6th CCP Phenogenomics Conference 2024

17–18 September 2024

Hybrid Conference

ABSTRACT BOOK

IMG

Czech Centre for Phenogenomics

BIOCEV

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Czech Academy of Sciences

STRATEGY AV21

Top research in the public interest

T A
Č R

6th CCP Phenogenomics Conference 2024: Abstract Book
1st edition
Prague: Czech Academy of Sciences, 2024
Publisher: Institute of Molecular Genetics of the Czech Academy of Sciences
Graphic & DTP: Tonda Pátek | spolehlivygrafik.cz
ISBN: 978-80-907896-5-4

WE THANK TO ALL OUR SUPPORTERS AND SPONSORS FOR THEIR CONTRIBUTION TO THE CONFERENCE

Content

Sponsors and supporters 3
Welcome message 7
Programme 9

KEYNOTE SPEAKERS **12**
Jef D. Boeke, PhD - Engineering mammalian genomes 12
Cathleen Lutz, Ph.D., M.B.A. - After the Diagnosis: Gene Based Therapies and the Road to Treatments 13
David Sabatini - Control of growth and metabolism by the mTOR pathway 13

KEYNOTE LECTURES **14**
Jef Boeke - Engineering mammalian genomes 15
Cathleen Lutz - After the Diagnosis: Gene Based Therapies and the Road to Treatments 16
David Sabatini - Control of growth and metabolism by the mTOR pathway 17

SESSION 1 – MODELS TO UNDERSTAND GENE FUNCTION IN VIVO I **18**
David Sabatini - Control of growth and metabolism by the mTOR pathway 19

SESSION 2 – MODELS TO UNDERSTAND GENE FUNCTION IN VIVO II **20**
Eun Young Kim - Peptidyl prolyl isomerase fine-tunes circadian rhythm 21
Manuel Goncalves - Large gene-editing systems targeting the largest human gene: Duchenne muscular dystrophy as a target disease model 22
Jan Prochazka - Beyond the Tooth: The Multifunctional Roles of Enamel Matrix Proteins in Cardiovascular Physiology 23

SESSION 3 – TEAMING PROJECTS IN GENE AND CELL THERAPY **24**
Mojca Benčina - Centre for the Technologies of Gene and Cell Therapy: Advancing Healthcare Through Synthetic Biology 25
Luis Pereira de Almeida - Gene Therapy: the time is now 26
Regina Demlova - Advanced Therapy Medicinal Products and Academia's Evolving Role: From Clinical Trials to Patient Access 27

SESSION 4 – NON-CODING & THERAPY **28**
Judith B Zaugg - How do cells integrate extrinsic signals and intrinsic state? A systems epigenetics approach 29
Martin Kircher - Scoring functional impact of genetic variation in coding and non-coding sequences 30
Jef Boeke - Engineering mammalian genomes 31

SESSION 5 – MODELS FOR GENE THERAPY & GENOME EDITING **32**
Hyunji Lee - Strategies for Creating Mitochondrial Genetic Engineering Mouse Model 33

SESSION 6 – SHORT PRESENTATIONS: SELECTED POSTER PRESENTATIONS & TECHNOLOGY TALKS **34**
Ladislav Vyklicky - Cellular and molecular consequences of nonsense and missense GRIN gene variants in animal models: Gain of function or loss of function, that is the question 35
Lillian Garrett - Decoding Schizophrenia genetics: insights from mouse models and human brain expression data 36
Silvia Mandillo - Muscle-specific gene editing improves molecular and phenotypic defects in a mouse model of Myotonic Dystrophy type 1 37
Filip Dámek - New insights by comprehensive phenotyping of the Idua knockout mouse model 38
Katarzyna Kowalczyk - BIO-RAD - DDPCR ADVANCED APPLICATIONS - EASY WAY FOR DELIVERY SOLUTION FOR DETECTING RARE DISEASES, CREATE THERAPIES AND QUALITY CONTROL 39

Jessica Hartman - Automate and simplify cell line development using the CellRaft AIR® System 40

SESSION 7 – RARE DISEASES & BOTTOM-UP EFFORTS TO DEVELOP (GENE) THERAPIES 41
Kacper Lukasiewicz - Insights into PACS2–related Syndrome: Lessons from In Vitro and In Vivo Models 42
Piotr Kosla - New model to cure ultra rare: Patient-led collaborative research networks 43
Duško Lainšček, Špela Miroševič - Finding a Cure for CTNNB1 Syndrome: The Role of the Foundation and Research Presentation 44
Tanja Zdolsek Draksler - Solving Rare Diseases: How Global Collaboration and Data are Advancing Kleefstra Syndrome Research 45
Pauline Larqué - Understanding the cell-type specific function of EHMT1 in neuronal network function 46

SESSION 8 – CLOSING 47
Cathleen Lutz - After the Diagnosis: Gene Based Therapies and the Road to Treatments 48

POSTER SESSION 1 49
(PO–01) Igor Varga - Automatic Skull Sutures Detection for Mouse Phenotyping 51
(PO–02) Michaela Šímová - DECIPHERING THE EMERGENCE OF ERYTHRO-MYELOID PROGENITORS IN THE MOUSE YOLK SAC 52
(PO–03) Olha Pyko - Deciphering the Impact of ZNF644 Deletion: Investigating the Role of C2H2 Zinc Finger Protein in Mouse Female Phenotype 53
(PO–04) Rodolfo Favero - Development and Characterization of a Conditional Spink5 Knockout Mouse Model for Netherton Syndrome 54
(PO–05) Hirotoshi Shibuya - Development of high-throughput, high-resolution soft tissue imaging methods using new contrast-enhanced micro-CT 55
(PO–06) Matilde Vale - Development of therapeutic exosomes and gene therapy for Diamond Blackfan Anemia (DBA) 56
(PO–07) Sabina Cerulová - Dysregulation of calcium-phosphate metabolism in originally created mouse model with a rare GALNT3 mutation 57
(PO–08) Zhenni Liu - Exploring the Role of GPR45 in Metabolic Regulation and Its Implications for Obesity and Related Diseases 58
(PO–09) Eni Tomovic - Genetic and functional analysis of GRIN variants detected in Czech pediatric patients 59
(PO–10) Ben Davies - Human genomic humanization of the Grem1 (88 kb) and Taf1 (166kb) genes 60
(PO–11) Federica Gambini - Characterization of a Novel Inducible hACE2 Mouse Model for SARS-CoV–2 Research: Insights into Acute Infection and Long COVID 61
(PO–12) Klevinda Fili - Characterization of mice carrying neurodevelopmental disease-associated variants 62
(PO–13) Vera Abramova - Characterization of Zebrafish Larvae with Knockouts of NMDA Receptor grin2Aa and grin2Ab Genes: Gene Expression and Swimming Behavior 63
(PO–14) Hana Kolesová - Morphology and physiology of Jagged1 conditional deletion and patient-based single variant mouse models 64
(PO–15) Petr Nickl - Multistep allelic conversion in mouse pre-implantation embryos by AAV vectors 65
(PO–16) Silvia Mandillo - Muscle-specific gene editing improves molecular and phenotypic defects in a mouse model of Myotonic Dystrophy type 1 66
(PO–17) Kristýna Neffeová - Physiological and Morphological Consequences of Jagged1 Deletion in Mouse Model of Tetralogy of Fallot 67
(PO–18) Tomasz Kowalczyk - Proteomic analysis of soft tissues from mice with PACS2 gene mutation 68
(PO–19) Dominik Cysewski - Proteomic and Metabolomic Profiling of Brain Tissues in PACS2 E209K Mutant Mice: Insights into Molecular Dysregulation 69
(PO–20) Betul Melike Ogan - ROLE OF FAM83H IN IMMUNE SYSTEM HOMEOSTASIS 70
(PO–21) Maximilián Goleňa - Seasonality of measured parameters in C57Bl/6NCrl mice 71
(PO–22) Tobiáš Ber, Kateryna Nemesh - Terrestrial Slugs as Prospective Animal Models for Studying RNA Silencing Pathways 72
(PO–23) Gunay Akbarova-Ben-Tzvi - The impact of modified TGF- ß family on Integrin-ß1 Synthesis of Chondrocyte Cell Sheets 73
(PO–24) Arkadiusz Żbikowski - The impact of PACS2 Syndrome on lung and kidney structure in mice 75
(PO–25) Viktor Kostohryz - The promise of episomal gene therapy 76
(PO–26) Miles Joseph Raishbrook - The significance of Fam84b in retinal homeostasis 77
(PO–27) JI XU - Transcriptional corepressor TLE1 is a positive factor in adipocyte differentiation 78
(PO–28) Sylvie Dlugosova - Skeletal dysmorphology and mineralization defects in Fgf20 KO mice 79

(PO–29) Eliška Pavelková - Assessing the reproductive phenotypes in mice – current methods and room for improvement 80

POSTER SESSION 2 **81**
(PO–30) Juraj Labaj - Biochemistry and Haematology Unit (CCP, Phenotyping Module) 82
(PO–31) Micheala Prochazkova - Bioimaging & Embryology Unit (CCP, Phenotyping Module) 84
(PO–32) Eva Nekvindova - Cardiovascular Unit (CCP, Phenotyping Module) 86
(PO–33) Jiri Lindovsky - Hearing & Electrophysiology Unit (CCP, Phenotyping Module) 87
(PO–34) Olha Fedosieieva - Histopathology Unit (CCP, Phenotyping Module) 89
(PO–35) Jana Balounová - Immunology Unit (CCP, Phenotyping Module) 92
(PO–36) David Pajuelo Reguera - Metabolism Unit (CCP, Phenotyping Module) 94
(PO–37) Karel Chalupský - Metabolomics Unit (CCP, Phenotyping Module) 97
(PO–38) Kateryna Pysanenko - Neurobiology & Behaviour Unit (CCP, Phenotyping Module) 98
(PO–39) Silvia Magalhaes Novais - PDX & Cancer Models Unit (CCP, Phenotyping Module) 99
(PO–40) Marcela Palkova - Vision Unit (CCP, Phenotyping Module) 100
(PO–41) Dominik Arbon - Animal Biosafety Level 3 facility for preclinical testing and research of infectious diseases 101
(PO–42) Vendula Novosadova - Bioinformatician Unit (CCP, Phenotyping Module) 102
(PO–43) Lucie Dufkova - Transgenic and Archiving Module (CCP) 104
(PO–44) Gizela Koubkova - Preclinical testing at the Czech Centre for Phenogenomics 107
(PO–45) Libor Kopkan, Anna Pilařová - Animal Facility Module (CCP, Vestec) 108

List of participants 109

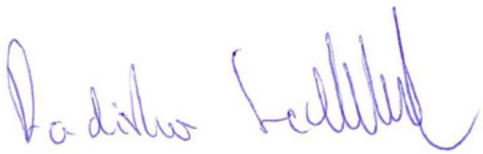
Dear Colleagues,

It is my great pleasure to welcome you to the 6th CCP Phenogenomics Conference.

Similarly to the last year, the conference is devoted to the topic of **rare diseases & their therapies** this year, with special focus on **experimental models** in research of rare diseases and **non-coding elements**.

We believe that the Conference will provide again an excellent opportunity to support networking and interactions among the researchers, CCP staff, users and experts from the commercial sector.

Yours sincerely,



On behalf of the CCP Organizing Committee,
Radislav Sedláček
Director of the Czech Centre for Phenogenomics

Photo © Jana Plavec / Czech Academy of Sciences

ORGANIZER – CZECH CENTRE FOR PHENOGENOMICS

The Czech Centre for Phenogenomics (CCP) is a large research infrastructure unique in combining genetic engineering capabilities, advanced phenotyping and imaging modalities, SPF animal housing and husbandry, as well as cryopreservation and archiving, all in one central location – at BIOCEV campus.

CCP is the only specialized place in the Czech Republic that, at the level of the world's best centres, creates genetically modified mouse and rat models for indispensable biomedical research and at the same time uses standardized but the most advanced phenotyping to characterize the expression of gene functions. CCP outputs are utilized solving the role of genes in the development and treatment of human diseases. CCP provides unique comprehensive preclinical research services in the Czech Republic. With the quality of service and publication results, CCP has gained a worldwide reputation, it has a strong position in international consortia such as the global IMPC (to determine the role of all genes), the European Infrafrontier, and EuroPDX. CCP is involved in several international scientific projects.

www.phenogenomics.cz

ACKNOWLEDGEMENTS:

MINISTRY OF EDUCATION, YOUTH AND SPORTS

The Czech Centre for Phenogenomics is supported by the Czech Academy of Sciences RVO 68378050 and by the project LM2023036 Czech Centre for Phenogenomics provided by the Ministry of Education, Youth and Sports of the Czech Republic.

Czech Academy of Sciences

STRATEGY AV21
Top research in the public interest

"Towards precision medicine and gene therapy" research programme of the Strategy AV21 is coordinated by the Institute of Molecular Genetics of the Czech Academy of Sciences.

T A
Č R
Tento projekt je spolufinancován se státní podporou Technologické agentury ČR v rámci Programu Národní Centra kompetence
www.tacr.cz
Výzkum užitečný pro společnost.

The project TN02000132/National Centre for New Methods of Diagnosis, Monitoring, Treatment and Prevention of Genetic Diseases is co-financed with state support from the Technology Agency of the Czech Republic under the National Centres of Competence Programme. The Centre aims to improve diagnostic quality beyond the identification of mutations in the genome and to create a base for monitoring of disease progression and therapy effectiveness as well as for testing and development of cell and gene therapies.

TUESDAY 17TH SEPTEMBER 2024

9:00 – 10:00	Registration

OPENING + SESSION 1 – MODELS TO UNDERSTAND GENE FUNCTION IN VIVO I *(Chair: Radislav Sedláček)*

9:30 – 9:45	Radislav Sedlacek, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic "Welcoming Lecture: CCP 2024: Leveraging the Mouse Models to Understand Gene Function and their Role in Human Diseases"
9:45 – 10:30	David Sabatini, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic (keynote speaker) "Control of growth and metabolism by the mTOR pathway"
10:30 – 10:55	Yann Herault, Institute of Genetics and Molecular and Cellular Biology, France "Liver Disease in Down Syndrome"
10:55-11:15	Coffee break/poster session

SESSION 2 – MODELS TO UNDERSTAND GENE FUNCTION IN VIVO II

11:15 – 11:40	Eun Young Kim, Ajou University, School of Medicine, Republic of Korea "Peptidyl Prolyl Isomerase Fine-tunes Circadian Rhythm"
11:40 – 12:05	Manuel Goncalves, Leiden University Medical Centre, Department of Cell and Chemical Biology, Netherlands "Large Gene-Editing Systems Targeting the Largest Human Gene: Duchenne Muscular Dystrophy as a Target Disease Model"
12:05 – 12:30	Jan Prochazka, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic "Beyond the Tooth: The Multifunctional Roles of Enamel Matrix Proteins in Cardiovascular Physiology"
12:30 – 13:00	Discussion with speakers
13:00-14:15	Lunch break/poster session

SESSION 3 – TEAMING PROJECTS IN GENE AND CELL THERAPY

14:15 – 14:35	Mojca Bencina, National Institute of Chemistry, Slovenia "Centre for the Technologies of Gene and Cell Therapy: Advancing Healthcare Through Synthetic Biology"
14:35 – 14:55	Luis Pereira de Almeida, Center for Neuroscience and Cell Biology, University of Coimbra, Portugal "Gene Therapy: the time is now"
14:55 – 15:15	Regina Demlova, Central European Advanced Therapy and Immunotherapy Center (CREATIC) + Faculty of Medicine, Masaryk University, Czech Republic "Advanced Therapy Medicinal Products and the Changing Role of Academia"
15:15 – 15:30	Discussion with speakers
15:30 – 16:00	Coffee break / poster session

SESSION 4 – NON-CODING & THERAPY

16:00 – 16:25	Judith Zaugg, European Molecular Biology Laboratory, Germany "How do cells integrate extrinsic signals and intrinsic state? A systems epigenetics approach"
16:25 – 16:50	Martin Kircher, Professor of Regulatory Genomics, Institute of Human Genetics, Campus Lübeck, Germany "Scoring Genetic Variation in Coding and Non-coding Sequences"
16:50 – 17:35	Jef D. Boeke, NYU Langone Health, United States (keynote speaker) "Engineering mammalian genomes"
17:35 – 18:00	Discussion with speakers

WEDNESDAY 18TH SEPTEMBER 2024

SESSION 5 – MODELS FOR GENE THERAPY & GENOME EDITING

9:00 – 9:25	Martin Jinek, University of Zurich, Department of Biochemistry, Switzerland "CRISPR Genome Editors: Mechanisms, Engineering and Therapeutic Applications"
9:25 –9:50	Hyunji Lee, KMPC and College of Medicine, Korea University, Republic of Korea "Strategies for Creating Mitochondrial Genetic Engineering Mouse Model"
9:50 – 10:15	Pavel Krejci, Masaryk University, Faculty of Medicine, Czech Republic "FGFR3 in Skeletogenesis and Achondroplasia: New Treatment Development"
10:15 – 10:40	Jun Won Park, KMPC and Department of Laboratory Animal Medicine, College of Veterinary Medicine, Seoul National University, Republic of Korea "Mouse Models for Dynamics of Epithelial Cell Plasticity During Gastric Carcinogenesis and Metastasis"
10:40 – 11:00	Discussion with speakers
11:00 – 11:20	Coffee break /poster session

SESSION 6 – SHORT PRESENTATIONS: SELECTED POSTER PRESENTATIONS & TECHNOLOGY TALKS

11:20 – 11:35	Ladislav Vyklicky, Institute of Physiology CAS, Czech Republic "Cellular and molecular consequences of nonsense and missense GRIN gene variants in animal models: Gain of function or loss of function, that is the question"
11:35 – 11:50	Lillian Garrett, Helmholtz Center Munich, Germany "Decoding Schizophrenia genetics: insights from mouse models and human brain expression data"
11:50 – 12:05	Silvia Mandillo, Institute of Biochemistry and Cell Biology, CNR-National Research Council, Italy "Muscle-specific gene editing improves molecular and phenotypic defects in a mouse model of Myotonic Dystrophy type 1"
12:05 – 12:20	Filip Dámek, Institute of Experimental Genetics and German Mouse Clinic, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764, Neuherberg, Germany "New insights by comprehensive phenotyping of the Idua knockout mouse model"
12:20 – 12:35	Katarzyna Kowalczyk, Field Application Specialist Genomics, CEE (Bio-Rad sponsored talk) "ddPCR Advanced Applications – Easy Way for Delivery Solution for Detecting Rare Diseases, Create Therapies and Quality Control"

12:35 – 12:50	Jessica Hartman, Cell Microsystems, USA “Automate and simplify cell line development using the CellRaft AIR® System”
12:50 – 13:10	Discussion with speakers
13:10 – 14:10	Lunch break / poster session

SESSION 7 – RARE DISEASES & BOTTOM-UP EFFORTS TO DEVELOP (GENE) THERAPIES

14:10 – 14:35	Kacper Lukasiewicz, Medical University of Bialystok, Psychiatry Clinic, Poland “Insights into PACS2-related Syndrome: Lessons from In Vitro and In Vivo Models” Piotr Kosla, PACS2 Research Foundation, Poland “New model to cure ultra rare: Patient-led collaborative research networks”
14:35 – 15:00	Spela Mirosevic, CTNNB1 Foundation, Slovenia “Finding a Cure for CTNNB1 Syndrome: The Role of the Foundation” Dusko Lainscek, National Institute of Chemistry, Department of Synthetic Biology and Immunology, Slovenia “Finding a Cure for CTNNB1 Syndrome: Research Presentation”
15:00 – 15:25	Tanja Zdolsek Draksler, Jožef Stefan Institute, International Research Centre on Artificial Intelligence under the Auspices of UNESCO and IDefine Europe Foundation, Slovenia “Solving Rare Diseases: How Global Collaboration and Data are Advancing Kleefstra Syndrome Research” Pauline Larqué, Radboud University, The Netherlands “Understanding the Cell-type Specific Function of EHMT1 in Neuronal Network Function”
15:25 – 15:50	Discussion with speakers
15:50 – 16:15	Coffee break/poster session

SESSION 8 – CLOSING

16:15 – 17:00	Cathleen Lutz, The Jackson Laboratory, United States (concluding keynote lecture) “After the Diagnosis: Gene Based Therapies and the Road to Treatments”
17:00 – 17:10	Radislav Sedlacek, Czech Centre for Phenogenomics Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic “Closing remarks”

Keynote speakers

Jef D. Boeke, PhD

Sol and Judith Bergstein Director, Institute of System Genetics + Professor, Department of Biochemistry and Molecular Pharmacology, NYU Grossman School of Medicine, United States of America

"Engineering mammalian genomes"

Jef D. Boeke, PhD, DSc, founded and directs the Institute for Systems Genetics at NYU Langone Health. From 1985–2013, Dr. Boeke was on the faculty at Johns Hopkins University School of Medicine. Dr. Boeke received a BS in biochemistry summa cum laude in 1976 from Bowdoin College, and then earned a PhD in molecular biology from Rockefeller University in 1982, where he worked with Peter Model and Norton Zinder on the genetics of the filamentous phage. He did his postdoctoral work at The Whitehead Institute of MIT as a Helen Hay Whitney Postdoctoral Fellow with Gerald Fink.

Dr. Boeke discovered a major form of mobile DNA, based on reverse transcription of RNA. He coined the term "retrotransposition" to describe this process, common to virtually all eukaryotic genomes and now studied by a worldwide scientific community. His systems-level studies helped elucidate intricate molecular mechanisms involved in retrotransposition in yeasts, mice and humans.

In the area of synthetic biology, Dr. Boeke leads the international team synthesizing an engineered version of the yeast genome, Sc2.0, the first synthetic eukaryotic genome. In 2018, he launched the "Dark Matter Project" designed to better understand the "instruction manuals" that specify how human genes are expressed, using big DNA technology.

Source: NYU Grossman School of Medicine, https://med.nyu.edu/research/boeke-lab/team

Cathleen Lutz, Ph.D., M.B.A.

Vice President, Rare Disease Translational Center, The Jackson Laboratory (JAX), United States of America

"After the Diagnosis: Gene Based Therapies and the Road to Treatments"

Cat Lutz, Ph.D., M.B.A. is the Vice President of the Rare Disease Translational Center at The Jackson Laboratory (JAX). With 25 years of experience in mouse genetics, Dr. Lutz has focused her research efforts on patient organizations and families diagnosed with rare diseases. The JAX Rare Disease Translational Center incorporates precision mouse models and broad-based drug efficacy testing to support IND enabling studies. She serves as the Principal Investigator of multiple NIH sponsored programs including the Center for Precision Genetics, The Somatic Cell Genome Editing Center, and Mouse Mutant Research and Resource Center. As a neuroscientist by training, Dr. Lutz has worked on models of the central nervous system such as Spinal Muscular Atrophy, Amyotrophic Lateral Sclerosis and Friedreich's Ataxia. Dr. Lutz was recently awarded a 2021 Rare Impact Award by the National Organization for Rare Disorders.

Source: The Jackson Laboratory,
https://www.jax.org/research-and-faculty/faculty/research-scientists/cat-lutz

David Sabatini

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic + Boston, United States of America

"Control of growth and metabolism by the mTOR pathway"

David Sabatini is an expert in cell signaling, cancer metabolism, and growth regulation, and he trained dozens of graduate students and post-doctoral fellows who now run their own labs in academia. He is known most notably for the co-discovery of mTOR. Currently, he leads the research group focusing on molecular analysis of growth regulation in animals at the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, located in Prague and Boston. Previously, he was a professor at the Massachusetts Institute of Technology (MIT).

Source: Institute of Organic Chemistry
and Biochemistry of the Czech Academy of Sciences,
https://sabatini.group.uochb.cz/en

Keynote lectures

- **Tuesday, 17th September 2024 (16:50 – 17:35)**

Jef D. Boeke, NYU Langone Health, United States
"Engineering mammalian genomes"

- **Wednesday, 18th September 2024 (16:15 – 17:00)**

Cathleen Lutz, The Jackson Laboratory, United States
"After the Diagnosis: Gene Based Therapies and the Road to Treatments"

- **Tuesday, 17th September 2024 (9:45 – 10:30)**

David Sabatini, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic
"Control of growth and metabolism by the mTOR pathway"

Engineering mammalian genomes

Jef Boeke [1]

1. NYU Langone Health

Email of the presenting author: jef.boeke@nyulangone.org

Rapid advances in DNA synthesis techniques have made it possible to engineer diverse genomic elements, pathways, and whole genomes, providing new insights into design and analysis of systems. The synthetic yeast genome project, Yeast2.0 is well on its way with the 16 synthetic S. cerevisiae chromosomes now completed by a global team. We have automated a big DNA synthesis and assembly pipeline, and can readily assembly and deliver human genomic regions of 100-200+ kb along with multiple designer synthetic variants thereof. Using precision delivery strategies such as Big-IN and mSwAP-In, we can precisely deliver DNA segments to stem cells, and use these methods to transplants of specific human genomic regions to animal genomes, and endow human or animal cells, or even animals, with new capabilities. Less than 2% of our genome is protein-coding DNA. The vast expanses of non-coding DNA make up the genome's **"dark matter"**, where introns, repetitive and regulatory elements reside. Surprisingly, variation between individuals in non-coding regulatory DNA is emerging as a major factor in the genetics of numerous diseases and traits, yet very little is known about how such variations contribute to disease risk. Studying genetics of regulatory variation is technically challenging, as regulatory elements can affect genes located tens of thousands of bp away, and often, multiple distal regulatory variations, each with a very small effect, combine in unknown ways to significantly modulate expression of genes. An example of extensive humanization using mSwAP-In is the "Covid mouse" made using mSwAP-In. Humanization of the ACE2 gene led to an improved mouse model for human Covid-19. Interestingly, including additional 5' sequences had significant effects in terms of tissue distribution of expression as well as efficiency of viral multiplication. This is an example of a Genomically Rewritten and tailored Genetically Engineered Mouse Model or GREAT GEMM (Zhang et al. Nature 2023).

After the Diagnosis: Gene Based Therapies and the Road to Treatments

Cathleen Lutz [1]

1. Rare Disease Translational Center, The Jackson Laboratory (JAX), United States of America

Email of the presenting author: cat.lutz@jax.org

Genome sequencing has ended the diagnostic odyssey for many families, but the research and development of treatments for individual diseases is lagging. At the same time, technologies surrounding gene-based therapies and delivery modalities are advancing at an unprecedented rate and offer new hope to patients. Therapeutic successes in diseases like Spinal Muscular Atrophy, Cystic Fibrosis, Duchenne Muscular Dystrophy, Friedreich's Ataxia and Sickle Cell Anemia have galvanized the rare disease community, creating a vision of success for families and a revolution of family-based organizations trailblazing their path to the clinic. With the support of NIH funding mechanisms, the Rare Disease Translational Center (RDTC) at The Jackson Laboratory works with patient organizations to create mouse models for these rare diseases, foraging research collaborations along the way for their expedient characterization and use. These mouse models not only serve as patient avatars that increase our understanding of the underlying pathophysiology of a given disease, but also allow us to test newly emerging small molecule and gene-based therapies. With over 50 patient programs in the pipeline, the RDTC at The Jackson Laboratory has created a systemized process for the expedient development and testing of therapeutics to the clinic for the rare disease community.

Control of growth and metabolism by the mTOR pathway

David Sabatini [1]

1. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

Email of the presenting author: david.sabatini@uochb.cas.cz

The mTOR pathway, particularly its mTORC1 branch, is a central regulator of growth in animals and regulates the balance between anabolism and catabolism in response to nutrients and growth factors. For some time we have worked to discover and understand the function of components of the upstream pathway that conveys nutrient levels to mTORC1, a large protein kinase. This work has led to the identification the lysosome as a signaling organelle and to the discovery of a complex signaling pathway anchored by the Rag GTPases at the lysosomal surface. In recent years we have discovered the regulators of the Rag GTPases, the GATOR complexes, and, most interestingly, the sensors that directly bind metabolites, such as amino acids. I will focus on our work in vivo in mice and in flies and discuss the physiological functions of the amino acid sensors in response to dietary conditions as well as the logic of their cell type-specific expression.

