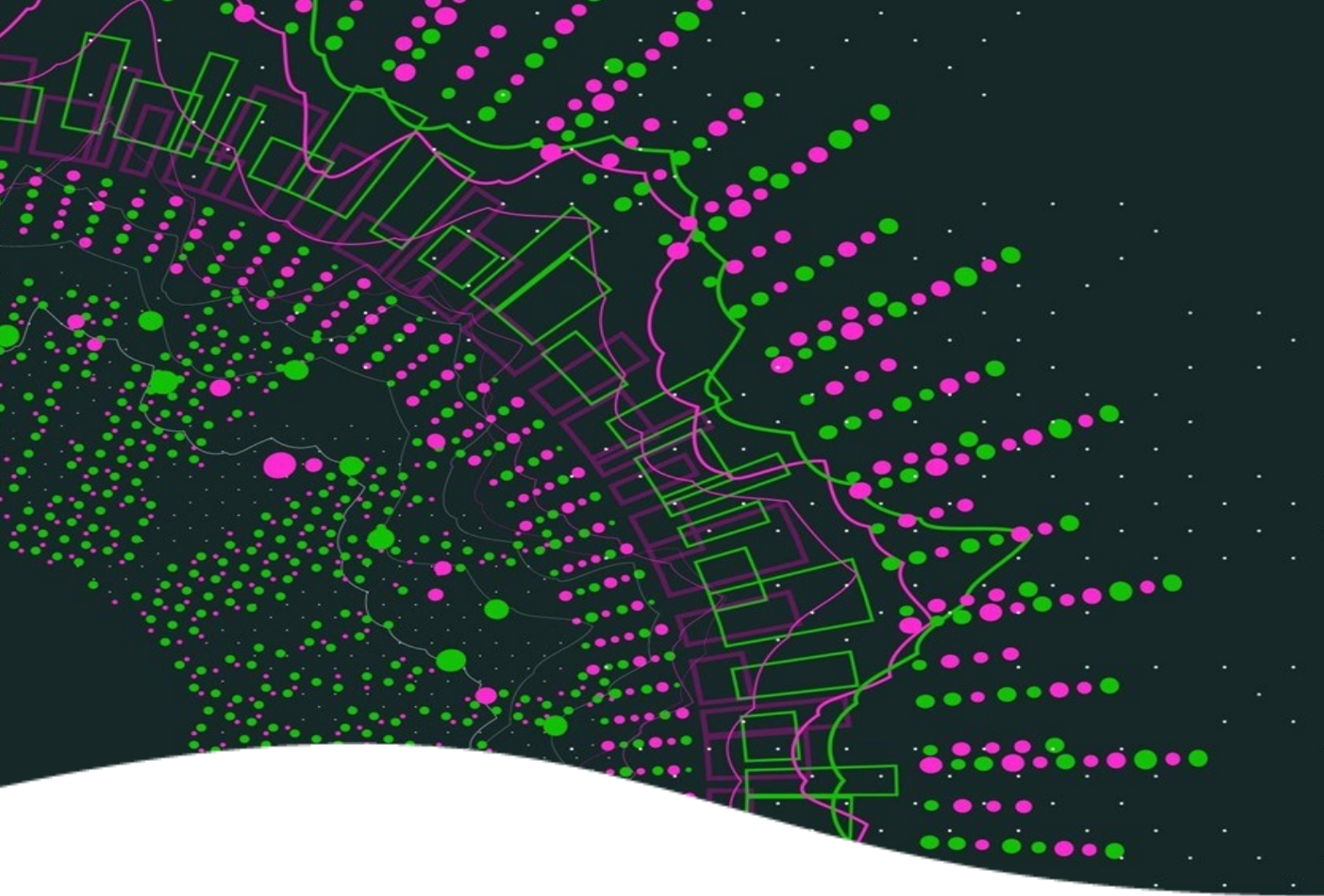


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