Session 1 – Models to understand gene function in vivo I

Tuesday, 17th September 2024 (9:30 – 10:55)

9:30 – 9:45

Radislav Sedláček, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
"Welcoming Lecture: CCP 2024: Leveraging the Mouse Models to Understand Gene Function and their Role in Human Diseases"

9:45 – 10:30

David Sabatini, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic (keynote speaker)
"Control of growth and metabolism by the mTOR pathway"

10:30 – 10:55

Yann Herault, Institute of Genetics and Molecular and Cellular Biology, France
"Liver Disease in Down Syndrome"

Control of growth and metabolism by the mTOR pathway

David Sabatini [1]

1. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

Email of the presenting author: david.sabatini@uochb.cas.cz

The mTOR pathway, particularly its mTORC1 branch, is a central regulator of growth in animals and regulates the balance between anabolism and catabolism in response to nutrients and growth factors. For some time we have worked to discover and understand the function of components of the upstream pathway that conveys nutrient levels to mTORC1, a large protein kinase. This work has led to the identification the lysosome as a signaling organelle and to the discovery of a complex signaling pathway anchored by the Rag GTPases at the lysosomal surface. In recent years we have discovered the regulators of the Rag GTPases, the GATOR complexes, and, most interestingly, the sensors that directly bind metabolites, such as amino acids. I will focus on our work in vivo in mice and in flies and discuss the physiological functions of the amino acid sensors in response to dietary conditions as well as the logic of their cell type-specific expression

Session 2 – Models to understand gene function in vivo II

Tuesday, 17th September 2024 (11:15 – 13:00)

11:15 – 11:40

Eun Young Kim, Ajou University, School of Medicine, Republic of Korea
"Peptidyl Prolyl Isomerase Fine-tunes Circadian Rhythm"

11:40 – 12:05

Manuel Goncalves, Leiden University Medical Centre, Department of Cell and Chemical Biology, Netherlands
"Large Gene-Editing Systems Targeting the Largest Human Gene: Duchenne Muscular Dystrophy as a Target Disease Model"

12:05 – 12:30

Jan Prochazka, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
"Beyond the Tooth: The Multifunctional Roles of Enamel Matrix Proteins in Cardiovascular Physiology"

Peptidyl prolyl isomerase fine-tunes circadian rhythm

Eun Young Kim [1]

1. Department of Brain Science, Ajou University School of Medicine, 164 Worldcup-ro, Suwon, Kyunggi-do 16499, Republic of Korea

E-mail of the presenting author: ekim@ajou.ac.kr

The circadian clock allows organisms to anticipate the 24-hour rhythmic changes in their environment and regulate behavior and physiology accordingly. This cell-autonomous molecular clock operates through a transcriptional-translational feedback loop (TTFL) involving clock proteins. The primary loop involves the activation of period (per) and timeless (tim) gene transcription by CLOCK (CLK) and CYCLE (CYC). The PER and TIM proteins then repress their own transcription, with PER acting as the core repressor. Core clock proteins undergo significant changes in activity, subcellular-localization, and levels through posttranslational modifications, including phosphorylation, ubiquitination, and O-GlcNAcylation etc. Additionally, peptidyl-prolyl cis/trans isomerization can induce conformational changes that serve as molecular timers and impact cellular processes. Our research aimed to explore the potential role of peptidyl-prolyl cis/trans isomerases (PPIases) in regulating the circadian clock using Drosophila as a model system. Our previous findings showed that overexpression of Dodo (Dod), a mammalian PIN1 homolog in clock cells, led to a lengthening of the circadian period and regulated the stability of PER through its association with phosphorylated isoforms of PER in Drosophila. We further conducted an RNAi knockdown screen of 21 annotated PPIases to evaluate their impact on circadian locomotor behavior. The results showed that all PPIase knockdowns resulted in a lengthened period and reduced rhythmicity, with one PPIase leading to a period of approximately 28 hours. In this talk, I will delve into the mechanism behind the regulation of the circadian rhythm by this PPIase in Drosophila

Large gene-editing systems targeting the largest human gene: Duchenne muscular dystrophy as a target disease model

Manuel Goncalves [1]

1. Leiden University Medical Center, Dep. Cell and Chemical Biology, Leiden, The Netherlands

E-mail of the presenting author: m.f.v.goncalves@lumc.nl

ADP-ribosylation, which is a widespread and transient post-translational protein modification, plays a crucial role in numerous essential cellular and biological functions. These include DNA damage repair, cellular proliferation and differentiation, metabolic processes, stress responses, and immune reactions. Our research is focused on unraveling the complexities of ADP-ribosyl transferases, a subclass of DNA repair enzymes responsible for detecting DNA single-strand breaks (SSBs). These enzymes signal the presence of such breaks by catalyzing the rapid generation of mono(ADP-ribose) and poly(ADP-ribose). Furthermore, our investigations extend to hydrolases, enzymes tasked with eliminating specific ADP-ribosyl modifications from proteins.

SSBs constitute some of the most prevalent DNA lesions that emerge within cells. These lesions have the potential to disrupt replication, RNA processing and transcription, posing threats to both genetic stability and cellular viability. Significantly, deficiencies in DNA single-strand break repair and ADP-ribose metabolism are intimately linked to hereditary neurodevelopmental and neurodegenerative disorders in humans. This underscores the critical importance of these processes, particularly in neurons characterized by extended lifespans and post-mitotic properties.

We investigate the molecular mechanisms by which SSBs are detected and aim to identify and characterize the protein factors and pathways that establish connections between aberrant ADP-ribose metabolism and neurodegenerative diseases including a use of CRISPR/Cas9 gene-edited mouse models. Our ultimate goal is to find whether the perturbed ADP-ribose metabolism at SSBs extends beyond rare DNA repair-deficient conditions to more common dementia.

Beyond the Tooth: The Multifunctional Roles of Enamel Matrix Proteins in Cardiovascular Physiology

Jan Prochazka [1]

1. Institute of Molecular Genetics, Czech Centre for Phenogenomics

E-mail of the presenting author: jan.prochazka@img.cas.cz

Enamel matrix proteins (EMPs), traditionally associated with dental enamel formation, have recently been implicated in various non-dental physiological processes, extending their relevance to cardiovascular health. These proteins, including amelogenin, ameloblastin, and enamelin, are fundamental to developing tooth enamel, the hardest tissue in the human body, through complex epithelial-mesenchymal interactions and cellular processes such as secretion and mineralization. Beyond their dental roles, our research reveals that ameloblastin (AMBN) also expresses in non-dental tissues like the heart, where it significantly impacts physiological functions. AMBN interacts with L-type voltage-dependent calcium channels (Cav1.2), suggesting a regulatory role in calcium flux dynamics in cardiomyocytes. This interaction has implications for cardiac muscle response under stress, influencing heart contraction rates and ventricular repolarization during pharmacological challenges. Additionally, the evolutionary analysis shows that the pleiotropic effects of EMPs and their conserved motifs suggest functions that predate teeth, possibly related to primitive calcium channel regulation. These findings open new avenues for understanding EMPs' evolutionary history and multifunctionality, providing a foundation for future biomedical applications in both dental and general physiological contexts.

Session 3 – Teaming projects in gene and cell therapy

Tuesday, 17th September 2024 (14:15 – 15:30)

14:15 – 14:35

Mojca Bencina, National Institute of Chemistry, Slovenia
"Centre for the Technologies of Gene and Cell Therapy: Advancing Healthcare Through Synthetic Biology"

14:35 – 14:55

Luis Pereira de Almeida, Center for Neuroscience and Cell Biology, University of Coimbra, Portugal
"Gene Therapy: the time is now"

14:55 – 15:15

Regina Demlova, Central European Advanced Therapy and Immunotherapy Center (CREATIC) + Faculty of Medicine, Masaryk University, Czech Republic
"Advanced Therapy Medicinal Products and Academia's Evolving Role: From Clinical Trials to Patient Access"

Centre for the Technologies of Gene and Cell Therapy: Advancing Healthcare Through Synthetic Biology

Mojca Benčina [1,2]

1. Centre for the Technologies of Gene and Cell Therapy; National Institute of Chemistry, Ljubljana, Slovenia
2. Department of Synthetic Biology and Immunology; National Institute of Chemistry, Ljubljana, Slovenia

Email of the presenting author: mojca.bencina@ki.si

The Centre for Technologies of Gene and Cell Therapy (CTGCT) addresses the unmet need for treating rare genetic diseases, affecting 5-8% of the European population. Despite advancements in biomedicine, including CRISPR and CAR T technologies, translating these innovations into clinical therapies has been slow, especially in less research-intensive EU countries. Supported by European funding and the Slovenian government, the CTGCT aims to bridge this gap by developing novel therapies for rare genetic diseases and cancer immunotherapy, focusing on the translation from preclinical testing to clinical application.

A crucial aspect of the CTGCT's mission is leveraging synthetic biology to improve the safety and efficacy of gene and cell therapies. By integrating tools like engineered regulatory circuits and synthetic promoters, the Centre will fine-tune gene expression and control therapeutic gene activity with greater precision. These advancements will facilitate the development of next-generation therapies that are more effective, with fewer off-target effects and improved safety profiles.

In collaboration with leading institutions such as University College London, University Medical Centre Utrecht, Charité - Universitätsmedizin Berlin, and the Technical University Dresden, the CTGCT will establish a state-of-the-art facility in Ljubljana. The Centre will utilize cutting-edge technologies, including CRISPR, RNA technology, CAR-T cells, and synthetic biology, to develop targeted, patient-specific therapies. Organized around four core areas—research, GMP, translational research, and technology transfer—the CTGCT will ensure seamless translation of research into clinical practice.

Additionally, the CTGCT will foster collaboration among academia, industry, and patient organizations, enhancing research innovation and access to advanced therapies for rare genetic diseases in Slovenia and beyond. This effort will bring together experts from various fields to translate scientific results into the clinic, ultimately benefiting patients with rare genetic diseases through access to life-changing therapies.

Gene Therapy: the time is now

Luis Pereira de Almeida [1]

1. Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

E-mail of the presenting author: luispa@cnc.uc.pt

Gene therapy holds immense promise for revolutionizing modern medicine. It represents a paradigm shift in disease treatment by addressing the root causes of diseases rather than merely alleviating symptoms. With unparalleled potential for both cures and economic impact, this field of medical biotechnology demands cutting-edge research and innovation to tackle the thousands of severe human diseases. Success stories will be highlighted during the presentation.

However, there remains a significant unmet medical need to treat the numerous rare and severe genetic disorders. To address this challenge, we have conceived the GeneT project. GeneT is a Teaming Project designed to establish a Gene Therapy Center of Excellence (CoE) in the central region of Portugal. This CoE will leverage the existing R&D and innovation capacity at the University of Coimbra, a national hub for innovation potential and knowledge transfer in Red Biotechnology. It aims to serve as an (inter)national enabler of scientific and business excellence in gene therapy innovation and manufacturing. GeneT collaborates with the University of Sheffield (Gene Therapy Innovation and Manufacturing Centre), the University of Eastern Finland (Finnish National Virus Vector Laboratory), and nationally with the Coimbra University Hospitals and the Biotechnology Park BIOCANT, a pivotal center for the biotech industry in Portugal. GeneT is founded on three pillars: (1) a research center in gene therapy, (2) a vector production center, and (3) a clinical trials unit. We anticipate that GeneT will become a beacon of excellence in gene therapy R&I, unlocking the transformative power of gene therapy to improve health and transform lives.

Advanced Therapy Medicinal Products and Academia's Evolving Role: From Clinical Trials to Patient Access

Regina Demlova [1]

1. Masaryk University, Faculty of Medicine, Central European Advanced Therapy and Immunotherapy Center (CREATIC)

Email of the presenting author: demlova@med.muni.cz

Progress in biomedical science has enabled scientists to use an entirely new toolbox to treat a significant group of diseases, such as inherited rare diseases and various rare cancers. Traditional treatments based on chemical drugs do not always work for rare diseases, often focusing on symptom management rather than reversing the course of the disease itself. Advanced therapy medicinal products (ATMPs), which include cell and gene therapies, have the potential to reverse the negative prognosis for patients with unmet medical needs for whom no conventional treatment is currently available.

ATMPs, often referred to as “living drugs,” leverage recent advances in genomics, cell biology, and gene editing to offer truly personalized curative options, such as somatic-cell and gene therapies. Somatic-cell therapies (e.g., dendritic cell-based anti-cancer vaccines, expanded tumor-infiltrating T cells) hold the promise of curing cancers with otherwise poor prognoses. Gene therapies have enormous potential to be a game-changer for monogenic rare diseases, as somatic genome editing can repair, deactivate, or replace dysfunctional genes, potentially curing inherited rare diseases and some types of cancers. Since ATMPs are medicines based on genes, cells, or tissues, they present challenges regarding excellent research and Good Manufacturing Practice (GMP) in development and production.

CREATIC (Central European Advanced Therapy and Immunotherapy Centre) is a newly established Center of Excellence at Masaryk University, Faculty of Medicine, supported by the Horizon Europe Teaming for Excellence project. CREATIC is building upon the existing expertise at MU’s Advanced Cell Immunotherapy Unit (ACIU) in the research and innovation (R&I) of somatic cell therapies. The unit is already conducting Phase I/II clinical trials for two existing ATMP cell-based products: MyDendrix™ (for high-risk pediatric cancers) and FlyCellix™ (for Epidermolysis Bullosa). With a focus on undiagnosed and untreatable rare diseases, pediatric patients with high-risk tumors, and adult cancer patients suitable for ATMP treatment, CREATIC aims to translate R&I in cell and gene therapies into clinical practice to benefit patients.

Session 4 – Non-coding & therapy

Tuesday, 17th September 2024 (16:00 – 18:00)

16:00 – 16:25

Judith Zaugg, European Molecular Biology Laboratory, Germany
"How do cells integrate extrinsic signals and intrinsic state? A systems epigenetics approach"

16:25 – 16:50

Martin Kircher, Professor of Regulatory Genomics, Institute of Human Genetics, Campus Lübeck, Germany
"Scoring Genetic Variation in Coding and Non-coding Sequences"

16:50 – 17:35

Jef D. Boeke, NYU Langone Health, United States
"Engineering mammalian genomes"

How do cells integrate extrinsic signals and intrinsic state? A systems epigenetics approach

Judith B Zaugg [1]

1. European Molecular Biology Laboratory Heidelberg

E-mail of the presenting author: judith.zaugg@unibas.ch

Our group aims to understand how cells integrate genetic and epigenetic information with extrinsic signals from their microenvironment, and how these molecular layers jointly contribute to cellular states that ultimately define complex disease phenotypes. Our main systems of interest are the hematopoietic system and immune cells, for which we aim to understand how they interact with their microenvironment, and how these interactions are disrupted in hematological malignancies and common immune-related traits and diseases. To address these questions, we are developing experimental technologies and computational approaches. Specifically, we have developed SUM-seq to jointly profile transcriptome and chromatin accessibility in single cells across many samples and developed a systems epigenetics framework to integrates genetic, epigenetic and transcription factor-mediated mechanisms from single cell RNA/ATAC-seq data. In this talk I will briefly introduce our technology and computational framework, and present our most recent work where we use these technologies to understand molecular and cellular mechanisms in autoimmune diseases, and hematological malignancies.

Scoring functional impact of genetic variation in coding and non-coding sequences

Martin Kircher [1,2]

1. Institute of Human Genetics, University Medical Center Schleswig-Holstein, University of Lübeck, Germany
2. Exploratory Diagnostic Sciences, Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Germany

E-mail of the presenting author: martin.kircher@bih-charite.de

The identification of disease-causing mutations is crucial in both research and clinical settings, yet the interpretation and ranking of these variants remains challenging.

The Combined Annotation Dependent Depletion (CADD) framework addresses this by integrating annotations and contrasting variants that survived purifying selection with simulated mutations to score short sequence variants (SNVs, InDels, multi-allelic substitutions). Since its inception, CADD has been widely adopted and continuously improved, including support for GRCh37 and GRCh38 assemblies and the integration of deep neural network (DNN) models for splice effects. Recently available updates (CADD v1.7, https://cadd.bihealth.org/) incorporate state-of-the-art protein language model-derived scores from Meta AI's Evolutionary Scale Modeling and convolutional neural networks for regulatory sequence effects, enhancing the scoring of coding sequence alterations as well as regulatory sequences. Additionally, the CADD framework has been previously extended to score structural variants (CADD-SV, https://cadd-sv.bihealth.org/), providing a comprehensive approach that leverages a large training dataset, covering diverse and rare feature annotations without major ascertainment biases.

In non-coding sequences, particularly regulatory regions, the NIH Impact of Genomic Variation on Function (IGVF) consortium employs Massively Parallel Reporter Assays (MPRAs) to investigate functional consequences of sequence perturbations. By testing thousands of candidate cis-regulatory regions (CREs), their common and rare variation within as well as predicted high-impact variants allows us to benchmark and develop better models for the interpretation of regulatory sequences. Our current results highlight the power of sequence-based models in identification of broadly active CREs, while also showing clear limitations for variant-level effects. This underlines the importance of experimental methods to perturb and explore the regulatory code.

Overall, the continuous development and integration of advanced models and annotations enables robust tools for the prioritization of genetic variants, aiding in diagnostics and research.

Engineering mammalian genomes

Jef Boeke [1]

1. NYU Langone Health

E-mail of the presenting author: jef.boeke@nyulangone.org

Rapid advances in DNA synthesis techniques have made it possible to engineer diverse genomic elements, pathways, and whole genomes, providing new insights into design and analysis of systems. The synthetic yeast genome project, Yeast2.0 is well on its way with the 16 synthetic *S. cerevisiae* chromosomes now completed by a global team. We have automated a big DNA synthesis and assembly pipeline, and can readily assembly and deliver human genomic regions of 100-200+ kb along with multiple designer synthetic variants thereof. Using precision delivery strategies such as Big-IN and mSwAP-In, we can precisely deliver DNA segments to stem cells, and use these methods to transplants of specific human genomic regions to animal genomes, and endow human or animal cells, or even animals, with new capabilities. Less than 2% of our genome is protein-coding DNA. The vast expanses of non-coding DNA make up the genome's **"dark matter"**, where introns, repetitive and regulatory elements reside. Surprisingly, variation between individuals in non-coding regulatory DNA is emerging as a major factor in the genetics of numerous diseases and traits, yet very little is known about how such variations contribute to disease risk. Studying genetics of regulatory variation is technically challenging, as regulatory elements can affect genes located tens of thousands of bp away, and often, multiple distal regulatory variations, each with a very small effect, combine in unknown ways to significantly modulate expression of genes. An example of extensive humanization using mSwAP-In is the "Covid mouse" made using mSwAP-In. Humanization of the *ACE2* gene led to an improved mouse model for human Covid-19. Interestingly, including additional 5' sequences had significant effects in terms of tissue distribution of expression as well as efficiency of viral multiplication. This is an example of a Genomically Rewritten and tailored Genetically Engineered Mouse Model or GREAT GEMM (Zhang et al. Nature 2023).

Session 5 – Models for gene therapy & genome editing

Wednesday, 18th September 2024 (9:00 – 11:00)

9:00 – 9:25

Martin Jinek, University of Zurich, Department of Biochemistry, Switzerland
"CRISPR Genome Editors: Mechanisms, Engineering and Therapeutic Applications"

9:25 –9:50

Hyunji Lee, KMPC and College of Medicine, Korea University, Republic of Korea
"Strategies for Creating Mitochondrial Genetic Engineering Mouse Model"

9:50 – 10:15

Pavel Krejci, Masaryk University, Faculty of Medicine, Czech Republic
"FGFR3 in Skeletogenesis and Achondroplasia: New Treatment Development"

10:15 – 10:40

Jun Won Park, KMPC and Department of Laboratory Animal Medicine, College of Veterinary Medicine, Seoul National University, Republic of Korea
"Mouse Models for Dynamics of Epithelial Cell Plasticity During Gastric Carcinogenesis and Metastasis"

Strategies for Creating Mitochondrial Genetic Engineering Mouse Model

Hyunji Lee [1]

1. Department of Convergence Medicine, Korea University College of Medicine, Seoul, 02708, Korea

Email of the presenting author: hjlee102@korea.ac.kr

Mitochondria are fundamentally important for programmed cell death, cellular metabolism, and the modulation of intracellular calcium concentrations. Within the mitochondria, there is DNA that contains crucial genetic information for mitochondrial function, known as mitochondrial DNA (mtDNA). Mutations in mtDNA can cause inherited mitochondrial disorders, affecting various organs and systems. However, mtDNA editing, which is vital for treating these disorders, faces several challenges. As a result, the development of animal models and treatments for mitochondrial genetic diseases has been quite limited. Recently, programmable editing tools such as cytosine base editors derived from DddA (DdCBE) and transcription activator-like effector-linked deaminases (TALED) for mtDNA base editing have shown considerable potential in correcting pathogenic mtDNA variants. I describe recent advances in structural biology and repair mechanisms, and introduces advanced strategies for applying mtDNA base editors to mice, including various mitochondrial DNA editing mouse models created using these methods. Ultimately, it discusses the potential medical applications and disease modeling of mtDNA editing for treating mitochondrial diseases.

Session 6 – Short presentations: Selected poster presentations & technology talks

Wednesday, 18th September 2024 (11:20 – 13:10)

11:20 – 11:35

Ladislav Vyklicky, Institute of Physiology CAS, Czech Republic
"Cellular and molecular consequences of nonsense and missense GRIN gene variants in animal models: Gain of function or loss of function, that is the question"

11:35 – 11:50

Lillian Garrett, Helmholtz Center Munich, Germany
"Decoding Schizophrenia genetics: insights from mouse models and human brain expression data"

11:50 – 12:05

Silvia Mandillo, Institute of Biochemistry and Cell Biology, CNR-National Research Council, Italy
"Muscle-specific gene editing improves molecular and phenotypic defects in a mouse model of Myotonic Dystrophy type 1"

12:05 – 12:20

Filip Dámek, Institute of Experimental Genetics and German Mouse Clinic, Helmholtz Zentrum München, German Research Center for Environmental Health, Germany
"New insights by comprehensive phenotyping of the Idua knockout mouse model"

12:20 – 12:35

Katarzyna Kowalczyk, Field Application Specialist Genomics, CEE (Bio-Rad sponsored talk)
"BIO-RAD - DDPCR ADVANCED APPLICATIONS - EASY WAY FOR DELIVERY SOLUTION FOR DETECTING RARE DISEASES, CREATE THERAPIES AND QUALITY CONTROL"

12:35 – 12:50

Jessica Hartman, Cell Microsystems, USA (Accela sponsored talk)
"Automate and simplify cell line development using the CellRaft AIR® System"

Cellular and molecular consequences of nonsense and missense GRIN gene variants in animal models: Gain of function or loss of function, that is the question

Ladislav Vyklicky [1]

1. Institute of Physiology CAS, Videnska 1083, 142 00 Prague-4, Czech Republic

E-mail of the presenting author: Ladislav.Vyklicky@fgu.cas.cz

Glutamate is the primary excitatory neurotransmitter in the central nervous system, interacting with AMPA, kainate, and N-methyl-D-aspartate receptors (NMDARs). NMDARs play a crucial role in most brain circuits. NMDARs are heteromeric cation channels comprising two obligatory GluN1 and two GluN2A-D/3A-B subunits. The glutamate-binding GluN2A-D subunits exhibit diverse spatial and temporal expression patterns and impart specific biophysical and pharmacological characteristics to the NMDAR. Genetic variations in GRIN genes encoding NMDAR subunits have been identified in patients with neurodevelopmental disorders, including intellectual disability, epilepsy, and autism. However, it is not well understood how the presence of specific GRIN gene variants leads to disease.

Using a combination of techniques, we characterized the consequences of missense and nonsense mutations in GRIN2A/B, encoding the GluN2A/B subunits. We analyzed variant NMDAR function and cell-surface expression using whole-cell patch-clamp recording and immunofluorescence microscopy. We quantified mRNA levels of different NMDAR subunits using qPCR, and protein expression using mass spectrometry. We analyzed the behavioral phenotypes in mouse and zebrafish GMO animal models. Our findings provide evidence of variant-dependent alterations in functional and pharmacological receptor properties. We also demonstrate a range of compensatory alterations brought on by the presence of GRIN gene variants, including changes in mRNA or protein levels of different NMDAR subunits, and we show the impact of compensatory substitution of GluN2A for GluN2B. Further, our findings suggest that it is the abundance of GluN1, rather than GluN2A/B, that is the primary determinant of the number of NMDARs in the adult mouse hippocampus and thus of the overall NMDAR function. We propose that the functional impact of different GRIN gene variants is a complex result of multiple changes at the molecular and cellular level.

Decoding Schizophrenia genetics: insights from mouse models and human brain expression data

Lillian Garrett [1], Dietrich Trümbach [1], IMPC Consortium [2], Valerie Gailus-Durner [1], Helmut Fuchs [1], Wolfgang Wurst [1], Martin Hrabe de Angelis [1], Sabine Hölter [1]

1. Helmholtz Center Munich, Germany
2. International Mouse Phenotyping Consortium (IMPC)

E-mail of the presenting author: lillian.garrett@helmholtz-munich.de

Objective: Schizophrenia (SZ) is a complex psychiatric disorder with significant genetic underpinnings and limited treatment options. Our research harnesses the power of large-scale mouse gene knockout (KO) model systems, combined with mouse and human brain expression data, to identify gene networks linked to SZ-related behavioral abnormalities. This approach not only advances our understanding of SZ genetics but also highlights the broader applicability of cross-species data integration in neuropsychiatric research.

Method: We utilized KO mouse lines from the International Mouse Phenotyping Consortium (IMPC), created using CRISPR/Cas9 mutagenesis, to investigate the impact of gene deletions on prepulse inhibition (PPI), a core SZ endophenotype. We thereby identified a candidate gene set, which we integrated with transcriptomic data from the Allen Brain Atlas, encompassing both mouse and human brain expression profiles. Using hierarchical clustering and weighted gene co-expression network analysis (WGCNA), we identified and functionally annotated co-expression modules relevant to SZ pathology.

Results: Our findings reveal two key gene modules enriched with both SZ risk and mouse PPI genes, primarily associated with synaptic function and neurotransmission. These modules show high expression in the cingulate cortex, hippocampus and amygdala, regions critically implicated in SZ. Within this framework, we identified potentially novel SZ-relevant genes with the post-synaptic scaffold protein TANC2 emerging as a novel SZ genetic driver affecting PPI in mice.

Conclusion: This research underscores the value of integrating mouse and human genetic data to understand the genetic architecture of schizophrenia (SZ). By identifying gene networks linked to SZ, we highlight the potential of cross-species data integration to uncover molecular mechanisms underlying complex psychiatric disorders, opening new avenues for targeted therapies.

Muscle-specific gene editing improves molecular and phenotypic defects in a mouse model of Myotonic Dystrophy type 1

Mariapaola Izzo [1], Jonathan Battistini [1], Elisabetta Golini [1], Claudia Provenzano [1], Tiziana Orsini [1], Georgios Strimpakos [1], Ferdinando Scavizzi [1], Marcello Raspa [1], Genevieve Gourdon [2], Silvia Mandillo [1], Beatrice Cardinali [1], Germana Falcone [1]

1. Institute of Biochemistry and Cell Biology, CNR-National Research Council, Monterotondo, 00015 Rome, Italy
2. Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, 75013 Paris, France

E-mail of the presenting author: silvia.mandillo@cnr.it

Therapeutic gene editing for treatment of monogenic diseases is a powerful technology that could in principle eliminate definitively the disease-causing genetic defect. The precision and efficiency of the molecular mechanisms are still under investigation in view of a possible use in clinical practice. Here we describe the application of the CRISPR/Cas9 strategy to remove the CTG-expansion in the DMPK gene causing myotonic dystrophy type 1 (DM1) in a mouse model carrying the human transgene from a DM1 patient. To optimize the editing efficiency in vivo, we identified new tools that allowed to improve the expression levels and the activity of the CRISPR/Cas9 machinery. Newly designed guide RNA pairs were tested in DM1-patient derived cells prior to in vivo application. Edited cells expressing the selected pair were analyzed to assess the occurrence of off-target and the accuracy of on-target genomic events. Systemic delivery of CRISPR/Cas9 components through myotropic adeno-associated viral vectors led to significant improvement of molecular alterations in the heart and skeletal muscle. Importantly, a persistent increase of body weight, improvement of muscle strength and body composition parameters were observed in treated animals.

New insights by comprehensive phenotyping of the Idua knockout mouse model

Filip Dámek [1], Patricia da Silva-Buttkus [1], Julia Calzada-Wack [1], Adrián Sanz-Moreno [1], Susan Marschall [1], GMC consortium[1], Valérie Gailus-Durner [1], Helmut Fuchs [1], Martin Hrabě de Angelis [1,2,3]

1. Institute of Experimental Genetics and German Mouse Clinic, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764, Neuherberg, Germany
2. Chair of Experimental Genetics, TUM School of Life Sciences, Technische Universität München, Freising, Germany
3. German Center for Diabetes Research (DZD), Neuherberg, Germany

E-mail of the presenting author: filip.damek@helmholtz-munich.de

Introduction: The alpha-L-iduronidase (Idua) gene is crucial for the degradation of dermatan sulfate and heparan sulfate glycosaminoglycans in lysosomes. Mutations in this gene can cause mucopolysaccharidosis type I (MPS I), a rare autosomal recessive disorder with various skeletal and organ abnormalities. This study aimed to characterize the function of the Idua gene in mice to enhance insights into human diseases and support therapeutic development.

Methods: A targeted Idua gene knockout was created using CRISPR/Cas9 genome editing. Homozygous C57BL/6N mutant and control mice were generated and subjected to systemic phenotype characterization across 14 disease areas at the German Mouse Clinic (GMC), using the International Mouse Phenotyping Consortium (IMPC) screening pipeline.

Results: Idua mutant mice displayed multiple phenotypic abnormalities, including decreased locomotor activity, exploratory behavior, and acoustic startle reactivity, alongside elevated auditory thresholds and reduced grip strength. Metabolic changes included increased plasma urea, iron, liver enzymes, and reduced cholesterol, glucose, and albumin. Hematological findings showed anemia, altered blood cell profiles, and increased platelets. Mice also had increased lean mass, spleen weight, with reduced fat and heart weight, and mitral valve and m. trachealis vacuolization. Ocular changes included retinal and corneal thinning, reduced eyelid openings, and retinal aggregates. Mice exhibited increased bone density and zygomatic arch thickness, with some showing a compressed snout.

Conclusion: This study provides a comprehensive phenotypic characterization of Idua knockout mice, confirming known abnormalities while uncovering novel findings in m. trachealis and iron metabolism. Additionally, unexpected heart and hematological findings were observed, which differ from previous patient observations. These results advance the understanding of lysosomal storage disorders and highlight potential avenues for therapeutic development.

BIO-RAD - DDPCR ADVANCED APPLICATIONS - EASY WAY FOR DELIVERY SOLUTION FOR DETECTING RARE DISEASES, CREATE THERAPIES AND QUALITY CONTROL

Katarzyna Kowalczyk [1]

1. Kowalczyk Katarzyna

E-mail of the presenting author: katarzyna_kowalczyk@bio-rad.com

Droplet Digital PCR (ddPCR) provides ultrasensitive nucleic acid detection and direct absolute quantification without the need for standard curves. Not only does this simplify experiment set-up but it also increases reproducibility. In ddPCR, the PCR reaction is partitioned by droplet generator into 20,000 uniform sized droplets, which has been externally validated and shown to be highly reproducible.

In summary, this technology stands out by simplified quantification - no calibration standards or reference (ΔΔCq method) are required for absolute quantification. We could reduce consumable costs - reaction volumes range from pico to nanolitres, reducing reagent consumption and the amount of sample required for each data point. The emulsion-based reaction system means that PCR reactions can be performed in a standard thermocycler without complex chips or microfluidics. ddPCR technology allows 20,000 droplets per 20 µl sample, or almost two million partitioned PCR reactions in a 96-well plate which increases precision and sensitivity compared to qPCR or even dPCR.

This direct method of quantification can be applied to any application that uses primers or primers/ probes to detect nucleic acid sequences and we have tools to help you switch any qPCR assay over to ddPCR. Some but not all applications include environmental monitoring of pathogens, species determination, residual host cell contamination assessment, viral load analysis, mutation detection, copy number variation (CNV), minimal residual disease (MRD), microbial quantification, NGS library quantification, genome editing assessment (HDR and NHEJ), small-fold change gene expression analysis, miRNA quantification and methylation sensitive restriction enzyme (MSRE) ddPCR without the need for bisulfite conversion. Probe based analysis using a one-step kit for RT-ddPCR provides minimal hands-on time for reliable 4-plex (QX200) gene expression analysis.

The Bio-Rad system is a well-established platform (>8300 publications across a wide range of applications), with easy-to-use software, high resolution data, experienced technical support, and continued product development to broaden the scope and applications of ddPCR. You can also search Bio - Rad publication list to look for applications of interest.

Automate and simplify cell line development using the CellRaft AIR® System

Jessica Hartman [1]

1. Cell Microsystems, USA

E-mail of the presenting author: jhartman@cellmicrosystems.com

The CellRaft AIR System is a fully automated, one-platform solution for culturing, phenotyping, and isolating genetically modified cells in both 2D and 3D culture. The AIR® System improves these complex workflows by maintaining cell viability, enabling rapid screening of thousands of cells, and gently retrieving the characterized colonies. With the AIR System, users can grow and maintain cells on CellRaft arrays and serially image over time, enabling track-and-trace imaging for proof of clonality. The CellRaft Cytometry software allows users to define parameters to identify clones of interest, and then the desired edited clones are retrieved without fluidics or enzymatic dissociation for downstream analysis and propagation. These features increase the success rate of cell line development, even with rare edits or the most difficult to culture cells.

Session 7 – Rare diseases & bottom-up efforts to develop (gene) therapies

Wednesday, 18th September 2024 (14:10 – 15:50)

14:10 – 14:35

Kacper Lukasiewicz, Medical University of Bialystok, Psychiatry Clinic, Poland
"Insights into PACS2-related Syndrome: Lessons from In Vitro and In Vivo Models"

Piotr Kosla, PACS2 Research Foundation, Poland
"New model to cure ultra rare: Patient-led collaborative research networks"

14:35 – 15:00

Spela Mirosevic, CTNNB1 Foundation, Slovenia
"Finding a Cure for CTNNB1 Syndrome: The Role of the Foundation"

Dusko Lainscek, National Institute of Chemistry, Department of Synthetic Biology and Immunology, Slovenia
"Finding a Cure for CTNNB1 Syndrome: Research Presentation"

15:00 – 15:25

Tanja Zdolsek Draksler, Jožef Stefan Institute, International Research Centre on Artificial Intelligence under the Auspices of UNESCO and IDefine Europe Foundation, Slovenia
"Solving Rare Diseases: How Global Collaboration and Data are Advancing Kleefstra Syndrome Research"

Pauline Larqué, Radboud University, The Netherlands
"Understanding the Cell-type Specific Function of EHMT1 in Neuronal Network Function"

Insights into PACS2-related Syndrome: Lessons from In Vitro and In Vivo Models

Kacper Lukasiewicz [1,2]

1. Experimental Medicine Centre, Medical University of Bialystok
2. Department of Psychiatry, Medical University of Bialystok

Email of the presenting author: kacper.lukasiewicz@gmail.com

PACS2-related syndrome is an ultra-rare disease and its' true prevalence is not known. As of now, about 32 cases have been reported in the literature and about 100 in total diagnosed world-wide. This disease is caused by mutations in the PACS2 gene encoding PACS2 protein. c.625 G > A (p.Glu209Lys) de novo variant in the PACS2 gene has been predominantly identified among the PACS2 syndrome patients. PACS2 (phosphofurin acidic cluster sorting protein 2) is a multifunctional cytosolic membrane trafficking protein which plays crucial role in mitochondria - endoplasmic reticulum (ER) membranes tethering and modulating communication between the ER and mitochondria especially by influencing calcium ion homeostasis.

The phenotype of PACS2-related syndrome is unspecific. Typical disease symptoms are: seizures, global developmental delay, hypotonia, behavioral abnormalities, dysmorphic features, ophthalmologic defects, cerebellar dysgenesis, hematologic abnormalities, distal limb abnormalities. Currently there is no treatment available.

First, I will present recent findings from in vitro study on PACS2 syndrome patients' fibroblasts run by Charles River Laboratories. Drug repurposing screen using CellPainting and machine learning approach revealed potential drug candidates.

Next I will focus on the results of Pacs2+/E209K mouse model phenotyping performed at Czech Centre for Phenogenomics. Pilot screen using IMPC early adult phenotyping pipeline was followed by custom advanced test selection, which confirmed model face validity in multiple domains. Tests covered behavioral, metabolic, vision, skull morphology, biochemistry and histological features. Strong phenotype was found in the eye fundus using optical coherence tomography and was further confirmed using histological stainings.
We believe that these results paved a way to create a transgenic mouse model-based assay for the advanced drug screening for PACS2-related syndrome.

This research was supported by Visegrad Fund (Fellowship #62410114 to KL)

New model to cure ultra rare: Patient-led collaborative research networks

Piotr Kosla [1], Malgorzata Kosla [1]

1. PACS2 Research Foundation

E-mail of the presenting author: piotrkosla@gmail.com

Out of ~10 000 rare diseases vast majority constitutes long tail of ultra rare disorders each affecting less than 1000 people globally. There is limited scientific coverage of those diseases – hence, no hope for a cure.

Back in February 2022, we were faced with devastating news – one of our twin girl, Lena, was diagnosed with PACS2 syndrome (DEE66 - Developmental and epileptic encephalopathy 66), ultra-rare disease with < 50 patients diagnosed worldwide back then. Syndrome firstly described in 2018 with unknown pathomechanism and very limited interest in therapy development.

In May 2022, building on our entrepreneurial skills and many years of experience in managing projects in multinational companies, together with my wife we have decided to establish patient-driven foundation with full focus on research and therapy development. During presentation, I will show what progress we've made in the last 2 years, how we've managed to increase interests among scientists from 1 project and 1 scientist to 10+ projects on PACS2 syndrome and 50+ scientists involved in them.

I will also present how, as research foundation, we establish and coordinate cooperation between scientists, physicians and families with focus simultaneously on basic, translational and clinical research, how we reduce administrative bottlenecks and monitor all works on PACS2 syndrome across the globe.

We believe that we have created new model to cure ultra-rare in which relentless families without or with limited scientific background drive toward cures while being capital efficient, moving fast and bursting with motivation to help a loved one. New model in which patient-driven organizations play role of real partners in the research project.

Finding a Cure for CTNNB1 Syndrome: The Role of the Foundation and Research Presentation

Duško Lainšček [1,2], Špela Miroševič [3], Damjan Osredkar [4], Petra Sušjan [1,4], Rodney Samaco [5], Ana Gonzalez Hernandez [3], Roman Jerala [1,2], Andrea Perez-Iturralde [6], Leszek Lisowski [6,7,8]

1. Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, 1000, Slovenia
2. Centre for Technologies of Gene and Cell Therapy, Ljubljana, 1000, Slovenia
3. CTNNB1 Foundation, The Gene Therapy Research Institute, Dalmatinova ulica 5, 1000 Ljubljana, Slovenia
4. Department of Pediatric Neurology, University Children's Hospital Ljubljana, University Medical Centre Ljubljana, Bohoričeva 20, 1525 Ljubljana, Slovenia
5. Association of University Centers On Disabilities, Silver Spring, MD 20910, United States of America.
6. Translational Vectorology Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW 2145, Australia
7. Australian Genome Therapeutics Centre, Children's Medical Research Institute and Sydney Children's Hospitals Network, Westmead, NSW 2145, Australia
8. Laboratory of Molecular Oncology and Innovative Therapies, Military Institute of Medicine - National Research Institute, Warszawa, Poland

Email of the presenting author: spela@ctnnb1-foundation.org

Background: CTNNB1 syndrome is a rare neurodevelopmental disorder caused by loss-of-function mutations in the CTNNB1 gene, resulting in the reduced function of beta-catenin, a protein critical for neuronal development and synapse formation. The lack of effective treatments underscores the need for innovative therapeutic strategies. The CTNNB1 Foundation has taken a leading role in fostering research collaborations, raising awareness and securing over 2 million euros for funding to advance the development of potential therapies. These funds have enabled the development of cell and animal models of CTNNB1 syndrome and the exploration of various therapeutic options, including gene-replacement therapy.

Methods: Our research team has developed six AAV-CTNNB1 gene therapy candidates (C1-C6), each containing different regulatory elements to optimize expression and functionality. These candidates were initially tested in brain organoids from a CTNNB1 patient to assess their ability to restore beta-catenin expression. The most promising candidate was then tested in a CTNNB1 disease model (Ctnnb1+/-) in which different doses (4.24e9, 2.12e10 and 1.06e11 vg/animal) were administered via the intracerebroventricular route of administration. Safety was assessed in wild-type mice that received the highest doses (1.06e11 and 2.25e11 vg/animal) and were followed for eight months.

Results: The selected AAV-CTNNB1 vector showed significant therapeutic potential. In brain organoids, the therapy restored beta-catenin expression to near-normal levels and in vivo study with the highest dose (1.06e11 vg/mouse) corrected anxiety-like behaviors and improved locomotor function in CTNNB1-deficient mice. Importantly, safety testing showed no adverse effects at the doses tested, supporting the safety profile of the therapy.

Conclusions: AAV-CTNNB1 gene therapy has demonstrated promising results by restoring beta-catenin expression and improving disease phenotypes with a favorable safety profile. The vector is being produced for GLP toxicology studies, with a clinical trial set for Q3 2025 to evaluate its safety and efficacy in treating CTNNB1 syndrome. This work is an example of the power of patient-centered initiatives and collaborative research to advance potential cures for rare genetic diseases

Solving Rare Diseases: How Global Collaboration and Data are Advancing Kleefstra Syndrome Research

Tanja Zdolsek Draksler [1,2]

1. Jožef Stefan Institute, International Research Centre on Artificial Intelligence under the Auspices of UNESCO, Ljubljana, Slovenia
2. IDefine Europe Foundation, Slovenia

E-mail of the presenting author: tanja.zdolsek@ijs.si

Kleefstra syndrome (KLEFS1) is a rare genetic syndrome caused by haploinsufficiency of the EHMT1 gene, which leads to a wide range of clinical manifestations. This presentation gives an in-depth exploration of the KLEFS1 global community, highlighting its global nature and the pivotal role of collaboration in advancing research and data collection. The Kleefstra Syndrome Global Community—a diverse network spanning Europe, the USA, Canada, Australia, South America and even Asia. Several data collection initiatives that are crucial for KLEFS1: (1) Kleefstra Syndrome Worldwide Map, (2) GENIDA registry - by caregiver-reported data, (3) RARE-X, a collaborative platform for global data sharing and analysis by caregiver-reported data, (4) Citizen Health - collecting, organizing and harmonizing clinical data from patient medical records to deliver a cost-effective natural history dataset (active in USA). Alongside these long-term data collection activities, there were/are also several short-term activities, collecting specific data, mostly in a clinical setting. The data collection initiatives mostly focus on gathering caregiver-reported data, which contributes to a more accurate understanding of KLEFS1 clinical symptoms and prevalence. That is why there is an increased need for advocacy activities for heighten awareness and increase efforts in promoting the importance of data collection to both families and clinicians. Additionally, the Rare Diseases Observatory [1] was developed and introduced, an innovative project aimed at monitoring rare diseases globally through diverse data sources, including global media, social media, and scientific publications. The Rare Diseases Observatory is focusing on rare neurodevelopmental disorders, one of them being also KLEFS1.

This presentation encapsulates my journey, the challenges I've faced, and the ongoing efforts to bridge gaps in data collection for rare diseases. Through global collaboration and advanced data initiatives, I aim to underscore the critical steps being taken to improve health outcomes and societal well-being for those affected by KLEFS1 and other rare diseases.

[1] https://rarediseases.ijs.si/.

Understanding the cell-type specific function of EHMT1 in neuronal network function

Pauline Larqué [1,2], Nael Nadif Kasri [1,2], Dirk Schubert [1,2]

1. Radboudumc - Nijmegen, The Netherlands
2. Donders Institute for Brain, Cognition and Behaviour - Nijmegen, The Netherlands

E-mail of the presenting author: paulinesophie.larque@radboudumc.nl

EHMT1, a histone methyltransferase implicated in Kleefstra Syndrome, plays a pivotal role in maintaining the excitatory/inhibitory (E/I) balance within neuronal networks. While previous research has highlighted that mutations in EHMT1 disrupt this balance and alter network activity, the specific contributions of distinct neuronal cell types have not been thoroughly investigated.

To address this gap, we used human-induced pluripotent stem cells (iPSCs) to explore the cell-type specific functions of EHMT1. Our prior research demonstrated that EHMT1 deficiency in excitatory neurons leads to significant disruptions in neuronal network dynamics, including reduced network bursting rate, prolonged burst duration, and increased temporal irregularity. These abnormalities have been linked to NMDAR hyperfunction in EHMT1-deficient neurons (Frega et al., 2019). However, the impact of EHMT1 deficiency on inhibitory neurons remains largely unknown. I will discuss our recent advancements in developing protocols to co-culture excitatory and inhibitory neurons, enabling the study of the combined and separate effects of EHMT1 deficiency on both neuronal types. This approach aims to provide a more comprehensive understanding of EHMT1's role in neuronal network function and its impact on E/I balance in the context of Kleefstra Syndrome.

Session 8 – Closing

Wednesday, 18th September 2024 (16:15 – 17:10)

16:15 – 17:00

Cathleen Lutz, The Jackson Laboratory, United States
"After the Diagnosis: Gene Based Therapies and the Road to Treatments"

17:00 – 17:10

Radislav Sedlacek, Czech Centre for Phenogenomics Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
"Closing remarks"

After the Diagnosis: Gene Based Therapies and the Road to Treatments

Cathleen Lutz [1]

1. Rare Disease Translational Center, The Jackson Laboratory (JAX), United States of America

Email of the presenting author: cat.lutz@jax.org

Genome sequencing has ended the diagnostic odyssey for many families, but the research and development of treatments for individual diseases is lagging. At the same time, technologies surrounding gene-based therapies and delivery modalities are advancing at an unprecedented rate and offer new hope to patients. Therapeutic successes in diseases like Spinal Muscular Atrophy, Cystic Fibrosis, Duchenne Muscular Dystrophy, Friedreich's Ataxia and Sickle Cell Anemia have galvanized the rare disease community, creating a vision of success for families and a revolution of family-based organizations trailblazing their path to the clinic. With the support of NIH funding mechanisms, the Rare Disease Translational Center (RDTC) at The Jackson Laboratory works with patient organizations to create mouse models for these rare diseases, foraging research collaborations along the way for their expedient characterization and use. These mouse models not only serve as patient avatars that increase our understanding of the underlying pathophysiology of a given disease, but also allow us to test newly emerging small molecule and gene-based therapies. With over 50 patient programs in the pipeline, the RDTC at The Jackson Laboratory has created a systemized process for the expedient development and testing of therapeutics to the clinic for the rare disease community.

Poster session 1 (on-site) - Tuesday 17th

10:55 – 11:15
13:00 – 14:15
15:30 – 16:00

Poster session 2 (on-site) –Wednesday 18th

11:00 – 11:20
13:10 – 14:10
15:50 – 16:15

A) Research poster presentations

(PO-01) **Igor Varga:** Automatic Skull Sutures Detection for Mouse Phenotyping

(PO-02) **Michaela Šímová:** DECIPHERING THE EMERGENCE OF ERYTHRO-MYELOID PROGENITORS IN THE MOUSE YOLK SAC

(PO-03) **Olha Pyko:** Deciphering the Impact of ZNF644 Deletion: Investigating the Role of C2H2 Zinc Finger Protein in Mouse Female Phenotype

(PO-04) **Rodolfo Favero:** Development and Characterization of a Conditional Spink5 Knockout Mouse Model for Netherton Syndrome

(PO-05) **Hirotoshi Shibuya:** Development of high-throughput, high-resolution soft tissue imaging methods using new contrast-enhanced micro-CT

(PO-06) **Matilde Vale:** Development of therapeutic exosomes and gene therapy for Diamond Blackfan Anemia (DBA)

(PO-07) **Sabina Cerulová:** Dysregulation of calcium-phosphate metabolism in originally created mouse model with a rare GALNT3 mutation

(PO-08) **Zhenni Liu:** Exploring the Role of GPR45 in Metabolic Regulation and Its Implications for Obesity and Related Diseases

(PO-09) **Eni Tomović:** Genetic and functional analysis of GRIN variants detected in Czech pediatric patients

(PO-10) **Ben Davies:** Human genomic humanization of the Grem1 (88 kb) and Taf1 (166kb) genes

(PO-11) **Federica Gambini:** Characterization of a Novel Inducible hACE2 Mouse Model for SARS-CoV-2 Research: Insights into Acute Infection and Long COVID

(PO-12) **Klevinda Fili:** Characterization of mice carrying neurodevelopmental disease-associated variants

(PO-13) **Vera Abramova:** Characterization of Zebrafish Larvae with Knockouts of NMDA Receptor grin2Aa and grin2Ab Genes: Gene Expression and Swimming Behavior

(PO-14) **Hana Kolesová:** Morphology and physiology of Jagged1 conditional deletion and patient-based single variant mouse models

(PO-15) **Petr Nickl:** Multistep allelic conversion in mouse pre-implantation embryos by AAV vectors

(PO-16) **Silvia Mandillo:** Muscle-specific gene editing improves molecular and phenotypic defects in a mouse model of Myotonic Dystrophy type 1

(PO-17) **Kristýna Neffeová:** Physiological and Morphological Consequences of Jagged1 Deletion in Mouse Model of Tetralogy of Fallot

(PO-18) **Tomasz Kowalczyk:** Proteomic analysis of soft tissues from mice with PACS2 gene mutation

(PO-19) **Dominik Cysewski:** Proteomic and Metabolomic Profiling of Brain Tissues in PACS2 E209K Mutant Mice: Insights into Molecular Dysregulation

(PO-20) **Betul Ogan:** ROLE OF FAM83H IN IMMUNE SYSTEM HOMEOSTASIS

(PO-21) **Maximilián Goleňa:** Seasonality of measured parameters in C57Bl/6NCrl mice

(PO-22) **Tobiáš Ber, Kateryna Nemesh:** Terrestrial Slugs as Prospective Animal Models for Studying RNA Silencing Pathways

(PO-23) **Gunay Akbarova-Ben-Tzvi:** The impact of modified TGF-β family on Integrin-β1 Synthesis of Chondrocyte Cell Sheets

(PO-24) **Arkadiusz Żbikowski:** The impact of PACS2 Syndrome on lung and kidney structure in mice

(PO-25) **Viktor Kostohryz:** The promise of episomal gene therapy

(PO-26) **Miles Raishbrook:** The significance of Fam84b in retinal homeostasis

(PO-27) **Ji Xu:** Transcriptional corepressor TLE1 is a positive factor in adipocyte differentiation

(PO-28) **Sylvie Dlugosova:** Skeletal dysmorphology and mineralization defects in Fgf20 KO mice

(PO-29) **Eliška Pavelková:** Assessing the reproductive phenotypes in mice – current methods and room for improvement

(PO–01) Automatic Skull Sutures Detection for Mouse Phenotyping

Igor Varga [1,2], Frantisek Spoutil [1], Sylvie Dlugosova [1], Vendula Novosadova [1], Jan Prochazka [1], Michaela Prochazkova [1]

1. Phenotyping Module, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences
2. Department of Cybernetics, Faculty of Electrical Engineering,Czech Technical University in Prague

E-mail of the presenting author: varhai@img.cas.cz

Phenotyping the mouse skull is a critical component of biomedical research, especially in the context of gene mapping. This process involves the comprehensive analysis of skull morphology, focusing on aspects such as size, shape, and density. Advanced imaging techniques, like micro-computed tomography (micro-CT) and histology, are commonly employed to capture detailed structural data. Quantitative analyses, including landmark-based geometric morphometrics, are then used to assess these morphological features.

Skull shape phenotyping has proven to be a highly reliable method for identifying outliers, achieving close to 99% accuracy rates. Despite its precision, this method is limited to shape-based differences and cannot detect non-shape-related variations that may be present in CT images. To address this limitation, an artificial detection method was developed to identify mouse sutures, which are not discernible through traditional shape analysis.

This advancement enhances the ability to detect subtle morphological differences that may be critical in gene mapping studies. By integrating this new technique with existing shape phenotyping methods, researchers can better understand skull morphology, leading to more accurate gene mapping and a deeper insight into the genetic factors influencing craniofacial development. This innovation represents a significant step forward in the field, offering a more nuanced approach to mouse skull phenotyping.
Our findings show accuracy of 84.4% of mouse sutures detection, concerning labellers accuracy above 90 %. This result could save a labeller's time and perform a vital analysis for mouse phenotyping.

(PO–02) DECIPHERING THE EMERGENCE OF ERYTHRO-MYELOID PROGENITORS IN THE MOUSE YOLK SAC

Michaela Šímová [1], Carlos Eduardo Madureira Trufen [1], Iva Šplíchalová [2], Jan Kubovčiak [3], Michal Kolář [3], Vendula Novosadová [4], Jan Procházka [1,4], Dominik Filipp [3], Jana Balounová [4], Radislav Sedláček [1,4]

1. Laboratory of Transgenic Models of Diseases, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
2. Laboratory of Immunobiology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
3. Laboratory of Genomics and Bioinformatics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
4. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

E-mail of the presenting author: michaela.simova@img.cas.cz

During embryogenesis, blood cell production is sustained by three independent hematopoietic waves. Hematopoietic stem cells, which emerge intra-embryonically, drive the third wave and postnatal hematopoiesis; however, they are dispensable prenatally. The first two hematopoietic waves emerge extra-embryonically in the yolk sac (YS) and are essential for embryonic development, as they are the primary source of red blood cells (RBCs) as well as the first myeloid cells in embryos. Given their role in RBC production, understanding the emergence of EMP-derived erythropoiesis is critical for future clinical applications in pre- and early postnatal therapy.

The goal of our project is to map the development of YS-derived hematopoietic lineages. We aim to validate the common endothelial origin of YS-derived waves *in vivo*, determine their contribution to myeloid cell production, and explore the role of selected genes in the specification of EMPs across various hematopoietic niches.

Based on scRNA-Seq analysis of YS-derived progenitors, we propose that all hematopoietic cells share a common hemato-endothelial ancestor. The first ("primitive") wave generates cells with a megakaryocytic-erythroid (MkE) fate, while the second wave, driven by EMPs, produces both MkE progenitors and the first myeloid cells during embryonic development. To further investigate EMP-derived blood cell production, we have identified genes differentially expressed in early EMPs and searched for potential surface markers for use in flow cytometry analysis. So far, we have identified the complement receptor CD88 as a marker of early EMPs. Additionally, we are actively developing a novel lineage-tracing model that will enable us to distinguish between individual hematopoietic waves in different niches.

We conclude that EMPs are responsible for the production of the first myeloid cells during embryogenesis. We propose that both YS-derived waves share a hemato-endothelial origin, and we are currently working on in vivo validation of this hypothesis.

(PO–03) Deciphering the Impact of ZNF644 Deletion: Investigating the Role of C2H2 Zinc Finger Protein in Mouse Female Phenotype

Olha Pyko [1,2], Prokop Černý [1,2], Jan Procházka [1,2], Radislav Sedláček [1,2]

1. Czech Centre for Phenogenomics ,Institute of Molecular Genetics of the Czech Academy of Sciences
2. Laboratory of Transgenic Models of Diseases and Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

E-mail of the presenting author: olha.pyko@img.cas.cz

Zinc finger proteins (ZNFs) are a poorly studied diverse group of transcription factors that play critical roles in gene expression regulation. They are involved in various cellular processes, including transcriptional regulation, ubiquitin-mediated protein degradation, signal transduction, DNA repair, and cell differentiations. ZNFs are classified based on their zinc-finger domain structure, with the most important types being C2H2, RING, PHD, and LIM. This study is focused on C2H2 type of transcription factor, particularly on ZNF644. Partial deletion ZNF644Δ8 caused an enigmatic female infertility phenotype. Our goal is to explain unusual phenotype of ZNF644Δ8, compare it to ZNF644 full KO and find possible

(PO–04) Development and Characterization of a Conditional Spink5 Knockout Mouse Model for Netherton Syndrome

Rodolfo Favero [1], Juraj Lábaj [1], Olha Fedosieieva [1], Ivana Buková [1], Petr Kašpárek [1], Jan Procházka [1], Radislav Sedláček [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Vestec, Czech Republic

E-mail of the presenting author: rodolfo.favero@img.cas.cz

Overactivation of proteases due to loss-of-function mutations in the protease inhibitor SPINK5/LEKTI is the cause of Netherton Syndrome (NS). Although traditionally described as a genetic skin disease, NS is a multisystemic life-threatening condition. This study introduces a novel preclinical mouse model of NS through conditional Spink5 knockout. R26-CreERT2 Spink5flox/flox mice were induced by either topical application of three doses of 4-hydroxytamoxifen (4-OHT) at 1, 5, 10, and 100 ng or intraperitoneal (i.p.) administration of a single dose of tamoxifen (TAM) at 0,25, 0,5, and 1 mg/40 g body weight. Skin and other organs were collected and subjected to histopathological analysis. Spink5 mRNA levels were evaluated by qPCR. Circulating leukocyte profiles ware examined with a hemocytometer. Both induction methods resulted in skin lesions within three days post-treatment across all dosage groups. Histopathological examination revealed characteristic NS skin features, including keratinocyte hyperplasia, hyperkeratosis (orthokeratosis and parakeratosis), and spongiosis. Topical 4-OHT doses ≥5 ng also promoted skin alterations distant from the application site. Leukocyte profile changes, encompassing neutrophils, lymphocytes, monocytes and eosinophils, varied depending on the induction protocol. Additionally, we observed thymic atrophy and histiocytic infiltration in lymph nodes, indicative of broader systemic involvement. Our study demonstrates that different administration routes and tamoxifen doses produce distinct local and systemic effects in this model. The conditional Spink5 knockout recapitulates key features of NS, offering a valuable experimental tool to study the different aspects of this complex disease. This model promises to advance our understanding of NS pathogenesis and may facilitate the development of therapeutic approaches.

(PO–05) Development of high-throughput, high-resolution soft tissue imaging methods using new contrast-enhanced micro-CT

Hirotoshi Shibuya [1], Masaru Tamura [1]

1. Mouse Phenotype Analysis Division, RIKEN BioResource Research Center

E-mail of the presenting author: hirotoshi.shibuya@riken.jp

Visualisation and morphometry of biological 3D tissue structures are essential techniques for histopathology. Bioimaging techniques using fluorescent proteins or luminescent tags are powerful tools for elucidating the pathophysiological mechanisms and understanding biological phenomena. However, a limitation of optical imaging of deep tissues using the visible light spectrum is that it can only be used for transparent samples. Over the past few years, genome editing technologies, such as CRISPR/Cas9, have made it possible to knock out specific genes in animals with unexpected simplicity and speed. This has shifted the bottleneck in disease model analysis from generating knockout mutant animals to phenotyping. Recently, a combination of iodine- or phosphotungstic acid-based contrast agents and X-ray computed tomography (CT), called contrast-enhanced CT, has been used for embryonic lethal phenotyping. This powerful 3D imaging tool facilitates high-throughput and high-resolution analyses of the morphology of animal disease models. However, the current contrast-enhanced CT imaging with iodine or phosphotungstic acid has low tissue specificity, making threshold-based segmentation difficult. We are currently developing novel contrast agents and image analysis methods for the efficient segmentation of contrast-enhanced CT images. In this presentation, we describe an efficient method for imaging the cartilage of mouse embryos using novel contrast agent images. Additionally, we describe a single-nephron segmentation technique for mouse kidneys using image analysis.

(PO–06) Development of therapeutic exosomes and gene therapy for Diamond Blackfan Anemia (DBA)

Matilde Vale [1], Jan Procházka [1,2], Radislav Sedláček [1,2]

1. Laboratory of Transgenic Models of Diseases, Institute of Molecular Genetics of the Czech Academy of Sciences
2. Czech Centre of Phenogenomics

E-mail of the presenting author: ana-matilde.vale@img.cas.cz

Gene therapy offers promising approaches for treating genetic disorders. This project focuses on developing gene therapies for Diamond-Blackfan Anemia (DBA), a rare inherited bone marrow failure syndrome characterized by erythroid hypoplasia and associated congenital malformations. DBA is classified as a ribosomopathy due to mutations within ribosomal protein (RP) genes, resulting in defective maturation of ribosomal RNA.

We are exploring two innovative delivery methods: Extracellular Vesicles (EVs) and lentiviral vectors. For EV-based therapy, we have successfully isolated EVs from human plasma using differential ultracentrifugation. These vesicles are characterized through TEM, CryoEM, and MADLS, revealing a homogeneous population with an average diameter of ~200 nm. Current efforts focus on optimizing the loading of these EVs with luciferase mRNA using electroporation techniques.

Parallel to our EV research, we are advancing the development of lentiviral vectors for gene therapy applications. Our initial approach involved the generation of an integrating lentivirus expressing luciferase as a reporter gene. This vector was successfully delivered to mouse bone marrow via intrafemoral injection (IF), demonstrating the feasibility of in vivo delivery and integration. Our current focus has shifted to the development of non-integrating lentiviral vectors. These vectors are designed to express luciferase transiently, providing a platform for utilizing the CRISPR-Cas9 system. This approach aims to minimize the long-term risks associated with permanent genomic integration while still allowing for effective gene editing.

This dual approach addresses the limitations of current DBA treatments. Existing therapies like corticosteroids and chronic blood transfusions have long-term side effects, while hematopoietic stem cell transplantation (HSCT) carries risks of rejection and infection. Our gene therapy strategies aim to provide a more targeted, effective, and potentially safer alternative for DBA patients with identifiable genetic mutations. This research contributes to the broader field of gene therapy by addressing key challenges in delivery methods and targeted genetic modifications, with potential applications beyond DBA to other genetic disorders.

(PO–07) Dysregulation of calcium-phosphate metabolism in originally created mouse model with a rare GALNT3 mutation

Sabina Cerulová [1], Jan Krivanek [2], Jan Prochazka [3], Radislav Sedlacek [3], Zuzana Marincak Vrankova [1], Marcel Schüller [4], Jan Bohm [1], Petra Borilova Linhartova [1]

1. RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic
2. Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
3. Institute of Molecular Genetics of the Czech Academy of Science, Czech Centre for Phenogenomics, Prague, Czech Republic
4. Department of Pediatrics, University Hospital, Brno, Czech Republic

E-mail of the presenting author: 498829@mail.muni.cz

Transferase ppGalNAcT3, encoded by the *GALNT3* gene, is involved in the regulation of serum/plasma phosphate concentration. Recently, two model organisms resulting in ppGalNAcT3 malfunction were created by the Czech Centre for Phenogenomics (CCP) i) the first with a point *GALNT3* mutation (so called RQ), which was previously found in a patient with hyperphosphatemia, and ii) the second with a complete deletion (with knock-out, KO) of the *GALNT3* gene.

Here we show similarities and differences between biochemical profiles, especially in relation to calcium-phosphate metabolism, of mouse models with modified *GALNT3* gene and the patient with RQ *GALNT3* mutation.

As a part of the phenotyping screening, biochemical analyses of plasma concentrations of selected parameters (phosphate, calcium, and alkaline phosphatase) were performed in each of the three study groups of models – with KO *GALNT3* gene, with RQ *GALNT3* mutation, and healthy controls (wildtype, WT *GALNT3*). Previously, biochemical profile of the patient was repeatedly determined during control examinations follow-up at the hospital.

Our data show that both RQ and KO *GALNT3* animals in comparison to WT *GALNT3* animals have increased plasma levels of phosphate and calcium. No significant differences in the concentrations of these analytes were observed between RQ *GALNT3* and KO *GALNT3* models, even after sex stratification. In addition, it was found out that concentration of alkaline phosphatase was lower in the plasma of both RQ and KO *GALNT3* animals in comparison to controls. Phosphate levels in the patient's serum was increased for a long time, however, both the calcium levels and alkaline phosphatase levels were repeatedly found in the physiological ranges.

Taken together, hyperphosphatemic phenotype was observed in animals with RQ *GALNT3* mutation and those with deletion of the *GALNT3* gene as well as in the patient with RQ *GALNT3* mutation. These data open new possibilities in monitoring of hyperphosphatemia associated with hereditary dysfunction of ppGalNAcT3 using animal models for research and potential treatment.

This work was carried out with the support of RECETOX Research Infrastructure (ID LM2023069) and by the Ministry of Health, the Czech Republic (FNBr, 65269705). We used services of the Czech Centre for Phenogenomics (project LM2023036).

(PO–08) Exploring the Role of GPR45 in Metabolic Regulation and Its Implications for Obesity and Related Diseases

Zhenni Liu [1,2], Xiaorui Xie [2], Wenqiu Wang [2], Dejing Pan [2]

1. The Second Affiliated Hospital of Soochow University, Suzhou,China
2. Suzhou Medical College ,Soochow University,Suzhou,China

E-mail of the presenting author: 1104946735@qq.com

GPR45 is an orphan G protein-coupled receptor whose precise functions remain inadequately defined. Previous research has demonstrated that systemic knockout of Gpr45 in mice leads to an obesity phenotype, accompanied by a notable reduction in POMC protein expression. POMC is well-established as a key neuronal component involved in integrating hormonal and nutritional signals to sustain energy balance, which implies that GPR45 may be integral to metabolic regulation. Considering that POMC and AgRP are well-characterized neuropeptides essential for regulating feeding and fasting behaviors, we specifically investigated the role of GPR45 within AgRP neurons of the hypothalamus, given their critical function in energy balance regulation. Our data reveal that mice with a specific knockout of Gpr45 in AgRP neurons continue to exhibit obesity, suggesting that GPR45 may operate through a more intricate mechanism in metabolic control. Ongoing research will further elucidate the role of GPR45 across different neuronal populations and explore its potential impact on metabolic disorders, potentially offering novel insights for the understanding and treatment of related diseases.

(PO–09) Genetic and functional analysis of GRIN variants detected in Czech pediatric patients

Eni Tomovic [1], Kateřina Hirschfeldova [1], Veronika Holubova [2], Tomas Honzik [2], Pavlina Danhofer [3], Tereza Smejkalová [4], Ladislav Vyklický [4], Aleš Balík [1]

1. Laboratory of Cellular Neurophysiology, Institute of Physiology CAS, Biocev, Vestec, Czech Republic
2. KPDPM VFN Praha
3. KDN FN Brno
4. Laboratory of Cellular Neurophysiology, Institute of Physiology CAS

E-mail of the presenting author: eni.tomovic@gmail.com

NMDA receptors, a subclass of ionotropic glutamate receptors, mediate excitatory neurotransmission in the CNS and are fundamental to learning and memory formation. The majority of native NMDARs is assembled as heterotetramers consisting of two obligatory GluN1 and two GluN2A or 2B subunits in various combinations. Dysfunction of NMDARs caused by de-novo genetic variants is associated with the emergence of various neurological disorders. In collaboration with pediatric departments (VFN Praha and FN Brno) we have started to analyze the *GRIN* gene variants identified in Czech pediatric patients. All *GRIN2A* variants were associated with epilepsy, while *GRIN2B* variants were found in patients with developmental delays. To understand the effects of these variants and to help in personalized therapy, we investigated the functional changes of NMDARs. Here we show the analysis of three variants, GluN2B(A827V), GluN2B(T691A), and GluN2B(L612M). Using electrophysiological techniques, we found a significant decrease in current responses to glutamate in GluN2B(A827V) variant with an average amplitude of 23.9 pA/pF compared to the average amplitude of 47.4 pA/pF in the WT variant, indicating a lower number of NMDARs on the cell membrane. A decrease of EC50 value for glutamate was observed in GluN2B(L612M), while GluN2B(T691A) showed a shortened deactivation time course compared to WT, with the average deactivation being 473 ms and 120 ms, respectively. In addition, GluN2B (A827V) and GluN2B(T691A) exhibited lower channel open probability, while GluN2B(L612M) increased its value. Moreover, immunocytochemical staining of NMDAR surface expression showed that GluN2B(A827V) variant significantly decreased the surface expression of NMDARs. These results suggest the complex effects of the identified rare variants on receptor function, which still require more detailed pharmacological evaluation for efficient personalized therapy.

(PO–10) Human genomic humanization of the Grem1 (88 kb) and Taf1 (166kb) genes

Xiang Li [1], Emma Jaeger [2], Daniel Biggs [2], Cherie Lai [2], Elizabeth Fisher [3], Ben Davies [1]

1. Francis Crick Institute, London, UK
2. Wellcome Centre for Human Genetics, University of Oxford, UK
3. University College London, UK

E-mail of the presenting author: ben.davies@crick.ac.uk

As we learn more about disease mechanisms, it is becoming clear that simple genetically altered mouse models may be of limited use for studying certain human pathologies. Disease-associated mutations in non-coding regions of the human genome cannot be easily modelled in the mouse, and often the human pathology is the result of splicing variations that may not be conserved in the mouse. Genomic humanization, where mouse genomic sequences are replaced with the equivalent human sequences, provides a means of addressing the effects of non-coding mutations and could allow more accurate models of human disease.

Two common methods are used for large-scale genome engineering: CRISPR-assisted gene targeting using BAC-based targeting vectors, or BAC recombinase-mediated cassette exchange (RMCE). Here, we explore the use of these two technologies in mouse ES cells to model duplications in the regulatory region of the GREM1 gene, which contribute to hereditary mixed polypolysis syndrome (HMPS), through genomic humanisation of the murine Grem1 locus (88-129 kb). CRISPR-associated gene targeting was found to result in a high percentage of aberrant targeting, with only parts of the BAC being integrated and integration of the BAC backbone being a very common result. No usable ES cell clones were obtained using this method.

In contrast, RMCE at the same locus yielded correctly targeted clones and, although targeted aberrations were still observed at a rate of approximately 50%, usable ES clones were available for mouse production.

The same Cre-recombinase based RMCE method was applied to the murine Taf1 locus, replacing the gene with 166 kb of the orthologous human genomic sequence. Correctly targeted ES cell clones were obtained and were used for mouse generation, resulting in a humanized Taf1 mouse. Further work is now exploring the integration of disease-associated non-coding mutations within the humanized mouse which are associated with X-linked dystonia parkinsonism (XDP).

In this preliminary work, RMCE appears to be a more reliable and accurate strategy for large-scale manipulation of the mouse genome. Future work will determine whether this conclusion holds true for further independent loci, and whether the efficiency of RMCE can be improved by using serine recombinases (integrases).

(PO–11) Characterization of a Novel Inducible hACE2 Mouse Model for SARS-CoV–2 Research: Insights into Acute Infection and Long COVID

Federica Gambini [1,2,3], Dominik Arbon [1,2], Jana Balounova [1,2], Petr Nickl [1,2,3], Jan Prochazka [1,2], Radislav Sedlacek [1,2]

1. Institute of Molecular Genetics of the Czech Academy of Sciences (IMG), Prague, Czech Republic
2. Czech Centre for Phenogenomics, Vestec, Czech Republic
3. Faculty of Science, Charles University, Prague, Czech Republic

E-mail of the presenting author: federica.gambini@img.cas.cz

The SARS-CoV-2 pandemic has highlighted the urgent need for effective animal models to study viral behavior, host responses, and potential treatments. Mouse models are especially valuable in COVID-19 research due to their genetic flexibility and physiological similarities to humans. We have generated a novel inducible humanized mouse model called Rosa26creERT2/chAce2. Rosa26creERT2/chAce2 mice express hACE2 in multiple organs when tamoxifen is administered, allowing controlled timing of viral susceptibility. This makes it a valuable positive control for the development of tissue-specific hACE2 models, enabling detailed studies on virus spead as well as organ-specific effects of SARS-CoV-2 infection.

During the acute phase of infection (Days 1-7 post-infection), Rosa26creERT2/chAce2 mice show clear disease symptoms, including weight loss. The RNA of the virus is primarily found in the lungs and brain. Immune response studies revealed significant increases in neutrophils and effector CD8+ T cells in periperal blood at Day 7 post-infection. Additionaly, preliminary data suggest that this model is also suitable for studying Long COVID disease, as animals presented persistent organ damage at 90 days post infection.

Overall, the Rosa26creERT2/chAce2 model enhances our understanding of COVID-19 pathogenesis and offers a powerful platform for testing new treatments and studying both the acute and long-term effects of the disease. This versatile model makes a valuable contribution to advancing COVID-19 research.

(PO–12) Characterization of mice carrying neurodevelopmental disease-associated variants

Klevinda Fili [1], Miriam Candelas Serra [1], Viktor Kuchtiak [1], Eni Tomovic [1], Agnieszka Kubik Zahorodna [2], Karel Harant [3], Paulina Bozikova [4], Ales Balik [1], Tereza Smejkalova [1], Ladislav Vyklicky [1]

1. Institute of Physiology AS CR, v. v. i.
2. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences
3. Proteomics Core Facility, Faculty of Science, Charles University, Biocev
4. Institute of Biotechnology CAS, 25050 Vestec, Czech Republic

E-mail of the presenting author: klevinda.fili@fgu.cas.cz

The N-methyl-D-aspartate receptor (NMDAR) is a calcium-permeable, glutamate-gated ion channel essential for synaptic transmission, synaptogenesis, and plasticity. Recent sequencing in patients with neurological and psychiatric disorders identified numerous variants in NMDAR subunit genes. We developed two transgenic mouse lines: one carrying a missense mutation (*Grin2b* L825V), a de-novo variant found in a patient with intellectual disability and autism, and another carrying a frameshift mutation (*Grin2b* L825Ffs*15, Grin2bΔ) that encodes a truncated GluN2B subunit missing about half of its sequence.

Using in vitro and in vivo methods—including patch-clamp recordings from HEK293 cells expressing recombinant receptors and from primary hippocampal neurons prepared from heterozygous (*Grin2b*+/Δ, *Grin2b*+/L825V) and wild-type (*Grin2b*+/+) mice, molecular analyses, and behavioral tests—we characterized these models.

The *Grin2b* L825V variant showed a reduced open probability in receptors containing the GluN2B(L825V) subunit. NMDA-induced currents in *Grin2b*+/L825V hippocampal neurons were smaller, with faster NMDAR-eEPSC deactivation and less sensitivity to the GluN2B-selective inhibitor ifenprodil. Behavioral tests revealed sex-dependent effects, with males, exhibiting hypoactivity, anxiety, impaired sensorimotor gating, and aggression, limiting cognitive assessment.

The *Grin2b* L825Ffs*15 variant led to reduced levels of GluN1 and GluN2B mRNA in hippocampal tissue and altered receptor surface expression. Surprisingly, mass spectrometry showed the GluN2BΔ subunit was almost absent in the hippocampal proteome of male *Grin2b*+/Δ mice. *Grin2b*+/Δ neurons had reduced NMDA-induced current amplitudes and faster NMDAR-eEPSC deactivation. *Grin2b*+/Δ mice displayed mild hypoactivity, anxiety, and altered sensorimotor gating.

(PO–13) Characterization of Zebrafish Larvae with Knockouts of NMDA Receptor grin2Aa and grin2Ab Genes: Gene Expression and Swimming Behavior

Vera Abramova [1,3], Ales Balik [1], Eni Tomovic [1], Tereza Smejkalova [1], Paulina Bozikova [2], Jiri Cerny [1], Ladislav Vyklicky [1]

1. Institute of Physiology CAS, Videnska 1083, 142 20 Prague 4, Czech Republic
2. Institute of Biotechnology CAS, Prumyslova 595, 252 50 Vestec, Czech Republic
3. 3rd Faculty of Medicine, Charles University, Ruska 2411, 100 00 Prague 10, Czech Republic

E-mail of the presenting author: vera.abramova@fgu.cas.cz

Danio rerio (zebrafish) is a model for studying early neurophysiological development and the consequences of gene mutations. The zebrafish possesses orthologs of human NMDA receptor subunit genes (e.g. two zebrafish paralogs *grin2Aa* and *grin2Ab* are orthologs of human *GRIN2A*). The *grin2Ab* knockout strain, created via CRISPR-Cas9, and the *grin2Aa* knockout strain were investigated for spatial and temporal gene expression patterns and for swimming behavior.

Whole-mount in situ hybridization (WISH) was performed on wild-type (WT) zebrafish larvae aged 1, 3, and 5 days post fertilization (dpf). The results indicated minimal expression of both genes at 1 dpf. By 3 dpf, *grin2Aa* and *grin2Ab* exhibited higher expression in the nervous system. At 5 dpf, both genes were expressed in the telencephalon, tectum, cerebellum, hindbrain, and retina, with low expression in the spinal cord.

The analysis by qPCR confirmed low expression at 1 dpf, with *grin2Aa* surpassing *grin2Ab* by 3 dpf, and increased expression of both genes seen by 5 dpf.

Swimming parameters of 6 dpf larvae were recorded for 60 minutes in a 20-well chamber, under constant temperature (28°C) and light (100 lux) conditions. Total swim distance, the number of swim bouts, and the average length of individual swim bouts for every 10-minute interval were analyzed. *grin2Aa*-/- as well as *grin2Ab*-/- larvae showed increased total swim distance due to an elevated bout frequency compared to WT controls. *grin2Aa*-/- *grin2Ab*-/- (*grin2A*-/-) larvae demonstrated an even longer swim distance, attributed to a higher number of swim bouts.

In conclusion, single and double grin2A zebrafish knockouts show altered swimming behavior compared to WT controls and may serve for future research on compounds rectifying these phenotype changes.

(PO–14) Morphology and physiology of Jagged1 conditional deletion and patient-based single variant mouse models

Hana Kolesová [1], Kristýna Neffeová [1], Eva Nekvindová [1,3], Jan Mašek [2], David Sedmera [1]

1. Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic
2. Faculty of Science, Charles University, Prague, Czech Republic
3. Institute of Molecular Genetics of Czech Academy of Sciences, CCP

E-mail of the presenting author: hana.kolesova@lf1.cuni.cz

Jagged 1 (Jag1) is known to play an important role in cardiac development, where partial deletion of Jag1 is causing severe congenital heart defects. In this study we are comparing embryonic and postnatal hearts with conditional deletion of Jag1 with hearts with single nucleotide variant in Jag1, which was prepared based on patient data.

We analyzed prenatal as well as postnatal hearts of Jag1 floxed, Islet1-cre mouse line and two lines with single nucleotide variant in Jag1. Morphology of hearts was analyzed on histological sections. Physiological functions were assessed using ultrasound in vivo imaging - Vevo and optical mapping.

We found that Jag1, Islet1-cre mouse line exhibits severe heart defects during embryonic development, with variable phenotype ranging from mild abnormalities to Tetralogy of Fallot – double outlet right ventricle, VSD, and valve defects. Surviving postnatal mice present with milder defects, especially valve defects and physiological abnormalities in ventricular activation and contraction. Patients-based mouse model with single nucleotide variant in postnatal stages exhibits valve defects and physiological abnormalities.

Our results show that Jag1 is an important player in heart development and its disruption is causing various congenital heart defects. The humanized mouse models help to understand etiology and pathogenesis of congenital heart disease.

Supported by Charles University Cooperatio 207029 Cardiovascular Science.

(PO–15) Multistep allelic conversion in mouse pre-implantation embryos by AAV vectors

Petr Nickl [1,3], Irena Jenickova [1], Jan Elias [1,3], Petr Kasparek [1,3], Cyril Barinka [2], Jana Kopkanova [1], Radislav Sedlacek [1,3]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prumyslova 595, 252 50 Vestec, Czech Republic
2. Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Prumyslova 595, 252 50 Vestec, Czech Republic
3. Laboratory of Transgenic Models of Diseases, Institute of Molecular Genetics of the Czech Academy of Sciences, Videnska 1083, 142 20 Prague, Czech Republic

E-mail of the presenting author: petr.nickl@img.cas.cz

Site-specific recombinases (SSRs) are critical for achieving precise spatiotemporal control of engineered alleles. These enzymes play a key role in facilitating the deletion or inversion of loci flanked by recombination sites, resulting in the activation or repression of endogenous genes, selection markers or reporter elements. However, multiple recombination in complex alleles can be laborious. To improve this, a new and efficient method using AAV vectors can simplify the conversion of systems based on Cre, Flpo, Dre and Vika recombinases. In this study, we present an effective method for ex vivo allele conversion using Cre, Flp (flippase), Dre, and Vika recombinases, employing adeno-associated viruses (AAV) as a delivery vector. AAVs enable efficient allele conversion with minimal toxicity in a reporter mouse line. Moreover, AAVs facilitate sequential allele conversion, essential for fully converting alleles with multiple recombination sites, typically found in conditional knockout mouse models. While simple allele conversions show a 100% efficiency rate, complex multiple conversions consistently achieve an 80% conversion rate. Overall, this strategy markedly reduces the need for animals and significantly speeds up the process of allele conversions, representing a significant improvement in genome engineering techniques.

(PO–16) Muscle-specific gene editing improves molecular and phenotypic defects in a mouse model of Myotonic Dystrophy type 1

Mariapaola Izzo [1], Jonathan Battistini [1], Elisabetta Golini [1], Claudia Provenzano [1], Tiziana Orsini [1], Georgios Strimpakos [1], Ferdinando Scavizzi [1], Marcello Raspa [1], Genevieve Gourdon [2], Silvia Mandillo [1], Beatrice Cardinali [1], Germana Falcone [1]

1. Institute of Biochemistry and Cell Biology, CNR-National Research Council, Monterotondo, 00015 Rome, Italy
2. Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, 75013 Paris, France

E-mail of the presenting author: silvia.mandillo@cnr.it

Therapeutic gene editing for treatment of monogenic diseases is a powerful technology that could in principle eliminate definitively the disease-causing genetic defect. The precision and efficiency of the molecular mechanisms are still under investigation in view of a possible use in clinical practice. Here we describe the application of the CRISPR/Cas9 strategy to remove the CTG-expansion in the DMPK gene causing myotonic dystrophy type 1 (DM1) in a mouse model carrying the human transgene from a DM1 patient. To optimize the editing efficiency in vivo, we identified new tools that allowed to improve the expression levels and the activity of the CRISPR/Cas9 machinery. Newly designed guide RNA pairs were tested in DM1-patient derived cells prior to in vivo application. Edited cells expressing the selected pair were analyzed to assess the occurrence of off-target and the accuracy of on-target genomic events. Systemic delivery of CRISPR/Cas9 components through myotropic adeno-associated viral vectors led to significant improvement of molecular alterations in the heart and skeletal muscle. Importantly, a persistent increase of body weight, improvement of muscle strength and body composition parameters were observed in treated animals.

(PO–17) Physiological and Morphological Consequences of Jagged1 Deletion in Mouse Model of Tetralogy of Fallot

Kristýna Neffeová [1,2] , Eva Nekvindová [1,3], Veronika Olejníčková [1,2], David Sedmera [1], Hana Kolesová [1]

1. Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic
2. Institute of Physiology, The Czech Academy of Sciences, Prague, Czech Republic
3. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

E-mail of the presenting author: Kristyna.Neffeova@lf1.cuni.cz

The Notch signaling pathway plays a crucial role in embryonic development and adult homeostasis. Mutations in the human Jagged1 (Jag1) gene, which encodes a ligand for the Notch receptor, are responsible for Alagille syndrome. Symptoms of this inherited disease may include various forms of Tetralogy of Fallot.

Here, we generated Jag1 flox/flox Islet1 Cre/+ mice with conditional Jag1 deletion in the cardiac outflow tract to investigate the impact of Jag1 mutations on cardiac morphology and physiology. Mice with conditional deletion exhibited severe cardiac malformations typical for Tetralogy of Fallot. Islet1 is also express in sinoatrial and atrioventricular nodes, therefore we used the optical mapping to visualize changes in patterning of the cardiac conduction system.

The analysis of E14.5, E16.5 embryos and adult mice showed changes in the activation pattern. In controls, we showed maturated activation from apex to base with two separate activation centres. Mutant embryonic hearts revealed activation only from the left ventricle, indicating a perturbed function of the right bundle branch. In mutant adult mice, activation occurred at additional activation centres, distinguishing them from controls where excitation is conducted from a single site at the apex. Vevo ultrasound imaging physiological analysis was performed only on adult heterozygotes, because of the postnatal mortality of the homozygotes. Most of the monitored hemodynamical parameters did not show significant differences. However, spackle-based strain analysis revealed vulnerable areas of contractile defect that generate mechanical dyssynchrony pronounced mostly at anterior wall. In our study, we demonstrated morphological and electrophysiological alterations resulting from conditional deletion of Jag1. Embryonic mice exhibited malformations and irregular activation patterns. Severe malformations were less prevalent in adult mice, primarily due to the survival of heterozygotes and an increased mortality rate among mice displaying severe congenital defects. Nevertheless, surviving animals exhibited abnormal electrophysiological changes along with physiological alterations resulting in dyssynchronous myocardial contractions observed during strain analysis.

(PO–18) Proteomic analysis of soft tissues from mice with PACS2 gene mutation

Tomasz Kowalczyk [1], Arkadiusz Żbikowski [1], Kacper Łukasiewicz [2,3], Michał Ciborowski [1], Dominik Cysewski [1]

1. Metabolomics and Proteomics Laboratory, Clinical Research Center, Medical University of Bialystok
2. Center for Experimental Medicine; Medical University of Bialystok
3. Departament of Psychiatry, Medical University of Bialystok

E-mail of the presenting author: tomasz.kowalczyk@umb.edu.pl

Phosphofurin acidic cluster sorting protein 2 (PACS2) is a multifunctional protein critical for maintaining cellular homeostasis. Together with PACS1, PACS2 plays a crucial role in the transport of proteins between cellular membranes, thereby contributing to the regulation of essential processes such as apoptosis, mitochondria-endoplasmic reticulum interactions, and, consequently, Ca2+ flux, lipid biosynthesis, and autophagy. The significance of the PACS2 gene becomes particularly evident when considering the biological effects of mutations in this gene. Missense mutations, such as E209K and E211K (glutamic acid-to-lysine substitutions), have been associated with PACS2 syndrome, an ultra-rare disease. To understand the molecular alterations occurring in soft tissues, proteomic analyses were performed using tissues from a Pacs2+/E209K mouse model (10-12 weeks old). Tryptically digested proteins were separated using a nano-LC system coupled with Orbitrap Fusion MS, and data collection was performed via Data Independent Analysis (DIA). We quantified over 7,000 proteins, depending on tissue type. Tissues from mice carrying the PACS2 gene mutation exhibited a greater propensity for potential fibrosis in the liver. The spleen also showed similar tendencies toward fibrosis; however, at this stage of mouse development, these findings are not definitive. Additionally, liver tissue displayed abnormalities in lipid metabolism and potential lipid accumulation, as well as disruptions in Na+ ion transport. In contrast, the kidneys, unlike the liver, showed a higher potential for localized inflammation in mice with the PACS2 gene mutation. The kidneys also exhibited potential abnormalities in intracellular transport and ion metabolism, particularly involving Ca2+, as well as possible changes in the structure of the cellular cytoskeleton. Notably, the PACS2 mouse heart did not exhibit any significant molecular changes compared to controls at this stage of development.

(PO–19) Proteomic and Metabolomic Profiling of Brain Tissues in PACS2 E209K Mutant Mice: Insights into Molecular Dysregulation

Dominik Cysewski [1], Tomasz Kowalczyk [1], Arkadiusz Żbikowski [1], Krystyna Grzesiak [1], Michał Ciborowski [1], Adrian Godlewski [1], Kacper Łukasiewicz [1]

1. Medical University of Białystok

E-mail of the presenting author: dominik.cysewski@umb.edu.pl

Background: Phosphofurin acidic cluster sorting protein 2 (PACS2) is a multifunctional protein essential for maintaining cellular homeostasis, playing a pivotal role in protein transport between cellular membranes, apoptosis, mitochondria-endoplasmic reticulum interactions, Ca2+ flux, lipid biosynthesis, and autophagy. The E209K mutation in the PACS2 gene has been associated with neurodegenerative and metabolic disorders, but the underlying molecular mechanisms remain unclear. This study aims to elucidate the molecular alterations induced by the PACS2 E209K mutation using an integrative omics approach.

Methods: Brain tissues from 10-12 week-old PACS2 E209K mutant and wild-type mice were analyzed using a combination of proteomics and metabolomics techniques. Proteomic analysis was performed using nano-LC coupled with Orbitrap Fusion mass spectrometry, and Data Independent Analysis (DIA) was employed to quantify over 7,000 proteins. Metabolomic profiling was conducted using the Biocrates 500xl metabolomics panel, focusing on key metabolic pathways and metabolites relevant to brain function.

Results: The proteomic analysis revealed significant dysregulation of proteins involved in synaptic function, mitochondrial dynamics, and cellular stress responses. Metabolomic profiling highlighted abnormalities in energy metabolism, lipid biosynthesis, and Ca2+ homeostasis in brain tissues from PACS2 E209K mutant mice. These molecular disruptions suggest broad impacts on brain function, potentially contributing to the neurodegenerative and metabolic phenotypes associated with the PACS2 E209K mutation.

Conclusion: This integrative proteomic and metabolomic study provides a comprehensive overview of the molecular changes in brain tissues associated with the PACS2 E209K mutation. The findings enhance our understanding of the pathophysiology underlying PACS2-related disorders and may guide future research into therapeutic strategies targeting these molecular pathways.

(PO–20) ROLE OF FAM83H IN IMMUNE SYSTEM HOMEOSTASIS

Betul Melike Ogan [1], Veronika Forstlová [2], Michaela Šímová [1,2], Kristina Vičíková [2], František Špoutil [2], Jolana Turečková [1], Jan Procházka [1,2], Jana Balounová [1,2], Radislav Sedláček [1,2]

1. Laboratory of Transgenic Models of Diseases, Institute of Molecular Genetics (IMG)
2. Phenotyping Module, Czech Centre for Phenogenomics (CCP)

E-mail of the presenting author: betul.ogan@img.cas.cz

FAM83H is primarily expressed in epithelial cells and has been suggested to play a role in intracellular transport, regulation of cytoskeletal networks, and enamel formation. A deficiency of FAM83H has been reported as the cause of amelogenesis imperfecta (AI), a condition characterized by soft enamel. To investigate the function of FAM83H in immune system homeostasis, we generated Fam83h knock-out (KO) animals. The Fam83h KO animals exhibit decreased body size, sparse and scruffy coats, scaly skin, weakness, and hypoactivity. Although we did not observe any remarkable dentin-related phenotype, the Fam83h KO pups show severe swelling of their forepaws, accompanied by bone deformation as early as 3 weeks of age. However, the soft tissue lesions resolve, and leukocyte levels, including increased neutrophil levels in peripheral blood, return to normal by 7 weeks of age.

Additionally, juvenile Fam83h KO animals display elevated myeloid cells and decreased lymphoid cells in their peripheral blood. Specifically, B and NK cell development is partially blocked in the bone marrow (BM) of Fam83h KO animals. Moreover, postnatal thymus growth is limited, and thymocyte development is also impaired in Fam83h KOs. At two weeks of age, the thymi of these animals show reduced capacity to generate T cells, with a partial arrest at the DN stage.

We found that stromal cells deficient in Fam83h are responsible for impaired T cell production as well as partial B and NK cell blockage in the thymic and BM microenvironments respectively. Additionally, mice deficient in Fam83h exhibited altered Wnt signaling. Altogether, our findings highlight the importance of FAM83H in lymphoid cell differentiation in their hematopoietic niches and contribute to the understanding of Fam83h's role in regulating the immune system.

(PO–21) Seasonality of measured parameters in C57Bl/6NCrl mice

Maximilián Goleňa [1], Vendula Novosadová [1], Jan Procházka [1], Radislav Sedláček [1]

1. Czech Centre of Phenogenomics, Institute of Molecular Genetics of Academy of Sciences

E-mail of the presenting author: vendula.novosadova@img.cas.cz

Seasonal behavior is a well-documented phenomenon in nature, influencing various biological processes such as fur shedding, body weight fluctuations, and metabolic changes, typically attributed to external factors like temperature and humidity. However, these seasonal effects are expected to be absent under controlled environmental conditions where external variables are kept constant year-round. In this study, we examined the presence of seasonal patterns in various parameters of C57Bl/6NCrl mice utilized in the IMPC phenotyping pipeline. By applying multiple mathematical approaches, we rigorously analyzed data from these controlled environments to detect any residual seasonality. Our findings not only serve as a quality control measure for our environment but also contribute to a deeper understanding of how intrinsic biological rhythms may persist independently of external cues.

(PO–22) Terrestrial Slugs as Prospective Animal Models for Studying RNA Silencing Pathways

Tobiáš Ber [1,2], Kateryna Nemesh [1,2], Petr Svoboda [1]

1. Laboratory of Epigenetic Regulations, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
2. Faculty of Science, Charles University, Prague, Czech Republic

E-mail of the presenting author: kateryna.nemesh@img.cas.cz

Mollusca, the second largest animal phylum, has been rather neglected in molecular biology research. Our aim is to shed light on this phylum and understand how RNA silencing pathways evolved in molluscs. To achieve this, we propose two terrestrial slugs Deroceras laeve and Deroceras invadens as new model organisms for studying RNA silencing. The selected slugs are perfect for their small size (2-3 cm), relatively short generation cycle (3-5 generations a year), and established lab cultures. Additionally, we included the Spanish slug *Arion vulgaris* into our analysis, for it is an important pest and invasive species.

To study RNA silencing, we sequenced small RNAs from all three slugs. We identified 154 high confidence miRNA precursors in *A. vulgaris* genome. However, only about half of them exist in Deroceras spp., suggesting dynamic evolution of the miRNA pathway among slugs. Interestingly, piRNAs in *A. vulgaris* are ubiquitously expressed, targeting various types of repetitive elements (LINEs, LTRs, DNA transposons). Since genomes for *D. laeve* and *D. invadens* were unavailable, we sequenced them and so far completed an initial assembly for *D. laeve*: its genome is around 1.1 Gb and shows low heterozygosity rate. Genome of D. invadens is approximatelly 1 Gb. Furthermore, we sequenced transcriptomes of all three slugs, identifying genes involved in the RNA silencing pathway, including Dicer, two Argonaute, and two PIWI proteins. We identified the conserved DEDH tetrad motif in both Argonaute and PIWI proteins in the *Deroceras* species, confirming the presence of a functional RNA silencing machinery.

Presently, we are working on knocking-out Pax6 as a proof-of-concept experiment. Additional knock-outs will target genes involved in RNA silencing to extensively determine the role of small RNAs in all three slug species. Establishing genetic modifications will be crucial in developing terrestrial slugs into an experimental model system in molecular biology.

(PO–23) The impact of modified TGF- ß family on Integrin-ß1 Synthesis of Chondrocyte Cell Sheets

Gunay Akbarova-Ben-Tzvi [1], Seyed Ali (Behruz) Khaghani [2], Chin Fhong Soon [3], Gulrukh Dilbazi [4]

1. Baku State University, SABAH Centre
2. University of Bradford, Medical and Healthcare Technology, School of Engineering
3. Universiti Tun Hussein Onn Malaysia, Biosensor and Bioengineering Laboratory, MiNT-SRC Research Centre
4. The Veterinary Scientific-Research Institute, The laboratory of Veterinary preparations

E-mail of the presenting author: akperova_science@mail.ru

Regeneration of a damaged or non-functioning tissue requires adhesion of cells to their extracellular matrix (ECM). Adhesion of chondrocyte to a collagen type-II rich matrix, is dependent on cell adhesion molecules and integrins and cells adhere to ECM through integrins. Monolayer-expanded primary chondrocyte cells derived from forth passage were used in our work. The cells were isolated from knee joint of neonate Sprague-Dawley rat. Chondrocyte cells were cultured, expanded in monolayer culture system and 8x10(6) cells were resuspended in 40 ml DMEM media supplemented with 10% FCS. Remaining cell suspensions were aliquoted in 5 ml and supplemented with TGF-β1, TGF-β2, TGF-β3, TGF-β1&2, TGF-β1&3, TGF-β2&3, and TGF-β1&2&3. All cell cultures were incubated at 37°C for 24 hours. After 24 hours, cells were fixed by 1% formaldehyde and immunocytochemically stained for integrin β1 (CD29). Real-time PCR analysis confirmed not only the upregulation of β1 integrin mRNA by TGF-β1, TGF-β2 and TGF-β3 but also the upregulation of β1 integrin mRNA by TGF-β1&2, TGF-β2&3 and TGF-β1,2&3 (Fig. 1). We investigated the effects of TGF-β treatment on expression of the integrin β1 (Fig. 1). Among the TGF-β family β1-integrin were most prominently upregulated by TGF-β 1,2&3 after 24 hours. TGF-β1, TGF-β2, TGF-β3, TGF-β1&2 and TGF-β1&3 treatment increased the expression of β1-integrin, but the degree of upregulation was lower than that of TGF-β 1,2&3 (Fig. 2). Monolayer culture of the chondrocyte resulted in dedifferentiation of cells and production of stress fibres. This characteristic was prevented by high density and 3D multilayer chondrocyte culture. TGF-β2, TGF-β3, and manipulated TGF-β2 and TGF-β3 exhibited similar synthesis of integrin-β1 (CD29) to control, but TGF-β1, TGF-β1and TGF-β2, TGF-β1and TGF-β3, and TGF-β1, TGF-β2 and TGF-β3, decreased the expression of integrin-β. This is likely due to gene expression level of TGF-β and the chondrogenic transcription factors Sox-9, c-fos, or c-jun seen to be necessary for chondrogenesis (remains unchanged), and on the other hand, to high expression of β1 integrin, which plays major roles in cell–matrix interactions in chondrocytes.

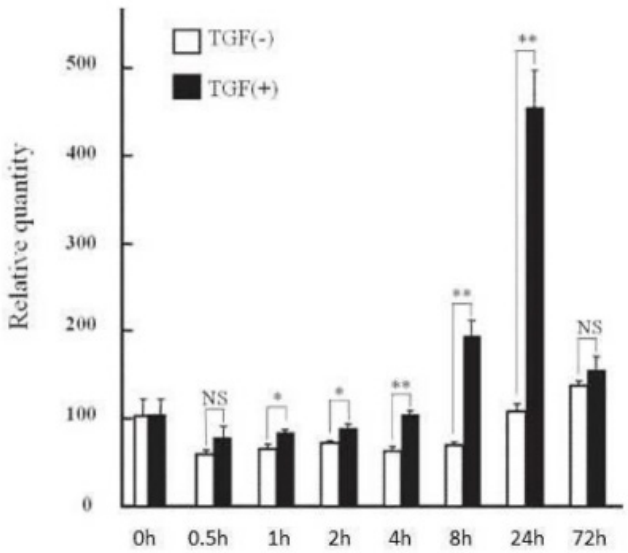


*Fig.1. Quantitative RT-PCR analysis of the expression of β1 integrin mRNA by TGF-β. Data are expressed means ± SEM (n = 3). NS, p > 0.05, *p < 0.05, **p < 0.01*

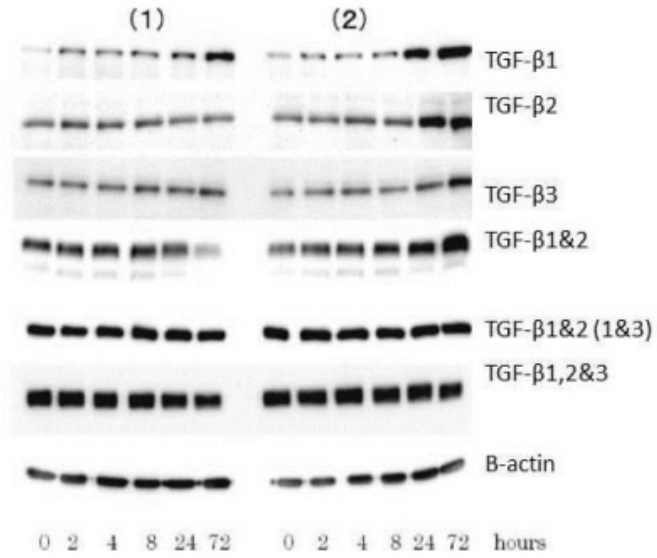


Fig.2. Western blot analysis of the TGF-β family. Cells were incubated without (1) or with (2) 1ng/ml of TGF-β. Blots were probed with the indicated antibodies.

(PO–24) The impact of PACS2 Syndrome on lung and kidney structure in mice

Arkadiusz Żbikowski [1], Tomasz Kowalczyk [1], Marlena Tynecka [2], Anna Pryczynicz [3], Katarzyna Guzińska-Ustymowicz [3], Michał Ciborowski [1], Dominik Cysewski [1], Kacper Łukasiewicz [4,5]

1. Metabolomics and Proteomics Laboratory, Clinical Research Centre, Medical University of Bialystok
2. Centre of Regenerative Medicine, Medical University of Bialystok
3. Department of General Pathomorphology, Medical University of Bialystok
4. Department of Psychiatry, Medical University of Bialystok
5. Experimental Medicine Centre, Medical University of Bialystok

E-mail of the presenting author: arkadiusz.zbikowski@umb.edu.pl

The protein PACS2 plays a crucial role in maintaining cellular balance, acting like a conductor trafficking of protein cargo within cells. This function impacts many cellular processes, including apoptosis, mitochondria-ER interaction, and autophagy. Moreover, recent research suggests that PACS2 might also play a role in tissue remodeling. PACS2 interacts with the TRPV1 protein, an ion channel. Disruptions in this partnership, particularly due to mutations in PACS2, have been linked to pulmonary fibrosis. Additionally, reduced levels of PACS2 have been associated with renal apoptosis and fibrosis, indicating potential involvement in kidney health.

Two specific mutations, PACS2E209K and PACS2E211K, alter the protein's structure, affecting its function. These mutations are linked to Developmental and Epileptic Encephalopathy-66 (DEE66), a neurological disorder known as PACS2 Syndrome. Interestingly, the PACS2E209K mutation seems to enhance the protein's interaction with TRPV1, hinting at potential changes in lung structure.

For now, the impact of PACS2E209K mutation on tissue remodeling is unknown. Therefore, we decided to evaluate the basic histology of the lungs and kidneys obtained from PACS2E209K mice. Furthermore, we assessed the expression of genes associated with tissue remodeling.
Adult mice +/E209K and +/+ (10-12 weeks) were sacrificed (n=6 for each group) and tissues were collected. For basic structural assessment hematoxylin and eosin staining were performed. Moreover, expression of remodeling-associated genes (collagen 1, 3, 5, 6, 15, 22 & 24; matrix metalloproteinase 2, 3, 9, 13, 19 & 25; tissue inhibitors of metalloproteinases (TIMP) 1, 2, 3 & 4; vimentin and smooth muscle α actin (Acta2)) were measured with qPCR. For statistical purpose, T tests were performed.

While some subtle changes were observed, the study found no significant differences in tissue structure or gene expression related to tissue remodeling between mice with PACS2 Syndrome and control mice. The only notable change was an increased expression of TIMP1 and MMP25 in lung tissue. While these findings suggest that PACS2 Syndrome might not cause significant tissue remodeling under the studied conditions, further research is needed to fully understand the potential effects of the PACS2E209K mutation on tissue structure.

(PO–25) The promise of episomal gene therapy

Viktor Kostohryz [1,2], Ondřej Slabý [1,2], Jiří Kohoutek [2]

1. CEITEC, Brno
2. Masaryk university

E-mail of the presenting author: 499598@mail.muni.cz

Modern day gene therapies are limited by a number of shortcomings that limit their practical use in the treatment of many key genetic diseases. The most significant limitations include 1) high immunogenicity of the delivery vector, 2) low stability in target cells and 3) mutagenicity of the construct. The answer to the aforementioned shortcomings is episomal gene therapy. Episomes are synthetic plasmid constructs containing regulatory sequences from mammalian chromatin. These sequences, the so-called "S/MARs", allow plasmids to stably segregate into daughter cells throughout many cell divisions, and help them prevent integration into the genome by a currently unknown mechanism (1). However, the stability of episomes in mammalian cell lines without selection is very low and currently requires significant improvement for further practical use. This problem goes hand in hand with the fact that the mechanisms of episomal retention and resistance to integration still remain poorly understood.

Therefore, we decided to practically study these mechanisms in order to improve the construct for future practical use. Our preliminary results show that episomes in part rely on the expression of the protein hnRNP U for their stability. This mechanism might be utilized in future versions of the episomal construct to further improve it for practical use.

(1) Grace Elizabeth M. et al. Human Gene Therapy 2021; 32(19–20), 1076–1095. doi:10.1089/hum.2020.310.

(PO–26) The significance of Fam84b in retinal homeostasis

Miles Joseph Raishbrook [1], Marcela Pálková [1], Jiři Lindovsky [1], David Liebl [2], Jan Procházka [1], Radislav Sedláček [1]

1. Institute of Molecular Genetics of the Czech Academy of Sciences
2. Imaging Methods Core Facility at BIOCEV, Charles University

E-mail of the presenting author: miles.raishbrook@img.cas.cz

Fam84b is an incompletely characterised protein. Despite reports associating its overexpression with breast, oesophageal and prostate cancer, its function is still unclear. The first FAM84B knockout mouse line was developed at CCP using CRISPR/Cas9 to create an indel in exon 2. FAM84B-/- mice display a degenerative retinal phenotype that becomes more severe with age. This phenotype, which bears similarities to the human retinal disease, age-related macular degeneration, has been extensively characterised in our lab. FAM84B-/- mice show thinner, and more disorganised retinal morphology from 12 weeks of age, as well as reducing electrical responses to light. Transmission electron microscopy revealed a presence of membrane-filled vacuole-like structures and a loss of basal infoldings in the retinal pigmented epithelial cells (RPE) of FAM84B-/- retinas, in addition to drusen-like deposits at the RPE-Bruch's membrane boundary. We show evidence that these retinal deposits may be due to dysregulation of autophagic processes in RPE cells lacking the Fam84b protein. The focus of this ongoing project is to elucidate the molecular function of Fam84b within the context of retinal homeostasis.

(PO–27) Transcriptional corepressor TLE1 is a positive factor in adipocyte differentiation

JI XU [1], XI YANG [1,2], JIALAN ZHANG [1], DEIJING PAN [1]

1. Suzhou Medical College, Soochow University, Suzhou, China
2. Faculty of Health and Sciences, University of Macau, Macau SAR, China

E-mail of the presenting author: 2308807520@qq.com

Adipocyte differentiation is a complex process that involves the activation of various signaling pathways and transcription factors. Transcriptional corepressor Transducin-Like Enhancer of Split 3 (*Tle3*) has been reported to promote adipocyte differentiation with Peroxisome proliferator-activated receptor gamma (Pparγ), a classical adipocyte differentiation factor. *Tle1*, a highly homologous of *Tle3*, is increased and subsequently decreased along adipocyte differentiation, unlike *Tle3* which is sustainedly increased. However, the role of *Tle1* in adipocyte differentiation remains elusive. We found that *Tle1* positively regulated adipocyte differentiation. Suppression of *Tle1* with siRNA knockdown significantly inhibited adipocyte differentiation in 3T3-L1 cells, a well-established cell line for studying adipocyte differentiation, evidencing less lipid droplet accumulation and decreased expression of adipocyte differentiation-related genes, such as Pparg, Cebpa, and Fabp4, while overexpression of *Tle1* reversed the inhibition effects. In addition, we demonstrated that *Tle1*, as a transcriptional corepressor, did not bind to known transcriptional factors in adipocyte differentiation, such as Pparγ, C/EBPα, C/EBPβ, CEBPδ. This suggests that *Tle1* may exert its effects on adipocyte differentiation through alternative mechanisms or pathways. The findings of this study highlight the importance of *Tle1* in adipocyte differentiation and provide insights into the development of adipose tissue. Further research on *Tle1* and its role in adipocyte differentiation could lead to a better understanding of metabolism-related diseases and potential therapeutic targets.

(PO–28) Skeletal dysmorphology and mineralization defects in Fgf20 KO mice

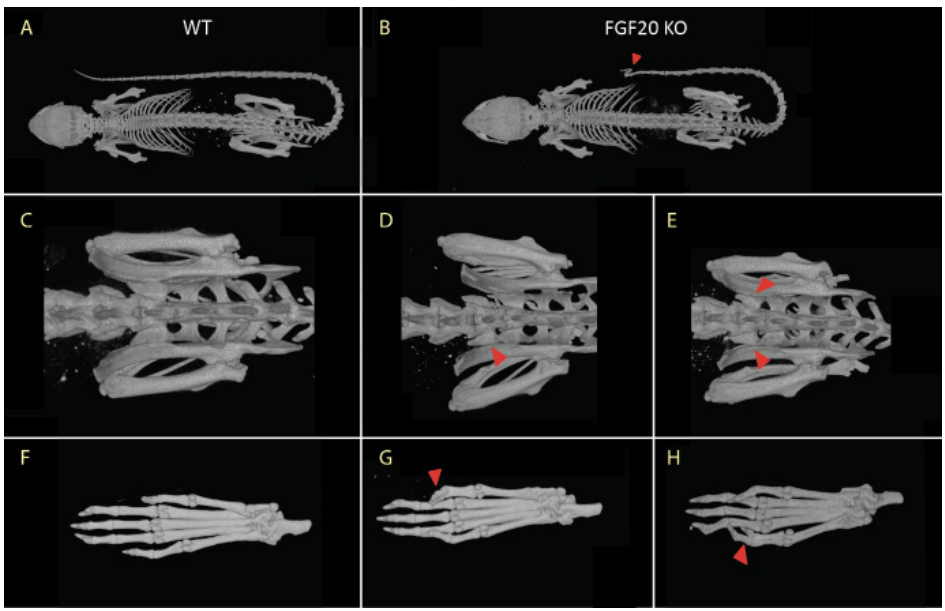
Sylvie Dlugosova [1,2], Frantisek Spoutil [1,2], Carlos Eduardo Madureira Trufen [1,2], Betul Melike Ogan [1,2], Michaela Prochazkova [1,2], Olha Fedosieieva [1,2], Petr Nick [1,2], Goretti Aranaz Novaliches [1,2], Radislav Sedlacek [1,2], Jan Prochazka [1,2]

1. Institute of Molecular Genetics of the Czech Academy of Sciences
2. Czech Centre for Phenogenomics

E-mail of the presenting author: sylvie.dlugosova@img.cas.cz

Fibroblast growth factor 20 (Fgf20), a member of the Fgf9 subfamily, was identified as an important regulator of bone differentiation and homeostasis processes. However, the role of Fgf20 in bone physiology has not been approached yet. Here we present a comprehensive bone phenotype analysis of mice with functional ablation of Fgf20. The bone phenotype could be detected especially in the area of the lumbar and caudal part of the spine and in fingers. Regarding the spine, Fgf20-/- mice exhibited adhesions of the transverse process of the sixth lumbar vertebra to the pelvis as well as malformations in the distal part of their tails. Preaxial polydactyly and polysyndactyly in varying degrees of severity were also detected. High resolution microCT analysis of distal femurs and the fourth lumbar vertebra showed significant differences in structure and mineralization in both cortical and trabecular bone. These findings were histologically validated and may be associated with the expression of Fgf20 in chondrocytes and their progenitors. Moreover, histological sections demonstrated increased bone tissue formation, disruption of Fgf20-/- femur cartilage, and cellular-level alterations, particularly in osteoclasts. We also observed molar dysmorphology, including root taurodontism, and described variations in mineralization and dentin thickness. Our analysis provides evidence that Fgf20, together with other members of the Fgf9 subfamily, plays a crucial regulatory role in skeletal development and bone homeostasis.

Presentation of most common dysmorphologies in Fgf20 KO mutans (B, D, E, G, H), highlighted with red arrowhead, and their comparison with standard morphology of WT (A, C, F).



(PO–29) Assessing the reproductive phenotypes in mice – current methods and room for improvement

Eliška Pavelková [1], Volodymyr Porokh [1], Michaela Procházková [2], Jan Procházka [2], Radislav Sedláček [2], Aleš Hampl [1]

1. Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
2. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic

E-mail of the presenting author: eliskapavelkova20@gmail.com

The mouse model is a powerful experimental system that allows gene functions to be studied at the organismic level. Introducing gene mutations or removing the gene from the genome using existing genome editing tools enables us to mimic human diseases and investigate their pathophysiology and treatment options. However, off-target effects of genome editing often result in suboptimal reproductive performance and hinder the generation of modified mouse strains. This is of great interest to developmental biologists, as such mouse lines provide a tool to study the molecular basis of infertility.

We propose a dual approach to assessing the developmental capacity of mutant mice before and after genome editing. We anticipate these approaches to enhance our understanding of gene roles in reproduction and contribute valuable insights for future studies. While many efforts are being made to improve genome editing techniques, we propose Cas13d-mediated RNAi as an alternative to assess early developmental phenotypes and provide guidance when choosing gene targets and allocating resources.

B) Infrastructure poster presentations

(PO-30) **Juraj Lábaj:** Biochemistry and Haematology Unit (CCP, Phenotyping Module)

(PO-31) **Michaela Procházková:** Bioimaging & Embryology Unit (CCP, Phenotyping Module)

(PO-32) **Eva Nekvindová:** Cardiovascular Unit (CCP, Phenotyping Module)

(PO-33) **Jiří Lindovský:** Hearing & Electrophysiology Unit (CCP, Phenotyping Module)

(PO-34) **Olha Fedosieieva:** Histopathology Unit (CCP, Phenotyping Module)

(PO-35) **Jana Balounová:** Immunology Unit (CCP, Phenotyping Module)

(PO-36) **David Pajuelo Regeura:** Metabolism Unit (CCP, Phenotyping Module)

(PO-37) **Karel Chalupský:** Metabolomics Unit (CCP, Phenotyping Module)

(PO-38) **Kateryna Pysanenko:** Neurobiology & Behaviour Unit (CCP, Phenotyping Module)

(PO-39) **Silvia Magalhaes Novais:** PDX & Cancer Models Unit (CCP, Phenotyping Module)

(PO-40) **Marcela Palková:** Vision Unit (CCP, Phenotyping Module)

(PO-41) **Dominik Arbon:** Models of Infection Diseases (BSL3) (CCP, Phenotyping Module)

(PO-42) **Vendula Novosadová:** Bioinformatician Unit (CCP, Phenotyping Module)

(PO-43) **Lucie Dufková:** Transgenic and Archiving Module (CCP)

(PO-44) **Gizela Koubková:** Preclinical testing at the Czech Centre for Phenogenomics

(PO-45) **Libor Kopkan - Anna Pilařová:** Animal Facility Module (CCP, Vestec)

(PO–30) Biochemistry and Haematology Unit (CCP, Phenotyping Module)

Juraj Labaj [1], Mariya Glushchenko [1], Eva Stefancova [1], Mariia Nehrych [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

E-mail of the presenting author: juraj.labaj@img.cas.cz

Clinical Chemistry (the study of the chemical composition of the blood plasma/serum), haematology (the study of the blood cellular components and acid-base balance), and urinalysis (analysis of chemical and cellular composition of urine) are integral part of clinical pathology which provides a quantifiable way to assess animal health and to diagnose disease and toxicity. Clinical chemistry analyses of plasma/serum and urine comprise of metabolites, ions, enzymes, and serological quantifications that could be used to assess metabolic and functional abnormalities of different organs of the body. Examination of whole blood for haematology may reveal pathologies or treatments that affect blood cell populations and coagulation.

We use advanced analytical platforms maintained at high standards with methodologies following robust screening protocol by the International Mouse Phenotyping Resource of Standardized Screens (IMPReSS). Furthermore, the Biochemistry and Haematology Unit is a GLP (Good Laboratory Practice) – certified, SUKL (State Institute for Drug Control, ČR) – audited laboratory capable of analyzing samples from pre-clinical studies. The Unit can likewise measure multitude of biomolecules from a single sample using different panels for multiplex immunoassays and tested kits for individual analytes. Multiplexing is done by a bead- and flow cytometry-based assay utilizing Luminex® xMAP® technology in a flexible analyzer.

More information at www.phenogenomics.cz/phenotyping/biochemistry-and-haematology/.

Instrumentation and Technologies:
Beckman Coulter AU480 Clinical Chemistry Analyzer, Siemens CLINITEK Advantus® Urine Chemistry Analyzer, Mindray BC-5300 Vet or BC-30 Vet analyzers, ABL90 FLEX PLUS blood gas analyzer, Bio-Plex® 200 Luminex

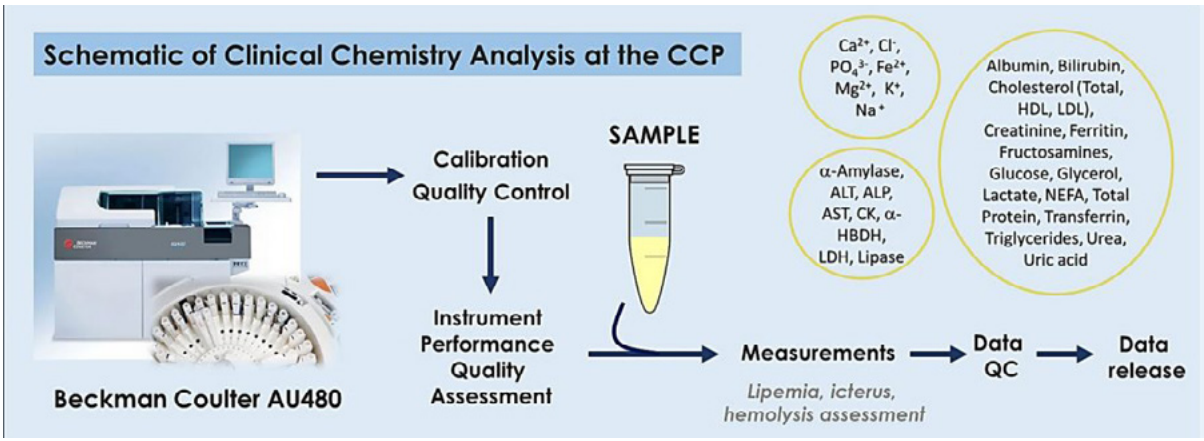


Fig 1. The Beckman Coulter AU480 Clinical Chemistry Analyzer

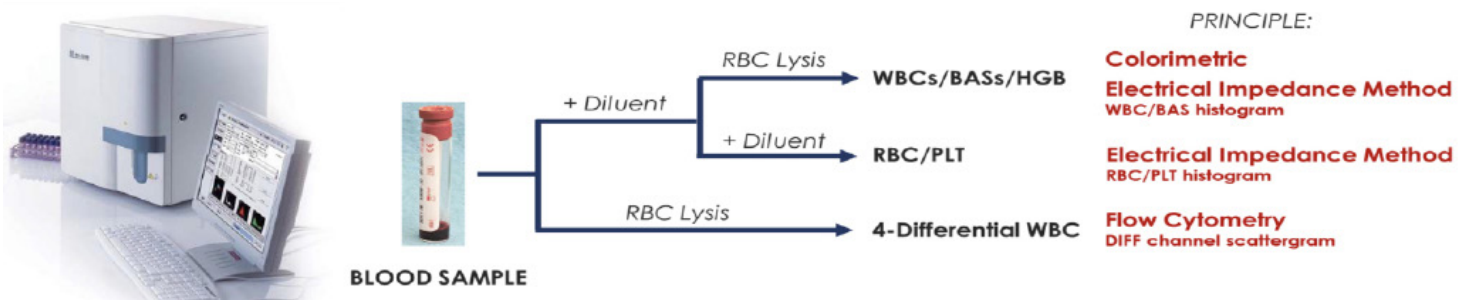


Fig 2. The Mindray BC-5300 Vet and BC-30 Vet Analyzer

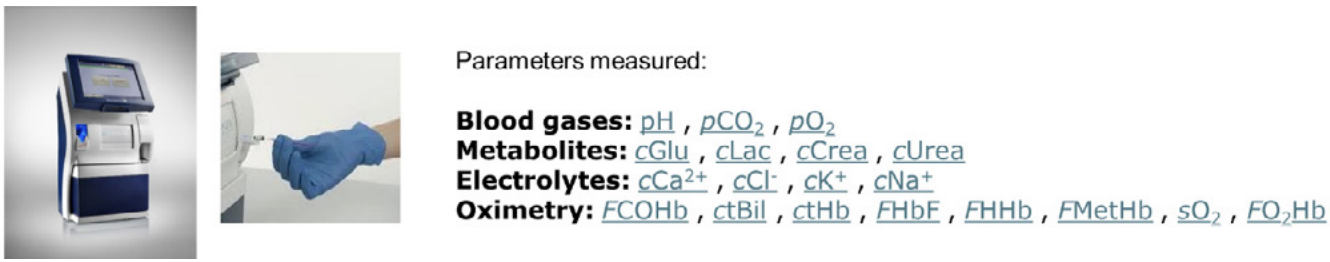


Fig 3. The ABL90 FLEX PLUS Blood Gas Analyzer

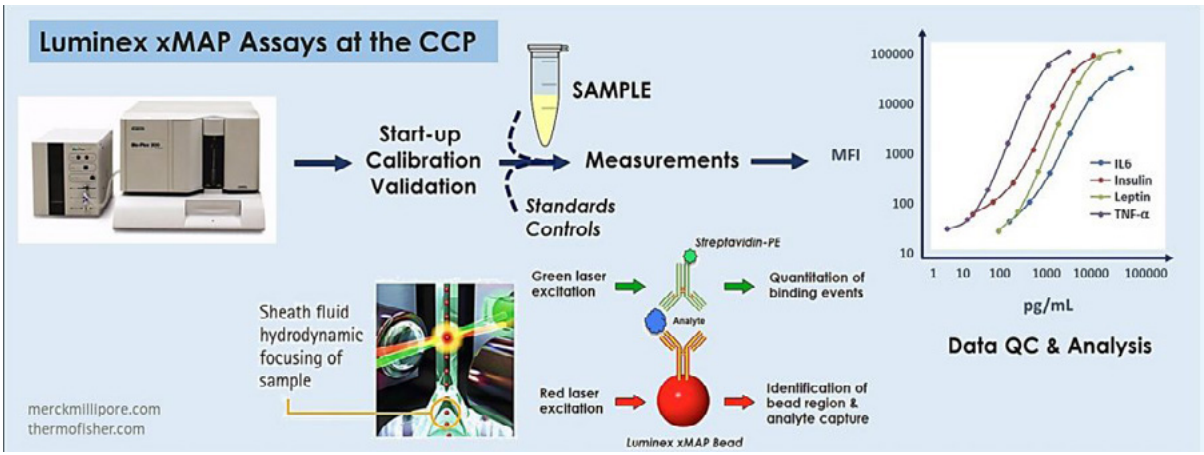


Fig 4. The Bio-Plex® 200 Luminex

(PO–31) Bioimaging & Embryology Unit (CCP, Phenotyping Module)

Micheala Prochazkova [1], Frantisek Spoutil [1], Sylvie Dlugosova [1], Barbora Kinska [1], Veronika Martinkova [1], Jan Prochazka [1], Radislav Sedlacek [1]

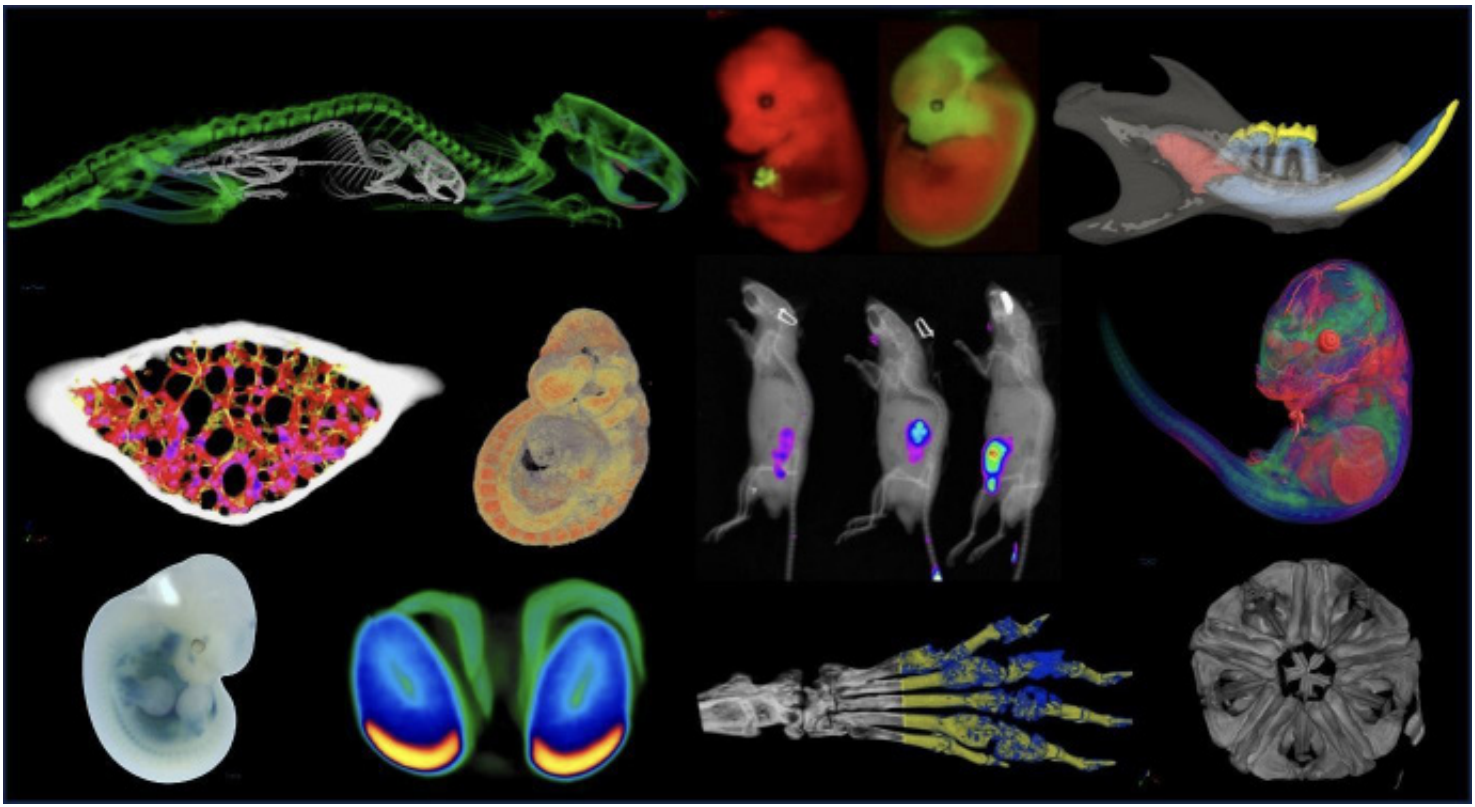
1. Laboratory of Transgenic Models of Diseases and Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

E-mail of the presenting author: michaela.prochazkova@img.cas.cz

The Bioimaging and Embryology (BIE) unit is focused on functional morphology projects using state of art 3D imaging technologies of adult mice and rats as well as murine embryos, specific tissues as enamel etc. The unit also provides the knowledge base for conditional gene inactivation, embryonic tissue isolation and dissections for OMICs or establishment of primary cell cultures.

The key technological base of our work lies in 3D imaging with µCT which allows visualization not only of mineralized tissues but also of soft tissues with use of appropriate contrast with resolution from 100 down to 0.5 µm. The BIE unit also provides comprehensive data analysis platform. Besides the 3D imaging, the unit is equipped with whole body imaging system for imaging of fluorescence and bioluminescence reporters in mice and rats in vivo. This technology is very advantageous especially for imaging of cancer models. Physiological processes like inflammation, kidney function or specific enzyme activity can be also non-invasively imaged and evaluated. The unit also provides functional assays on primary cells or their isolation for multiOMICs, offers dissection of embryonic tissues followed by primary cell line or organ culture setup, and delivers immortalized cell lines from KO phenotypes. We can also visualize gene expression by different methods both in whole mount and on sections. These approaches help to substantially accelerate the research of genes whose mutations cause severe developmental, often embryonic lethal phenotypes.

Examples of recent papers:
Early evolution of enamel matrix proteins is reflected by pleiotropy of physiological functions.
Spoutil F et al. **Scientific Reports, 2023**
Skeletal dysmorphology and mineralization defects in Fgf20 KO mice. Dlugosova et al. **Front. Endocrinol., 2024**



Examples of outputs of our work including µCT images, LacZ staining, transgenic fluorescent reporters and in vivo bioluminiscence.

(PO–32) Cardiovascular Unit (CCP, Phenotyping Module)

Eva Nekvindova [1], Jiri Lindovsky [1], Petr Macek [1], Sara Brilhante Viegas Dias [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

E-mail of the presenting author: eva.nekvindova@img.cas.cz

The cardiovascular system is a fundamental pillar of life. Within CCP, IMG's Cardiovascular Unit provides a comprehensive range of services aimed at uncovering cardiovascular traits in rodent models, including mice and rats.

We employ various non-invasive techniques, such as echocardiography, to utilize ultrasound for detailed exploration of heart and vascular structures. This approach allows us to analyze blood flow patterns, chamber efficiency and more. Our Cardiovascular Unit performs cardiovascular examinations on fetuses, pups, and adults, offering 4D visualization and strain analysis for a thorough cardiac evaluation. Electrocardiography enables us to monitor heart electrical activity in both conscious and anesthetized rodents. Additionally, we measure blood pressure to enhance our understanding of cardiovascular health.

We also address cardiovascular challenges by applying controlled physiological stress through treadmill-based assessments and inducing pharmacological stress with catecholamines to model pathological conditions. Our sonography services extend to gravidity checks, blood flow evaluations across various anatomical structures, and quantification of tumor size and vascularization. Our imaging expertise covers a broad range of anatomical locations.A notable feature of our services is the Image-Guided Injection technique, which uses ultrasound for precise delivery of small volumes of substances, such as viruses or drugs, into specific organ regions at all developmental stages.

Recently, we enhanced our unit's potential for studying individual cardiomyocytes through the adoption of the Langendorff cardiomyocyte isolation technique and the advanced xCELLigence Cardio ECR system from Agilent Technologies. This cutting-edge platform facilitates real-time assessment of cardiomyocyte contraction and electrical activity, enabling detailed analysis of the impact of genetic modifications, therapeutic interventions, and environmental variables on cardiac cellular function.

Our work aims to reveal gene functionality and understand disease mechanisms, contributing to the refinement of therapeutic strategies and the potential enhancement of human health.

(PO–33) Hearing & Electrophysiology Unit (CCP, Phenotyping Module)

Jiri Lindovsky [1], Kvetoslava Klajblova [1], Miles Raishbrook [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, v.v.i.

E-mail of the presenting author: jiri.lindovsky@img.cas.cz

The unit provides electrophysiological methods for functional testing of hearing and vision in mice and rats.
In principal, techniques used are based on recording of electric potentials of sensory pathways evoked by relevant stimuli. Presence or absence and size or form of the evoked potentials is then interpreted as a correlate of activity and functional status of individual structures of the nervous system.

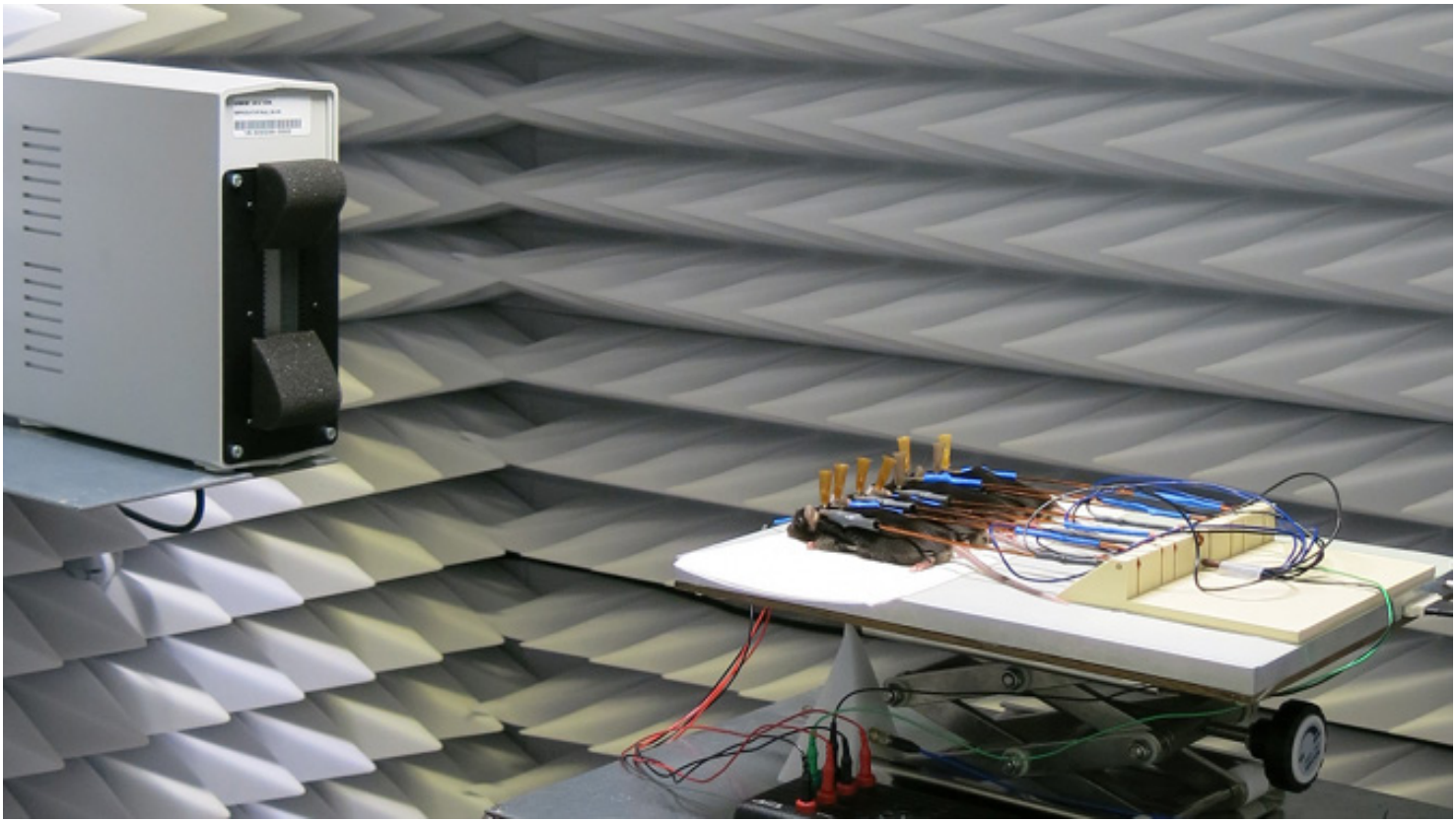
Services
- Auditory Brainstem Response (ABR)
- Electroretinography (ERG)
- Mutifocal Electroretinography (mfERG)
- Visual Evoked Potential (VEP)
- Force of isometric muscle contraction
- Wireless EEG

Devices and technologies
Hearing:
- 6 m3 sound-attenuated chamber
- Tucker-Davis Technologies System 6
- Custom-made scripts for data analysis (Matlab, Java Script)

Vision:
- Roland Consult RETIanimal
- Red IVC cages for dark adaptation (Tecniplast)
- Custom-made scripts for data analysis (Matlab)

Muscle force:
- Digitimer Neurolog System, custom made recording chamber, Matlab.

EEG:
- TSE Neurologger



Recording and analysis of the auditory brainstem responses.

(PO–34) Histopathology Unit (CCP, Phenotyping Module)

Juraj Labaj [1], Olha Fedosieieva [1], Hana Hola [1], Antonia Mastrangeli [1], Pavlina Mackova [1], Sarka Suchanova [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

E-mail of the presenting author: juraj.labaj@img.cas.cz

The Histopathology unit is one of the largest units of the CCP Phenotyping Module and provides service for a broad range of research community including users working with non-rodent material. The unit is particularly engaged in experimental pathology. The work flow of the histopathology laboratory covers all procedures from gross morphology through various staining techniques and fluorescent slide scanning to pathology description. Complete necropsy of mouse/rat is performed by veterinary pathologist and all macroscopic findings are documented. Almost all steps in tissue processing and slide preparation are automatized to achieve the highest levels of reproducibility and quality. The lab offers H&E staining done by automated stainer, wide range of special stains and immunohistochemistry. The microscopic evaluation of histological samples is done by pathologist and complex report with picture documentations is a standard. Most of activities are conformed to Good Laboratory Practices (GLP).

Instrumentation & technologies:
Tissue processing: Leica ASP6025 - The most modern vacuum tissue processor

Sectioning fresh specimens: Vibratom Leica 1200 - automated vibrating blade microtome

Slide staining: MultistainerLeica ST5020 in conjunction with Leica CV5030 Coverslipper - an exceptionally versatile stainer-coverslipper workstation; Ventana Benchmark Speial Stains - Automated slide stainer for special stains; Ventana Discovery ULTRA - Automated stainer for immunohistochemistry and in situ hybridization

Microscopy and analysis: Carl Zeiss Axio Imager.Z2 - motorized microscope imaging station, capable of both brightfield and fluorescence capture; Leica DM3000 - Semi automated high-throughput brightfield microscope system

Slide scanning:
Carl Zeiss Axio Scan.Z1 - Combined brightfield and fluorescence slide scanner with ability to also scan histotopograms. Equiped with ultra-fast LED fluorescent module and 7 different excitation/emission filters.
ConaPat - Tracking system.

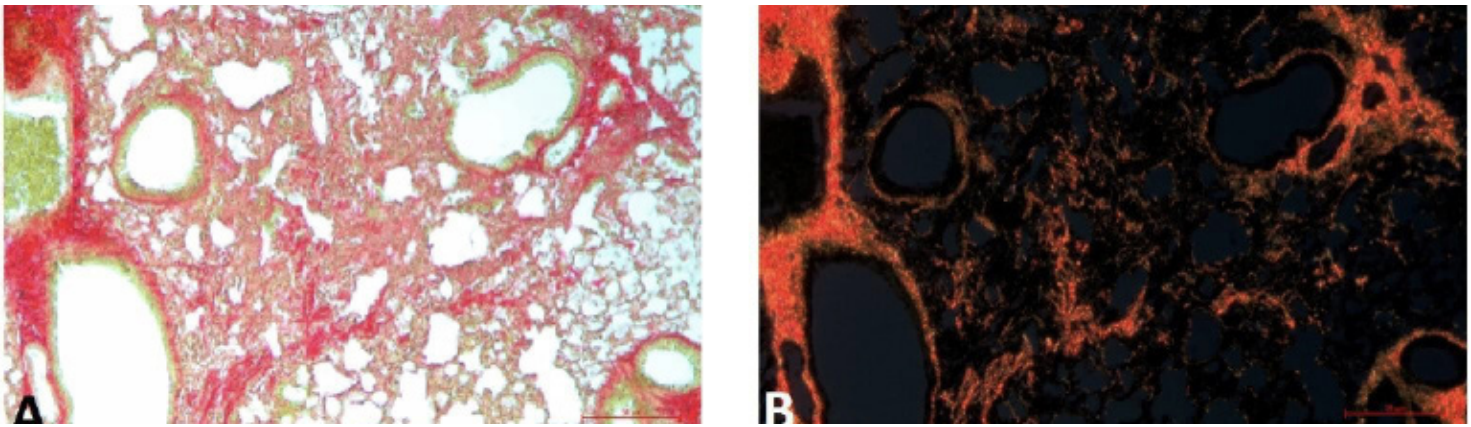


Fig 1. Picro Sirius Red staining. Lung fibrosis. A – light microscopy, B - polarized light microscopy. Magnification: x100.

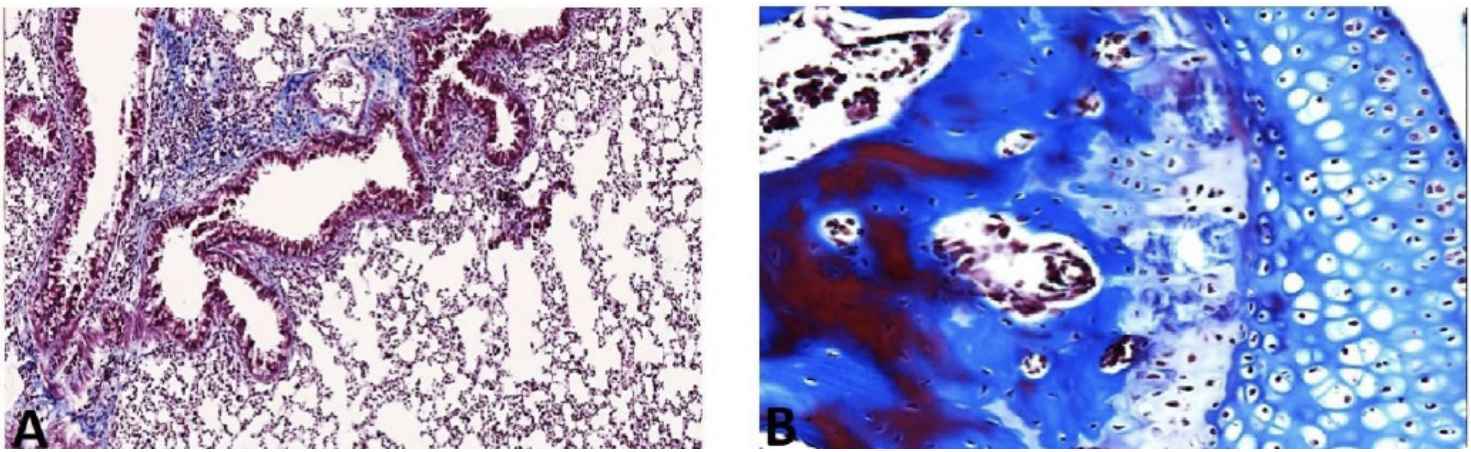


Fig 2. Masson Trichome staining. A - Lung fibrosis, magnification: x100. B – Bone with articular cartilage, magnification: x200.

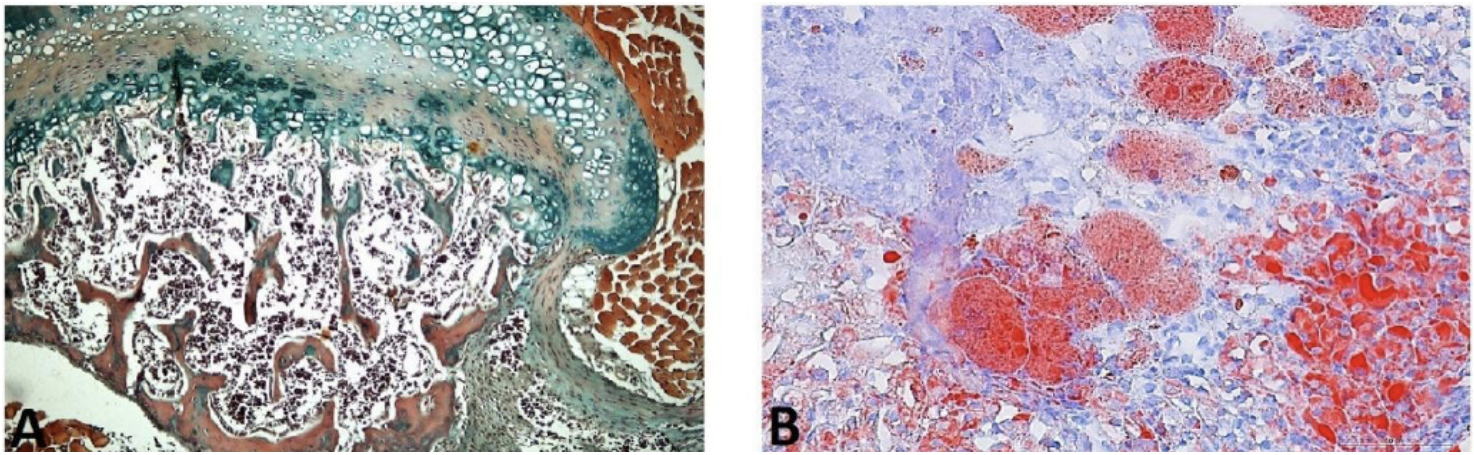


Fig 3. Special stainings. A - Alcian Blue Alizarin Red staining of bone with articular cartilage, magnification: x100. B - Oil Red staining of lipid inclusions in adrenal gland, magnification: x200.

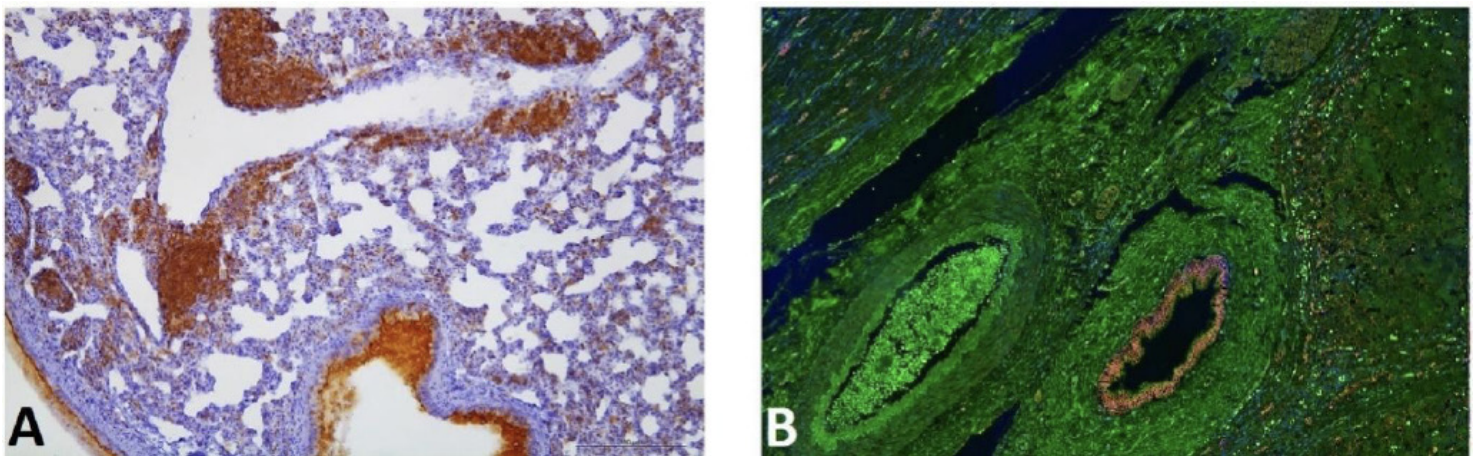


Fig 4. Immunohistochemical stainings. A - Paraffin-embedded mouse lung tissue stained for CD45, magnification: x200. B – Immunofluorescent staining of cryosections, magnification: x100.

(PO–35) Immunology Unit (CCP, Phenotyping Module)

Jana Balounová [1], Kristína Vičíková [1], Kamila Křížová [1], Michaela Šímová [1], Veronika Forstlová [1], Ondřej Honc [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

E-mail of the presenting author: jana.balounova@img.cas.cz

As an integral part of the terminal screen, immunophenotyping involves characterization of immune cell populations in terms of their cellularity and phenotype using multicolor flow cytometry (FCM). The procedures are based on standard immunophenotyping protocols of the Adult and Embryonic Phenotype Pipeline that has been agreed by the research institutions involved: IMPReSS -International Mouse Phenotyping Resource of Standardised Screens and beyond. Based on IMPC guidelines, we have developed a comprehensive immune-phenotyping panel enabling discrimination of various populations of lymphoid and myeloid cells in the mouse spleen or other tissues (peripheral blood, lymph nodes, thymus, bone marrow, peritoneal lavage, intestine) https://www.mousephenotype.org/impress/ProcedureInfo?action=list&procID=1463&pipeID=44. Additionally, we routinely use FCM assays to analyze cell populations in mouse blood, embryonic as well as adult hematopoiesis, thymus and tumor microenvironment. To characterize the PDX models developed at CCP, we have optimized FCM panels to determine human leukocyte populations in humanized mouse strains. Moreover, we can design a suitable FCM panel to detect, characterize or purify cell populations of interest.

Instrumentation & technologies

The Unit is equipped with Cytek Aurora spectral flow cytometer. With 5 lasers (355, 405, 488, 532, 635nm), three scattering channels, 64 fluorescence channels and automated sample loader, the Aurora system is suitable to acquire high dimensional flow cytometry data in hightroughput. The FCM data is then analyzed in FlowJo or Omiq software and statistically evaluated. Furthermore, the Immunology Unit is equipped with gentleMACS tissue dissociator (Miltenyi Biotec), EasySep cell separation magnet for column-free cell separation (StemCell Technologies), bright field automated cell counter for counting of viable cells (Cellometer Auto T4, Nexcelom Bioscience) and a microplate spectrophotometer - ELISA reader (BioTek Epoch).

Selected Publications:

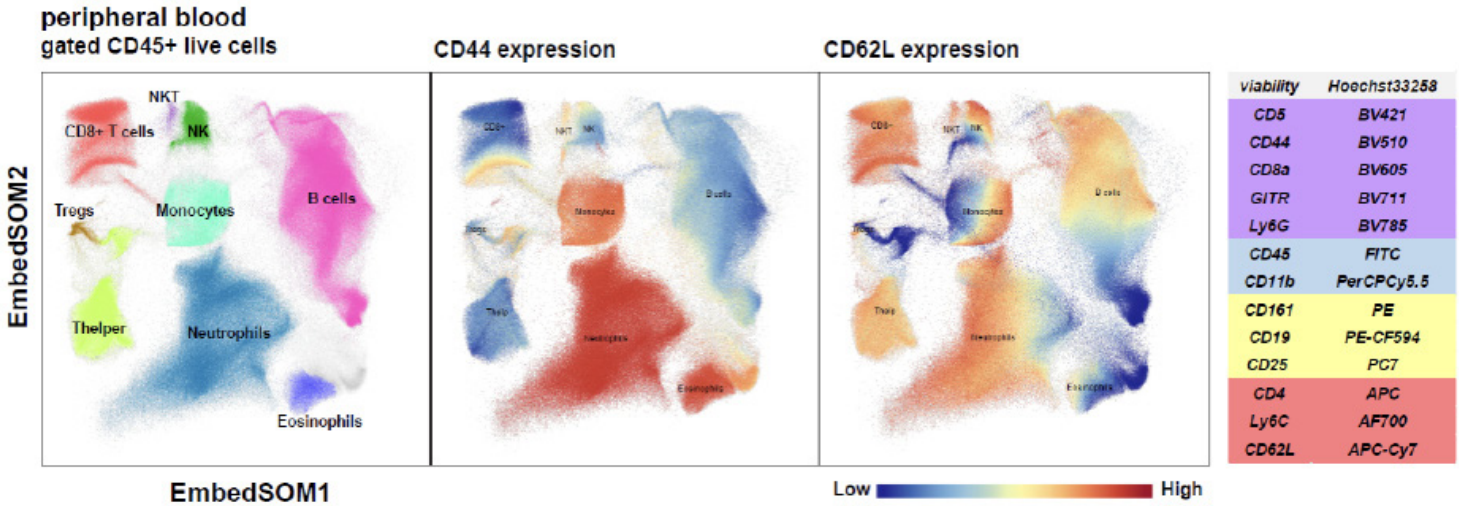
Self-reactivity of CD8 T-cell clones determines their differentiation status rather than their responsiveness in infections.
D Paprckova, V Niederlova, A Moudra, A Drobek, M Pribikova, S Janusova, K Schober, A Neuwirth, J Michalik, M Huranova, V Horkova, M Cesnekova, M Simova, J Prochazka, J Balounova, DH Busch, R Sedlacek, M Schwarzer, O Stepanek. Front Immunol. 2022 Oct 6;13:1009198. doi: 10.3389/fimmu.2022.1009198.

Deletion of TLR2 + erythro-myeloid progenitors leads to embryonic lethality in mice.
I Šplíchalová, J Balounová, M Vobořil, T Brabec, R Sedlacek and D Filipp. Eur J Immunol. 2021 Sep;51(9):2237-2250. doi: 10.1002/eji.202049142.

Regulation of Inflammatory Response by Transmembrane Adaptor Protein LST1.
M Fabisik, J Tureckova, N Pavliuchenko, J Kralova, J Balounova, K Vicikova, T Skopcova, F Spoutil, J Pokorna, P Angelisova, B Malissen, J Prochazka, R Sedlacek, T Brdicka. Front Immunol. 2021 Apr 27;12:618332. doi: 10.3389/fimmu.2021.618332

Toll-like receptor 2 expression on c-kit+ cells tracks the emergence of embryonic definitive hematopoietic progenitors.
Balounová J, Šplíchalová I, Dobešová M, Kolář M, Fišer K, Procházka J, Sedlacek R, Jurisicova A, Sung HK, Kořínek V, Alberich-Jorda M, Godin I, Filipp D. Nat Commun. 2019, 10(1):5176. (IF: 12.121)

SOM-based embedding improves efficiency of high-dimensional cytometry data analysis.
M Kratochvil, A Koladiya, J Balounova, V Novosadova, R Sedlacek, K Fiser, J Vondrasek and K Drbal. bioRxiv (2019): 496869.



(PO–36) Metabolism Unit (CCP, Phenotyping Module)

David Pajuelo Reguera [1], Pavlina Richtarechova [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics - IMG, Czech Academy of Sciences

E-mail of the presenting author: david.pajuelo-reguera@img.cas.cz

Genetically modified mouse models are indispensable for elucidating the specific gene function, including those involved in the energy metabolism and glucose homeostasis. Our research initiates with foundational phenotyping, including intraperitoneal glucose tolerance tests, non-invasive body composition analysis, and indirect calorimetry. These tests establish a baseline for subsequent in-depth, hypothesis-driven studies.

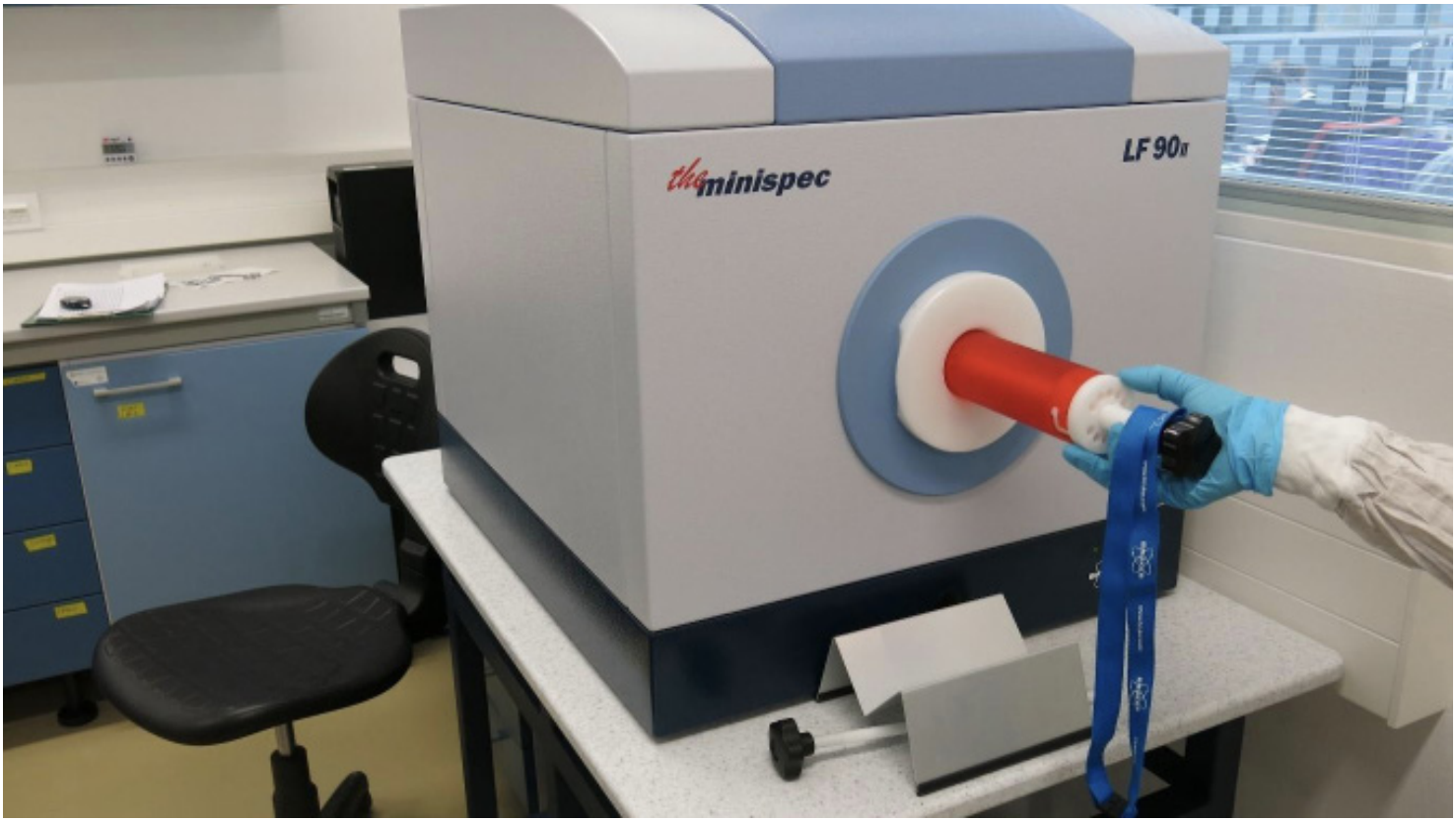
Environmental chambers equipped with adjustable light:dark cycles, humidity, and temperature settings facilitate the performance of cold challenges, thermoneutral studies, and alterations in light-dark patterns. We collect indirect calorimetric data from mice or rats undergoing these environmental challenges. Additionally, we can evaluate the impact of specialized diets, such as high-fat diets, on overall metabolism.

To investigate glucose metabolism in detail, we employ several tests: basal and maximal blood insulin concentrations are measured during glucose tolerance tests, while insulin sensitivity is assessed using insulin tolerance tests. These complementary methodologies aid in explaining potential defects in glucose metabolism resulting from genetic modification or specific treatments.

Our team has integrated telemetry of physiological parameters, including body temperature at two body locations and real-time blood sugar measurements. These parameters can be monitored in home-caged mice or in conjunction with indirect calorimetry. We utilize non-invasive body composition analysis based on TD-NMR technology, which offers a swift and accurate approach to determining lean and fat mass, as well as free fluids in mice and rats. The non-invasive nature and rapid analysis allow for repeated measurements of body composition over time.

Like all units at CCP, our metabolism services benefit from integration with other units of the centre, enabling the systemic and comprehensive characterization of experimental rodent models.

Telemetry System measuring 2 different temperatures in a non-restreined mouse



Body composition system based on TD-NMR technology and the restrainer for mouse

(PO–37) Metabolomics Unit (CCP, Phenotyping Module)

Klára Dohnalová [1], Kryštof Klíma [1], Marta Kaliaeva [1], Lukáš Kučera [1], Karel Chalupský [1], Jan Procházka [1], Radislav Sedláček [1]

1. Phenotyping Module, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the CAS, v. v. i

E-mail of the presenting author: karel.chalupsky@img.cas.cz

The Metabolomics unit is expanding the method portfolio of the Metabolic and Clinical Biochemistry Units. The analysis of blood is part of our standard first-line phenotyping. Measuring only a limited number of biochemical markers, increases the risk of missing the physiological impact of a studied gene or a treatment, or the early onset of a disease. Therefore, we implemented metabolomics and lipidomics technology to analyze blood, serum or tissue homogenates that may even give a hint to the mechanistic basis of a diseaserelevant phenotype. Using reverse and hilic chromatography we are able to detect and quantify about 300 metabolites. A specific MS/MS lipid library is designed for each lipidomics sample screen and usually consist of over 400 unique lipid species depending of sample type. Additionally, we can also track incorporation of labelled heavy carbon, delivered from 13C glucose, in cell culture samples. Our unit participates in preclinical screening in CCP by targeted detection of experimental compounds and provides stability and pharmacokinetics data. Besides analysis based on liquid chromatography we also provide the mass spectrometry analysis of tissue samples by MALDI imaging. Mass spectrometry imaging is mainly linked with histology and offer analysis of compounds in spatial context, which exceed the possibilities of classical histology. We are able to detect more than three hundreds of molecules on tissue slides. Our metabolomics unit has shown great potential in several biological applications. Discovery of diagnostic biomarkers, drug metabolization and their effects on whole metabolome, and progression of diseases are examples where studying metabolite profiles provided additional value also regarding translation to human disease. Using statistical methods allows to process and compare large data sets. Additional effort is put into the identification of unique metabolites and to map those to specific metabolic pathways which may be an important hint towards the molecular mechanism underlying the function of a gene.

(PO–38) Neurobiology & Behaviour Unit (CCP, Phenotyping Module)

Kateryna Pysanenko [1], Jiri Lindovsky [1], Pavel Jina [1], Andrea Sabotova [1], Katarina Kanasova [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics BIOCEV – Institute of Molecular Genetics

E-mail of the presenting author: kateryna.pysanenko@img.cas.cz

Genetic engineering opens an avenue of research opportunities to probe molecular bases of a variety of human diseases. Neurobehavioural tests using transgenic animal models make it possible to understand genetic mechanisms underlying neurological and psychiatric disorders including, but not limited to, anxiety, schizophrenia, mood disorders, and Parkinson's disease.

The Neurobiology and Behaviour Unit employs a number of tests to examine motor abilities, cognitive functions, emotion, sensory processing as well as neurological, and gait impairments in transgenic mice. We offer standardized primary and secondary phenotype screens based on IMPC (International Mouse Phenotyping Consortium) protocols (https://www.mousephenotype.org/impress). Primary/mandatory screens include modified SHIRPA and dysmorphology evaluation, Open Field, Grip Strength, Acoustic Startle and PPI, Light/Dark Box, and Fear Conditioning. The Unit also offers more specific secondary/optional screens that comprise tests evaluating animal emotionality and affect (Elevated Plus Maze, Forced Swim Test, Tail Suspension Test), cognitive function (Cued and Contextual Conditioning, Context Discrimination, Y-Maze Spontaneous Alternation, Barnes Maze, Novel Object Recognition), neuromotor abilities (RotaRod, Gait Analysis), pain sensitivity (Hot/Cold Plate, Tail Flick, Plethysmometer, von Frey Test), social preference, and last but not least evaluation of animal cognitive function and circadian activity in more natural conditions in IntelliCages. Social group housing in a large enclosure, free from human handling stress, equipped with multiple gadgets in IntelliCage provides environmental enrichment beyond typically employed protocols.

(PO–39) PDX & Cancer Models Unit (CCP, Phenotyping Module)

Silvia Magalhaes Novais [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Institute of Molecular Genetics of Czech Academy of Sciences

E-mail of the presenting author: silvia.novais@img.cas.cz

The PDX and Cancer Models Unit is dedicated to creating innovative oncology research models and committed to providing tools that propel effective treatments for cancer. We offer customizable study designs, access to a comprehensive collection of PDX mouse models, cancer cell line xenografted models, and immuno-oncology models to test the effectiveness of novel or existing immunotherapeutic compounds. Moreover, complementing our offerings, our in-house multidomain analysis spans an array of disciplines, including histopathology, hematology, biochemistry, immunology, bioimaging, and metabolomics. These cutting-edge approaches have the transformative potential to elevate any project to unparalleled heights of success. These sophisticated models and applications combine creativity, solid reliability, and a relentless commitment to customer satisfaction, ensuring access to clinically relevant mouse models and precision services.

(PO–40) Vision Unit (CCP, Phenotyping Module)

Marcela Palkova [1], Jiri Lindovsky [1], Kvetoslava Klajblova [1]

1. Institute of Molecular Genetics of the Czech Academy of Sciences - Czech Centre of Phenogenomics

E-mail of the presenting author: marcela.palkova@img.cas.cz

Vision unit is a part of phenogenomic center and it is mainly focused on imaging, analyzing morphological structures and assessing morphological abnormalities in rodent eyes. These primary examinations are routinely performed in all mice coming to our unit. In special cases such as obvious morphological pathology of retina or special requests (e.g. mouse model for the retinopathy, diabetic disease etc.), the function of the retina is proved by electroretinography (ERG).

Additional measurements of the intraocular pressure by rebound tonometer (IcareTonovet plus) provide us important information on the eye function and the health in the mice.

Imaging devices with high image quality and resolution are used to examine the anterior segment (Pentacam), retina and retinal vascular plexuses (Optical coherent tomograph Heidelberg Engineering - OCT). All procedures are non-invasive, painless and allow long-term studies with repeated ocular examination.

Pentacam scans the eye from 25-50 different angles and enables to measure many parameters of the cornea and the lens (e.g. surface, form, opacity, thickness and density) for each eye. The OCT scan quantifies reflections of a light beam from individual layers of the retina and composes virtual cross- sectional images of the retina. The OCT-A scan enables us to detect and analyze four retinal vascular plexuses (svc - superficial and dvc - deep vascular complex, choriocapillaris and choroid). Each cross-section is evaluated and a variety of parameters are measured, e.g. the thickness and the gross morphology of the retina (retinal layers), form and the position of the optic disc, structure and pattern of the superficial blood vessels and parameters of the blood plexuses, e.g. density, number of blood vessel junctions and endpoints per region. To prove any morphological changes in the retina at different time points of life in mice, the consecutive scans could be done. ERG measures electrical responses of different retinal cell types evoked by light stimulation. This examination enables us to assess the physiological relevance of the morphological abnormalities in the retina for the vision and it is described in detail in the Electrophysiological section.

Besides covering of the routine IMPC workflow, the unit also collaborates on many other research projects related to vision.

(PO–41) Animal Biosafety Level 3 facility for preclinical testing and research of infectious diseases

Dominik Arbon [1], Federica Gambini [1], Barbora Cechova [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre of Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Vestec, Czech Republic

E-mail of the presenting author: dominik.arbon@img.cas.cz

The Models of Infectious Diseases Unit includes an Animal Biosafety Level 3 (ABSL-3) laboratory, featuring a closed husbandry facility dedicated to researching infectious diseases using animal models. Equipped with advanced technologies and a broad range of research modalities, the facility supports both short- and long-term studies of viral, bacterial, and protozoan infections relevant to human and animal health. We offer comprehensive services, including project planning, licensing, and experimental design, supported by a team of skilled researchers experienced in a wide array of invasive and non-invasive techniques, as well as microbiological procedures. This infrastructure facilitates both primary academic research and preclinical testing, adhering to the highest scientific standards.

We offer advanced capabilities for customized humanized models, incorporating different gene editing techniques or transient induced expression systems of viral receptors to create organ-specific sensitized mice. Our expertise extends to transferring in vitro studies into in vivo drug screening, enabling comprehensive analysis of various solutions.

The laboratory is equipped with state-of-the-art safety technology and protection procedures, alongside advanced tools for complex in vivo analyses, such as lung function testing, immunology screening panels, histopathological evaluations, metabolic assessments, and whole-body imaging. Our goal is to provide accessible, high-quality infrastructure to advance basic research in infectious diseases and to support preclinical screening of therapeutic compounds.

Researchers equipped with BSL3 personal protective equipment

(PO–42) Bioinformatician Unit (CCP, Phenotyping Module)

Vendula Novosadova [1], Maximilian Golena [1], Lukas Drahnik [1], Igor Varga [1], Olha Pyko [1], Simon Trcka [1]

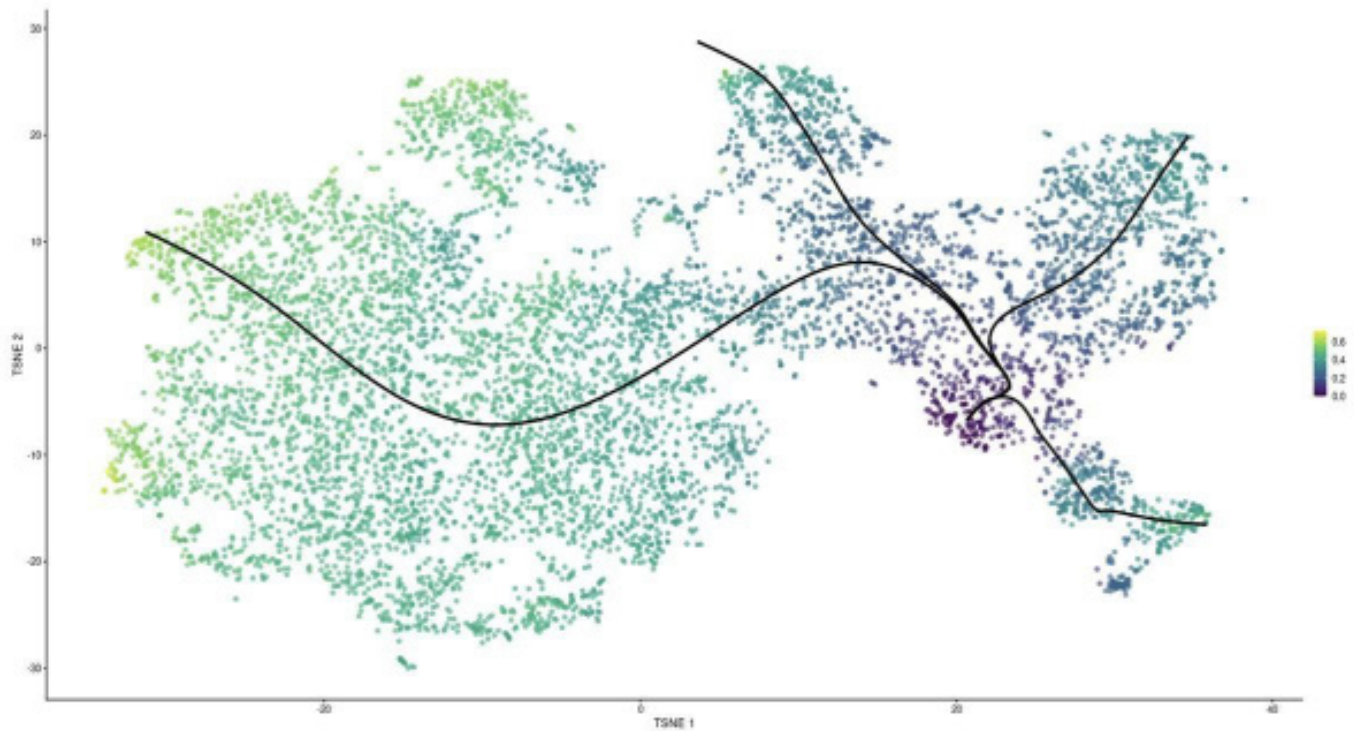
1. Czech Centre for Phenogenomics, IMG AS CR

E-mail of the presenting author: vendula.novosadova@img.cas.cz

Bioinformatics unit of CCP assists other CCP units with computational, statistical, and analytical analyses and provides these services in this field to CCP external customers. The unit focuses, principally, on data analysis, biostatistics, tool and application development and occasional organization of training workshops in biostatistics and programming. We endeavor towards the automation of various interdisciplinary enterprises leveraging such novel approaches as deep learning. The group also maintains a continuous and indispensable effort in integrative bioinformatics as part of its involvement in phenotyping research by large-scale analysis of phenotyping datasets and image analysis. The unit takes care about all phenotyping data including quality control, statistical analysis, their storage and placing them into public web. We are also developing LIMS system and help people with daily routine process automatization.

INSTRUMENTATION & TECHNOLOGIES

For big data analysis, we utilize our own Supermicro 1029GQ-TRT server. This server consists of two Intel Xeon Gold 5120 @ 2.2 GHz processors each with 14 cores, 128 MB RAM, and two SSD drives in RAID 1, each with 240GB memory. For computational acceleration of deep learning/neural network approaches, we use one graphics card NVIDIA Tesla P100 16GB. Especially long-term one threaded tasks are dislocated to MetaCentrum which provides free membership for researchers and students of academic institutions in Czech Republic. Our main used tools in our bioinformatics unit are R, Python.



Example of Trajectory analysis for NGS single cells data

Tools overview

(PO–43) Transgenic and Archiving Module (CCP)

Lucie Dufkova [1], Petr Nickl [1], Michaela Krupkova [1], Csilla Michalikova [1], Elena Vikhrova [1], Eliska Machalova [1], Markéta Joskova [1], Katerina Zelena [1], Anna Kolihova [1], Radislav Sedlacek [1]

1. The Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.

E-mail of the presenting author: lucie.dufkova@img.cas.cz

Transgenic and archiving module (TAM) is a key part of Czech Centre for Phenogenomics (CCP), responsible for generation of novel genetically modified mice and rats using state-of-the-art technologies. TAM offers a comprehensive, reliable and high-quality transgenic service to researchers.

OUR SERVICE COMPRISES:

Transgenic Mouse and Rat Production

- Mouse/rat model generation using programmable nucleases (TALEN, CRISPR/Cas9).
- Classical plasmid and BAC transgene generation using PNI (pronuclear injection).
- Mouse model generation using ES cells, including usage of ES cells from EUCOMM and KOMP repositories.
- CRE/FLP mediated allele conversions.

Rederivation

- Removing pathogens that exist in a breeding colony – importing live rodents for archiving and consequent rederivation of the strain for breeding in specific pathogen free (SPF) conditions.
- Reanimation of strains from frozen material - importing established lines when import of live animals is not an option.

There are several options for rederivation – In Vitro Fertilization (IVF) using fresh or frozen sperm and embryotransfers of fresh or frozen embryos.

Cryopreservation

- The successfully produced mouse/rat lines are cryopreserved.
- Long term storage repository of mouse and rats embryos and sperm.

Mouse breeding

- Mouse breeding to generate new genetically modified mouse strains.

Genotyping service

- Automatic capillary electrophoresis QIAxcel Advanced system.

Import/Export

- Import/Export arrangements (together with the Animal Facility Module).

Cooperation

TAM provides services to a broad national and international scientific community.
As a member of INFRAFRONTIER, we are contributing with mice generation to the IMPC project that aims to knockout all the mammalian genes.

TAM also represent a Czech node of EMMA (European Mouse Mutant Archive), a non-profit repository for the collection, archiving (via cryopreservation) and distribution of relevant mutant mouse strains essential for basic biomedical research.

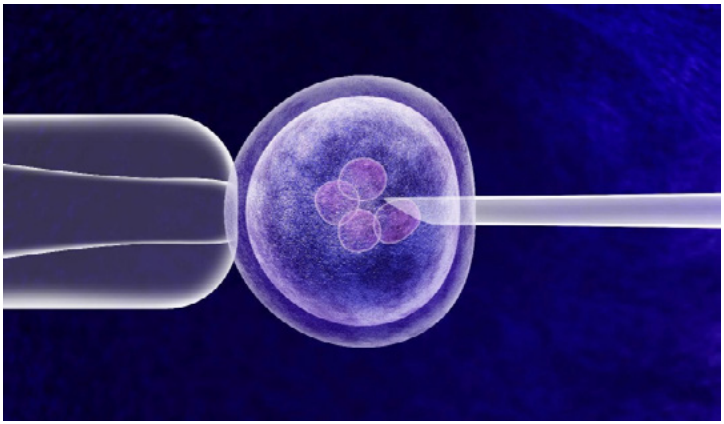


Fig.1: In Vitro Fertilization (IVF) using fresh or frozen sperm is one option for rederivation of mouse strains.

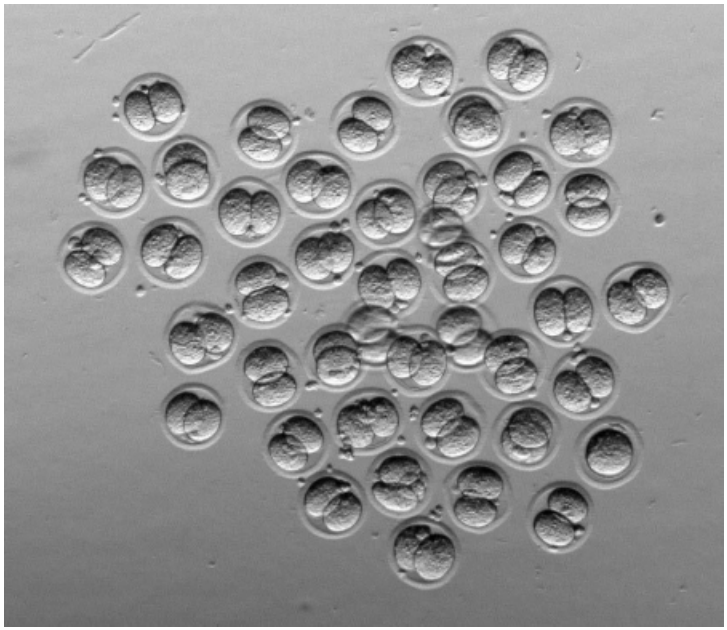


Fig.2: Embryotransfers (ETs) of fresh or frozen embryos (Rat 2-cell stage embryos in the picture) are used for rederivation of mouse strains.

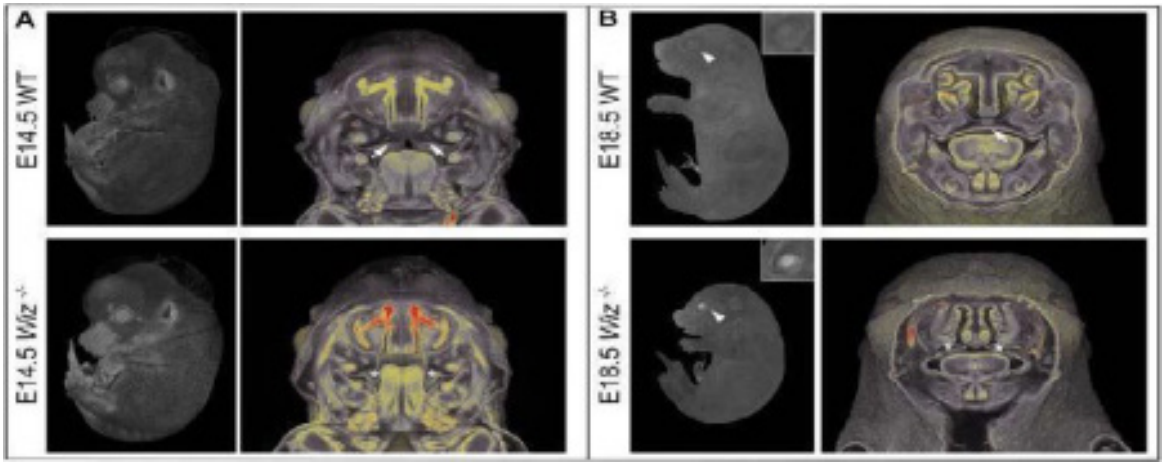


Fig.3: Published mouse models developed in TAM: Generation and Characterization of a Novel Angelman Syndrome Mouse Model with a Full Deletion of the Ube3a Gene (Syding et al., 2022, Cells).

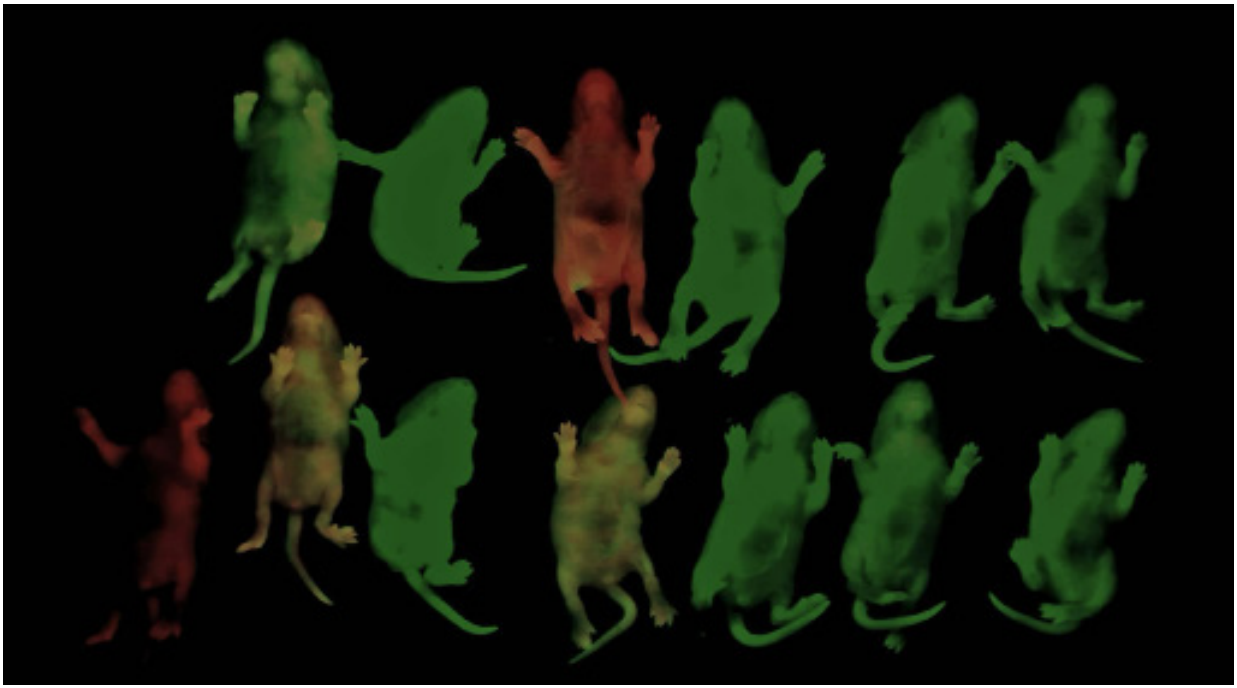


Fig.4: Published mouse models developed in TAM: Efficient allele conversion in mouse zygotes and primary cells based on electroporation of Cre protein (Jenickova et al., Methods, 2021).

(PO–44) Preclinical testing at the Czech Centre for Phenogenomics

Gizela Koubkova [1], Sarka Suchanova [1], Ivana Bukova [1], Vendula Novosadova [1], Juraj Labaj [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Institute of Molecular Genetics of Czech Academy of Sciences, CCP

E-mail of the presenting author: gizela.koubkova@img.cas.cz

The development of new drugs is an interdisciplinary, time-consuming, and costly process and critically depends on the selection of appropriate and predictive preclinical models. Developing safe and efficacious drugs requires thorough preclinical testing using in vitro, in vivo, and increasingly also in silico approaches. Based on the experiences from high throughput phenotyping of mouse models, the Czech Centre for Phenogenomics (CCP) offers a broad portfolio of highly standardized, state-of-the-art test assays (some in GLP mode) that can be applied in preclinical studies in experimental rodent models reproducing certain features of human disease. Established non-clinical tests comprise toxicity studies, hematological, and biochemical testing of samples taken from animals during toxicity studies, determination of active substances, and metabolites in plasma or other biological matrices, histopathology, ECG and echocardiography for effects on cardiovascular functions, body composition analysis, monitoring of energy fluxes, substrate utilization, feeding and drinking behavior, and locomotor activity, as well as various imaging modalities. The CCP has also implemented neurobehavioral testing and established model systems in the field of asthma and lung fibrosis, liver fibrosis, and induced colitis models. Furthermore, we offer efficacy testing in established CDX/PDX models and we can also provide new cancer model development starting with in vivo growth kinetics of the required cell line. Our CDX/PDX modality is strongly supported by the Bioimaging unit including services for in vitro experiments.We can offer genomic modification of provided cell line (e.g. to get luminescent cells or more sophisticated tasks). We can provide therapy testing also on several models for rare diseases using genetically modified animals (e.g. models of Prader-Willi and Angelman syndromes, Netherton syndrome) and also for some human infections – even when the wildtype mice are resistant (e.g. Covid-19 – using various GM mouse models). Further preclinical models are under development.

Link to CCP preclinical testing webpage | https://www.phenogenomics.cz/preclinical-testing/

(PO–45) Animal Facility Module (CCP, Vestec)

Anna Pilařová [1], Daniela Kučerová [1], Lucie Dufková [1], Libor Kopkan [1], Radislav Sedlacek [1]

1. The Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

E-mail of the presenting author: anna.pilarova@img.cas.cz

The Animal Facility Module (AFM) of the Czech Centre for Phenogenomics (CCP) located at the BIOCEV campus in Vestec is a modern and progressive facility regarding technologies, high quality laboratory animal husbandry and production that demands on animal health control and welfare.

Structure of AFM
The AFM contains four individual, fully separated breeding and experimental barrier areas, and an autonomous Biological Safety Level - 3 (BSL-3) laboratory with the strict personnel entry. The AFM holds laboratory mice and rats, with the total capacity of approximately 24 000 animals. Animals are kept in a controlled SPF (specific pathogen-free) environment in the barriers. All animals are housed in the individually ventilated cages (IVCs). All materials are decontaminated in autoclaves or hydrogen peroxide steamers. Conception of buildings and animal facility management is in accordance with the highest standards for laboratory animals.

Standard AFM services
- housing and husbandry of laboratory rodents,
- comprehensive veterinary care and expertise,
- import and export of animals,
- animal ethics and welfare recommendations etc.

New AFM services
- AFM now offers full support in the project management in Good Laboratory Practice mode. GLP encapsulates the practice using standardised guidelines to generate standardised and reproducible studies.
- BSL-3 facility allows experiments on live animals under BSL-3 conditions with breeding capacity of 2 880 mice in 480 IVCs and 168 rats in 56 IVCs.

Rodent colony management
AFM maintains breeding of the most common mouse strains C57Bl/6N, C57Bl/6J and BALB/cAn. There are differences in breeding performance between these strains. There were 429 litters with 2932 pups born to 79 females of the C57Bl/6N strain. Pre-weaning mortality was 11.8 % for this strain. In the C57Bl/6J strain with 72 females, 2623 pups were born in 437 litters, but the pre-weaning mortality was 14.3 %. In the BALB/cAn strain, 58 females gave birth to 2221 pups in 406 litters and the pre-weaning mortality rate was only 7.6 %.

Overall, the AFM provides an optimal breeding condition for small laboratory rodents and makes animal experiment expertise available for several scientific institutions in the highest feasible standards of the biomedical research.

Lastname	Name(s)	Organization	Country	E-mail
Abramova	Vera	Institute of Physiology	Czech Republic	vera.abramova@fgu.cas.cz
Ahmad	Parwez	New York University	United States of America	ahmad.parwez@gmail.com
Akbarova-Ben-Tzvi	Gunay	Baku State University	Azerbaijan	akperova_science@mail.ru
Arbon	Dominik	Czech Centre for Phenogenomics, IMG CAS	Czech Republic	dominik.arbon@img.cas.cz
Attri	Kriti	Institute of Molecular Genetics	Czech Republic	kriti.kriti@img.cas.cz
Aussel	Clotilde	MEDICAL CENTER – UNIVERSITY OF FREIBURG	Germany	clotilde.aussel@uniklinik-freiburg.de
Ayabe	Shinya	RIKEN BioResource Research Center	Japan	shinya.ayabe@riken.jp
Balík	Aleš	Institute of Physiology, Czech Academy of Sciences	Czech Republic	ales.balik@fgu.cas.cz
Balounova	Jana	Czech Centre for Phenogenomics/ Institute of Molecular Genetics	Czech Republic	jana.balounova@img.cas.cz
Basíková	Hana	Ecsimeda s.r.o	Czech Republic	basikova.h@eppendorf.cz
Beck	inken	Ulm University	Germany	inken.beck@uni-ulm.de
Bekhit	Fatma Elzahraa	Institute of Physiology CAS	Czech Republic	Fatma.Bekhit@fgu.cas.cz
Benčina	Mojca	National Institute of Chemistry	Slovenia	mojca.bencina@ki.si
Ber	Tobiáš	Institute of molecular genetics of the Czech Academy of Sciences	Czech Republic	tobias.ber@img.cas.cz
Bezginov	Alex	The Centre for Phenogenomics (TCP)	Canada	alex.bezginov@phenogenomics.ca
Bobek	Lumír	Animalab, s.r.o.	Czech Republic	lb@animalab.cz
Boeke	Jef	NYU Langone Health	United States of America	jef.boeke@nyulangone.org
Brakebusch	Cord	University of Copenhagen	Denmark	cord.brakebusch@bric.ku.dk
Braun	Robert	The Jackson Laboratory	United States of America	bob.braun@jax.org
Brechlerová	Dagmar	accela, s.r.o	Czech Republic	brechlerova@accela.eu
Brilhante Viegas Dias	Sara	CCP	Czech Republic	sara.dias@img.cas.cz
Buková	Ivana	Institute of Molecular Genetics of the ASCR, v. v. i.	Czech Republic	ivana.bukova@img.cas.cz
Cannizzo	Martina	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	martina.cannizzo@img.cas.cz
Capelletti	Sabrina	Lumc	Netherlands	c.sabr.z@gmail.com
Cerulová	Sabina	Masaryk University, Faculty of Science, RECETOX	Czech Republic	498829@mail.muni.cz
Cervena	Lenka	Institute of Molecular Genetics of CAS	Czech Republic	lenka.cervena@img.cas.cz
Cysewski	Dominik	Medical University of Białystok	Poland	dominik.cysewski@umb.edu.pl
Čechová	Barbora	Institute of Molecular Genetics, Academy of Sciences, Prague	Czech Republic	barbora.cechova@img.cas.cz
Černák	Michal	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	michal.cernak09@gmail.com
Černý	Prokop	Czech Centre for Phenogenomics- Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	prokop.cerny@img.cas.cz
Dámek	Filip	Helmholtz Zentrum München	Germany	filip.damek@helmholtz-munich.de
Daněk	Veronika	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	veronika.danek@img.cas.cz
Davies	Ben	Francis Crick Institute	United Kingdom	benjdav@gmail.com
Demlova	Regina	Masaryk University, Faculty of Medicine	Czech Republic	demlova@med.muni.cz
Desprat	vera	SANOFI	France	rdesprat@gmail.com
Dlugošová	Sylvie	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	sylvie.dlugosova@img.cas.cz

Dobbie	Michael	Phenomics Australia	Australia	michael.dobbie@anu.edu.au
Dohnalová	Klára	BIOCEV – Institute of Molecular Genetics	Czech Republic	klara.dohnalova@img.cas.cz
Dostálová	Pavla	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	pavla.dostalova@img.cas.cz
Dráber	Petr	Institute of Molecular Genetics	Czech Republic	draberpe@img.cas.cz
Drahník	Lukáš	IMG CAS CCP	Czech Republic	lukas.drahnik@img.cas.cz
Dufkova	Lucie	CCP/IMG	Czech Republic	lucie.dufkova@img.cas.cz
Favero	Rodolfo	Czech Centre for Phenogenomics	Czech Republic	rodolfo.favero@img.cas.cz
Fedosieieva	Olha	Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	olha.fedosieieva@img.cas.cz
Fessele	Sabine	INFRAFRONTIER GmbH	Germany	sabine.fessele@infrafrontier.eu
Fili	Klevinda	Fyziologický ústav AV ČR	Czech Republic	klevinda.fili@fgu.cas.cz
Flenniken	Ann	The Centre for Phenogenomics	Canada	flenniken@lunenfeld.ca
Forejt	Jiri	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	jforejt@img.cas.cz
Gambini	Federica	Czech Centre for Phenogenomics (IMG)	Czech Republic	federica.gambini@img.cas.cz
Garrett	Lillian	Helmholtz Center Munich	Germany	lillian.garrett@helmholtz-munich.de
Gercke	Daniel	Transnetyx	United States of America	dgercke@transnetyx.com
Glushchenko	Mariya	CCP	Czech Republic	mariya.glushchenko@img.cas.cz
Goleňa	Maximilián	Czech Centre for Phenogenomics	Czech Republic	maxo.golena@gmail.com
Golini	Elisabetta	CNR	Italy	elisabetta.golini@cnr.it
Goncalves	Manuel	Leiden University Medical Center	Netherlands	m.f.v.goncalves@lumc.nl
Gorgol	Anastasija	Institute of experimental medicine	Czech Republic	anastasija.gorgol@iem.cas.cz
Gulati	Pranav	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	pranav.gulati@img.cas.cz
Guo	Huijuan	NOVOGENE UK COMPANY LIMITED	United Kingdom	huijuan.guo@novogene-europe.com
Hajgajda	Radoslav	Asotiation of Gene Therapy	Czech Republic	hajgajda@asgent.org
Hartman	Jessica	Cell Microsystems Inc	United States of America	jhartman@cellmicrosystems.com
Havran	Jiří	AnLab, s.r.o.	Czech Republic	havran@anlab.cz
Hawko	Salwa	Institute of Molecular Genetics of the CAS, Czech Centre for Phenogenomics	Czech Republic	hawkos@img.cas.cz
Heikkinen	Anne	University of Oulu	Finland	anne.heikkinen@oulu.fi
HERAULT	Yann	University of Strasbourg-CNRS- INSERM	France	herault@igbmc.fr
Hinttala	Reetta	University of Oulu	Finland	reetta.hinttala@oulu.fi
Holá	Hana	Czech Centre for Phenogenomics	Czech Republic	hana.hola@img.cas.cz
Hölter-Koch	Sabine	Helmholtz Munich	Germany	sabine.hoelter-koch@helmholtz-munich.de
Huijuan	Guo (Cherry)	Novogene Europe	Germany	huijuan.guo@novogene-europe.com
Huylebroeck	Danny	Erasmus Univ Med Ctr Rotterdam	Netherlands	d.huylebroeck@erasmusmc.nl
Chalupsky	Karel	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	karel.chalupsky@img.cas.cz
Jansa	Petr	Institute of Molecular Genetics	Czech Republic	pjansa@img.cas.cz
Jeong	Eunyoung	KMPC	Republic of Korea	eyjeong.kmpc@snu.ac.kr
Jiménez	Jesús	Universitat Autònoma de Barcelona-CBATEG	Spain	jesus.jimenez@uab.cat
Josková	Markéta	Biocev	Czech Republic	marketa.joskova@img.cas.cz

Kaliaeva	Marta	Czech Academy of Sciences	Czech Republic	marta.kaliaeva@img.cas.cz
Kanásová	Katarína	Ústav molekulární genetiky AV ČR, v. v. i.	Czech Republic	katarina.kanasova@img.cas.cz
Karmakar	Shreyoshi	Institute of Molecular Genetics of the Czech Academy of Science	Czech Republic	shreyoshi.karmakar@img.cas.cz
Kašík	Petr	DIANA Biotechnologies a.s.	Czech Republic	petr.kasik@dianabiotech.com
Kerényi	Farkas	CCP	Czech Republic	farkas.kerenyi@img.cas.cz
Kertisová	Anna	UOCHB AV ČR	Czech Republic	anna.kertisova@uochb.cas.cz
Khadour	Ahmad	Universitätsklinikum Ulm	Germany	ahmad.khadour@uni-ulm.de
Kim	Eun Young	Ajou University, School of Medicine	Republic of Korea	ekim@ajou.ac.kr
Kim	Kyoungmi	Korea University College of Medicine	Republic of Korea	kim0912@korea.ac.kr
Kinská	Barbora	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	barbora.kinska@img.cas.cz
Kircher	Martin	Universität zu Lübeck/UKSH	Germany	martin.kircher@bih-charite.de
Klajblová	Květoslava	CCP, IMG	Czech Republic	klajblov@img.cas.cz
Klíma	Kryštof	Ústav molekulární genetiky AV ČR, v. v. i.	Czech Republic	klimak@img.cas.cz
Ko	Hyuk Wan	Yonsei University	Republic of Korea	kohw@yonsei.ac.kr
Kolesová	Hana	First Faculty of Medicine	Czech Republic	hana.kolesova@lf1.cuni.cz
Kolihová	Anna	CCP	Czech Republic	anna.kolihova@img.cas.cz
Kopkan	Libor	Institute of Molecular Genetics of CAS- Czech Centre for Phenogenomics	Czech Republic	libor.kopkan@img.cas.cz
Kosla	Piotr	PACS2 Research Foundation	Poland	piotrkosla@gmail.com
Kostohryz	Viktor	CEITEC	Czech Republic	499598@mail.muni.cz
Koubkova	Gizela	Institute of Molecular Genetics AS CR	Czech Republic	gizela@img.cas.cz
Kowalczyk	Katarzyna	Bio-Rad Sp. z o.o., 9510028953	Poland	katarzyna_kowalczyk@bio-rad.com
Kowalczyk	Tomasz	Medical University of Bialystok	Poland	tomasz.kowalczyk@umb.edu.pl
Kraus	Daniel	Institute of Molecular Genetics	Czech Republic	daniel.kraus@img.cas.cz
Kriška	Ján	Institute of Experimental Medicine of the CAS	Czech Republic	jan.kriska@iem.cas.cz
Krizova	Kamila	Ústav molekulární genetiky AV ČR, v. v. i.	Czech Republic	kamila.krizova@img.cas.cz
Krupková	Michaela	IMG	Czech Republic	michaela.krupkova@img.cas.cz
Kucera	Lukas	Czech Centre for Phenogenomics	Czech Republic	lukas.kucera@img.cas.cz
Kučerová	Daniela	Ústav molekulární genetiky AV ČR, v. v. i.	Czech Republic	daniela.kucerova@img.cas.cz
Kuehn	Ralf	Max delbrueck center	Germany	ralf.kuehn@mdc-berlin.de
Labaj	Juraj	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	juraj.labaj@img.cas.cz
Lainscek	Dusko	National institute of chemistry	Slovenia	Dusko.lainscek@ki.si
Lanoue	Louise	The Mouse Biology Program @ UC-Davis	United States of America	llanoue@ucdavis.edu
Larqué	Pauline	Radboudumc	Netherlands	PaulineSophie.Larque@radboudumc.nl
Lee	Hyunji	Korea University College of Medicine	Republic of Korea	hjlee102@korea.ac.kr
Lee	Ho	National Cancer Center, Korea	Republic of Korea	ho25lee@gmail.com
LEE	Nara	K-MEDI hub	Republic of Korea	domdomi101@kmedihub.re.kr
León	Xavier	Universitat Autònoma de Barcelona	Spain	xavier.leon@uab.cat
Li	Jun	IOCB	Czech Republic	li.jun@uochb.cas.cz
Lindovsky	Jiri	CCP, IMG	Czech Republic	jiri.lindovsky@img.cas.cz
Lipka	Jakub	Czech Centre for Phenogenomics (BIOCEV / IMG)	Czech Republic	jakub.lipka@img.cas.cz
Liu	Zhenni	Soochow University	China	1104946735@qq.com

Lloyd	Kent	University of California Davis	United States of America	kclloyd@ucdavis.edu
Löffler	Jessica	Uniklinik Ulm	Germany	jessica.loeffler@uni-ulm.de
Lukasiewicz	Kacper	Medical University of Bialystok	Poland	kacper.lukasiewicz@gmail.com
Lutz	Cathleen	The Jackson Laboratory	United States of America	Joyce.Billings@jax.org
Macková	Pavlína	CCP	Czech Republic	pavlina.mackova@img.cas.cz
Machalová	Eliška	BIOCEV – Institute of Molecular Genetics	Czech Republic	eliska.machalova@img.cas.cz
Mandillo	Silvia	CNR- Consiglio Nazionale delle Ricerche	Italy	silvia.mandillo@cnr.it
Marcó Costa	Sara	Universitat Autònoma de Barcelona	Spain	sara.marco@uab.cat
Martásek	Pavel	BIOCEV	Czech Republic	pavel.martasek@ibt.cas.cz
Martínková	Veronika	IMG	Czech Republic	veronika.martinkova@img.cas.cz
Mastrangeli	Antonia	CCP	Czech Republic	antonia.mastrangeli@img.cas.cz
Mihola	Ondrej	IMG	Czech Republic	mihola@img.cas.cz
Michalikova	Csilla	Institute of Molecular Genetics of CAS	Czech Republic	csilla.michalikova@img.cas.cz
Miklovicova	Sona	Institute of Biotechnology, Czech Academy of Sciences	Czech Republic	sona.miklovicova@ibt.cas.cz
Mirochnitchenko	Oleg	National Institutes of Health	United States of America	oleg.mirochnitchenko@nih.gov
Miroševič	Špela	CTNNB1 Foundation	Slovenia	spela@ctnnb1-foundation.org
Molas	Maria	Universitat Autònoma de Barcelona	Spain	maria.molas@uab.cat
Mrázková	Lucie	IOCB Prague CAS, Faculty of Science Charles University	Czech Republic	mrazkovluc@natur.cuni.cz
Murray	Steve	The Jackson Laboratory	United States of America	steve.murray@jax.org
Musil	Ing. Martin	TRIGON PLUS s.r.o.	Czech Republic	musil@trigonplus.cz
Neffeová	Kristýna	Institute of Anatomy Charles University, First Faculty of Medicine	Czech Republic	Kristyna.Neffeova@lf1.cuni.cz
Nehrych	Mariia	CCP	Czech Republic	mariia.nehrych@img.cas.cz
Nekvindová	Eva	Czech Centre for Phenogenomics, IMG CAS	Czech Republic	eva.nekvindova@img.cas.cz
Nemesh	Kateryna	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	kateryna.nemesh@img.cas.cz
Netušil	Jiří	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	jiri.netusil@img.cas.cz
Nickl	Petr	Institute of Molecular Genetics	Czech Republic	petr.nickl@img.cas.cz
Nouri	Farah	IMG	Czech Republic	nourisaf@natur.cuni.cz
Novais	Silvia	Institute molecular genetics of Czech Academy of Sciences	Czech Republic	silvia.novais@img.cas.cz
Novakova	Jana	Biorad	Czech Republic	jana_novakova@bio-rad.com
Nováková	Petra	INSTITUTE OF MOLECULAR GENETICS, CCP	Czech Republic	petra.novakova@img.cas.cz
Novosadova	Vendula	Institute of Molecular Genetics	Czech Republic	vendula.novosadova@img.cas.cz
Ogan	Betul	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	betul.ogan@img.cas.cz
Pajuelo Reguera	David	Czech Centre for Phenogenomics - IMG, Czech Academy of Sciences	Czech Republic	david.pajuelo-reguera@img.cas.cz
Paliwal	Neeraj	KPJ Healthcare University	Malaysia	neerajpaliwal.007@gmail.com
Pálková	Marcela	Institute of Molecular Genetics - Czech Centre for Phenogenomics	Czech Republic	marcela.palkova@img.cas.cz
PAN	DEJING	Soochow University	China	pandejing@suda.edu.cn
Park	Jun Won	Seoul National University	Republic of Korea	labanimal@snu.ac.kr
PARK	Changyeon	KMPC	Republic of Korea	cy.kmpc@snu.ac.kr

Pavelková	Eliška	Masarykova univerzita, Lékařská fakulta	Czech Republic	eliskapavelkova20@gmail.com
Pilařová	Anna	Ústav molekulární genetiky AV ČR, v. v. i.	Czech Republic	pilarova@img.cas.cz
Pincová Silberová	Petra	VELAZ, s.r.o.	Czech Republic	silberova@velaz.cz
Poonawala	Sakina	Institute of physiology	Czech Republic	sakina.poonawala@fgu.cas.cz
Popr	Martin	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	popr@img.cas.cz
Prex	Vivien	Ulm University Medical Center	Germany	vivien.prex@uni-ulm.de
Price	Elliott J.	Masaryk University	Czech Republic	elliott.price@recetox.muni.cz
Proft	Julie	IOCB	Czech Republic	proft@uochb.cas.cz
Prochazka	Jan	Instittute of Molecular Genetics	Czech Republic	jan.prochazka@img.cas.cz
Prochazkova	Michaela	Institute of Molecular Genetics, AS CR	Czech Republic	michaela.prochazkova@img.cas.cz
Pyko	Olha	IMG CCP	Czech Republic	olha.pyko@img.cas.cz
Pysanenko	Kateryna	Czech Centre for Phenogenomics BIOCEV – Institute of Molecular Genetics	Czech Republic	kateryna.pysanenko@img.cas.cz
Raess	Michael	INFRAFRONTIER ERIC	Germany	michael.raess@infrafrontier.eu
Raishbrook	Miles	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	miles.raishbrook@img.cas.cz
Rajendiran	Vignesh	University of Oulu	Finland	vignesh.rajendiran@oulu.fi
Rapaka	Naimisha	Institute of Molecular Genetics	Czech Republic	naimisha.rapaka@img.cas.cz
Rülicke	Thomas	University of Veterinary Medicine Vienna	Austria	thomas.ruelicke@vetmeduni.ac.at
Ryu	Ji-Hwan	Yonsei University	Republic of Korea	yjh@yuhs.ac
Sabatini	David	IOCB	Czech Republic	david.sabatini@uochb.cas.cz
Sacristán	Víctor	Universitat Autonoma de Barcelona	Spain	victor.sacristan@uab.cat
Salem	Ahmed	UPCE	Czech Republic	ahmedahmedahmed.salem@student.upce.cz
Salo	Miia	University of Oulu	Finland	miia.h.salo@oulu.fi
Sedlacek	Radislav	Institute of Molecular Genetics CAS	Czech Republic	radislav.sedlacek@img.cas.cz
SEONG	JE KYUNG	Seoul National University & KMPC	Republic of Korea	snumouse@snu.ac.kr
Setz	Corinna	Ulm University Medical Center	Germany	corinna.setz@uni-ulm.de
Shaikh	Mehak	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	mehak.shaikh@img.cas.cz
Shcherbak	Oksana	Institute of Animal Breeding and Genetics nd. a. M.V.Zubets of National Academy of Agrarian Science	Ukraine	ov19792006@gmail.com
SHibuya	Hirotoshi	RIKEN BioResource Research Center	Japan	hirotoshi.shibuya@riken.jp
Scheidhauer	Ellen	Uniklink Ulm Nuklearmedizin	Germany	ellen.scheidhauer@uniklinik-ulm.de
Schmoranz	Michal	Institute of molecular genetics ASCR	Czech Republic	michal.schmoranz@img.cas.cz
Smith-Prance	Joel	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	jsmithprance@outlook.com
Sorg	Tania	PHENOMIN- ICS	France	tsorg@igbmc.fr
Stará	Markéta	DIANA Biotechnologies	Czech Republic	marketa.stara@dianabiotech.com
Starosta	Jakub	Fisher Scientific, spol. s r.o.	Czech Republic	jakub.starosta@thermofisher.com
Suchanova	Sarka	Ústav molekulární genetiky AV ČR, v. v. i.- Centrum BIOCEV	Czech Republic	sarka.suchanova@img.cas.cz
Sürer	Ebra Ece	Kastamonu University	Turkey	ecesurer11@gmail.com
Svirska	Sofiia	IOCB	Czech Republic	sofiia.svirska@uochb.cas.cz

Šafránková	Jana	The Czech Centre for Phenogenomics at the Institute of Molecular Genetics	Czech Republic	jana.safrankova@img.cas.cz
Šímová	Michaela	Czech Centre for Phenogenomics	Czech Republic	michaela.simova@img.cas.cz
Šišková	Ingrid	Life Technologies Czech Republic s.r.o.	Czech Republic	ingrid.siskova@thermofisher.com
Špoutil	František	Institute of Molecular Genetics, CAS v.v.i.	Czech Republic	spoutil@img.cas.cz
Štefancová	Eva	CCP	Czech Republic	eva.stefancova@img.cas.cz
Tanieli	Giordano	Institute of Molecular Genetics of the CAS	Czech Republic	giordano.tanieli@img.cas.cz
Tomović	Eni	INSTITUTE OF PHYSIOLOGY CAS	Czech Republic	eni.tomovic@gmail.com
Trachtulec	Zdenek	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	zdenek.trachtulec@img.cas.cz
Tureckova	Jolana	Institute of Molecular Genetics/CCP	Czech Republic	turejo@img.cas.cz
Turon Lopez	Sandra	Universitat Autonoma de Barcelona	Spain	sandra.turon@uab.cat
Uhrová	Adéla	Institute of Molecular Genetics of the Czech Academy of Sciences- Czech Centre for Phenogenomics	Czech Republic	adela.uhrova@img.cas.cz
Vale	Matilde	Institute of Molecular Genetics (IMG)	Czech Republic	ana-matilde.vale@img.cas.cz
Valentová	Anna	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	anna.valentova@img.cas.cz
Varga	Igor	https://www.phenogenomics.cz/	Czech Republic	varhai@img.cas.cz
Vikhrova	Elena	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	elena.vikhrova@img.cas.cz
Vyklicky	Ladislav	Institute of Physiology CAS	Czech Republic	Ladislav.Vyklicky@fgu.cas.cz
Ward	Christopher	Baylor College of Medicine	United States of America	christow@bcm.edu
Wendlinger	Sarah	Charité Universitätsmedizin Berlin	Germany	sarah.wendlinger@charite.de
Wirth	Mareike	Ulm University Medical Center	Germany	mareike.wirth@uni-ulm.de
xu	ji	Soochow University	China	2308807520@qq.com
Zaugg	Judith	University of Basel	Switzerland	judith.zaugg@unibas.ch
Żbikowski	Arkadiusz	Medical University of Bialystok	Poland	arkadiusz.zbikowski@umb.edu.pl
Zdolsek Draksler	Tanja	Jožef Stefan Institute	Slovenia	tanja.zdolsek@ijs.si
Zelená	Kateřina	Institute of Molecular Genetics of the Czech Academy of Sciences	Czech Republic	katerina.zelena@img.cas.cz
Zeni	Pedro	CEITEC MU	Czech Republic	pedrozeni992@gmail.com

Local organizing committee:

Scientific committee:
Radislav Sedláček (chair)
Jan Procházka

Administration:
Lenka Červená
Petra Nováková

CONTACT
Czech Centre for Phenogenomics
Institute of Molecular Genetics of the Czech Academy of Sciences
BIOCEV campus
Prumyslova 595, 252 50 Vestec
Czech Republic

www.phenogenomics.cz
ccp@phenogenomics.cz
X.com (Twitter) CZphenogenomics

6th CCP Phenogenomics Conference 2024
www.ccp-conference.cz