4th CCP Phenogenomics Conference 2022

ABSTRACT BOOK

Hybrid Conference

15–16 September 2022

IMG

Czech Centre for Phenogenomics

BIOCEV

MINISTRY OF EDUCATION, YOUTH AND SPORTS

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We thank to all our supporters and sponsors for their contribution to the Conference

Seeing beyond

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Content

Dear Colleagues,

It is my great pleasure to invite you to the fourth CCP Phenogenomics Conference. The conference is held as a hybrid meeting in 2022. We have returned to BIOCEV campus in Vestec near Prague, where the Czech Centre for Phenogenomics is located, but we also want to give opportunity to join the conference on-line for those who cannot participate physically.

The scientific committee has selected the topic of rare diseases: experimental models & delivery of therapies as the main thematic focus of this year Conference.

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.

We are happy to meeting you again either in Vestec or virtually at CCP annual conference.

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 a number of international scientific projects.

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ACKNOWLEDGEMENTS:

MINISTRY OF EDUCATION, YOUTH AND SPORTS

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Operational Programme Research, Development and Education
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The Czech Academy of Sciences
Strategy AV21
Top research in the public interest

Strategy AV21 of the Czech Academy of Sciences responds to current social challenges through a sophisticated formulation of research programmes, based on cooperation of scientific fields and institutions. In January 2022, a new research programme "Towards precision medicine and gene therapy" coordinated by the Institute of Molecular Genetics of the Czech Academy of Sciences was approved.

Thursday 15 September 2022		
9:00 – 10:00	Registration	
SESSION 1 — OPENING + RARE DISEASES & MODELS I		
10:00 – 10:20	Welcoming lecture	Radislav Sedláček, Institute of Molecular Genetics Czech Academy of Sciences, Czech Republic
10:20 – 11:00	Keynote lecture – Developmental cues as a foundation for therapies	Ophir Klein, University of California, San Francisco, USA
SESSION 2 — RARE DISEASES & MODELS I		
11:00 – 11:20	Diamond-Blackfan anaemia model to understand the molecular pathology of the disease development	Tomas Stopka, First Faculty of Medicine, Charles University, Czech Republic
11:20 – 11:40	Efl1 mutant mice recapitulate Shwachman-Diamond syndrome	Murim Choi, Seoul National University College of Medicine, Korea
11:40 – 12:00	Models and preclinical gene therapy program in familial hypercholesterolemia	Seppo Ylä-Herttuala, University of Eastern Finland, Finland
12:00 – 12:20	Discussion with speakers	
12:20 – 13:30	**Lunch break**	
SESSION 3 — RARE DISEASES & THERAPY DELIVERY		
13:30 – 13:50	mTOR inhibitors alleviate proteinopathy resulting from OSBPL2 mutations	Heon Yung Gee, Yonsei University of College of Medicine, Korea
13:50 – 14:10	The same lentiviral platform for efficient DNA or RNA delivery	Florine Samain, Flash Therapeutics, France
14:10 – 14:30	Exosomes for CNS therapy	Saara Laitinen, Finnish Red Cross Blood Service, Finland
14:30 – 14:50	Discussion with speakers	
POSTER SESSION 1 — ON-SITE INCLUDING COFFEE BREAK		
14:50 – 16:00		
15:00 – 16:00	Satellite event: IVIM – IntraVital Microscopy Workshop	

SESSION 4 — SHORT AND TECHNOLOGY TALKS		
16:00 – 16:15	Stress reduction & catheter patency during infusion & blood sampling in rodents	Thomas Penning, Instech Laboratories, Germany
16:15 – 16:30	HaloTag and NanoBRET – Two Fusion Tag-based Technologies for Cellular Protein Analysis	Vojtech Ledvina, East Port Life Sciences, Czech Republic
16:30 – 16:45	Modern microscopy – high resolution, sensitivity, speed and easy to use	Pavel Krist, Carl Zeiss spol. s r.o., Czech Republic
16:45 – 16:55	Leveraging fully automated, highly multiplexed single-cell functional proteomics	Sara Bragado Alonso, IsoPlexis, Germany
16:55 – 17:05	Spatial Biology: multiplexed protein imaging and quantitation to understand and combat disease	Selena Larkin, RareCyte Inc., United States
18:00 – 21:00	Informal dinner	

Friday 16 September 2022	
8:30 – 9:30	Satellite event: IVIM — IntraVital Microscopy Workshop

SESSION 5 — RARE DISEASES & MODELS II		
9:30 – 9:50	Phenotype-based diagnostics and discovery in rare disease	Damian Smedley, Queen Mary University of London, United Kingdom
9:50 – 10:10	Geno-phenotype discoveries of rare diseases among the Finns	Reetta Hinttala, University of Oulu, Finland
10:10 – 10:30	Finding the genetic causes of chronic kidney disease	Stanislav Kmoch, First Faculty of Medicine, Charles University, Czech Republic
10:30 – 10:50	Discussion with speakers	
10:50 – 11:20	**Coffee break**	

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SESSION 6 — RARE DISEASES & NON-CODING ELEMENTS		
11:20 – 12:00	Keynote lecture In Vivo Studies of Human Genome Function	Len Pennacchio, Lawrence Berkeley National Laboratory, USA
12:00 – 12:30	Functional characterization and therapeutic targeting of gene regulatory elements	Nadav Ahituv, University of California, San Francisco, USA
12:30 – 12:50	The application of SuRE, a massive parallel reporter assay, for the identification of gene regulatory elements and their functional sequence variants	Joris van Arensbergen, Annogen B.V., Netherlands
12:50 – 13:10	Discussion with speakers	
13:10 – 14:10	**Lunch break**	
SESSION 7 — SHORT TALKS SELECTED FROM POSTER PRESENTATIONS		
14:10 – 14:20	An update on disease models from the IMPC: Can we identify which features impact the ability of mouse knockouts to recapitulate human phenotypes?	Pilar Cacheiro, William Harvey Research Institute, Queen Mary University of London, UK
14:20 – 14:30	An antibody gene transfer approach for therapeutic treatment of rare genodermatoses in a mouse model of KID syndrome	Chiara Peres, Institute of Biochemistry and Cell Biology, Italian National Research Council, Italy
14:30 – 14:40	Modelling of a rare mutation in the GALNT3 gene found in patient with teeth and bone defects using genetically engineered mice	Jan Krivanek, Faculty of Medicine, Masaryk University, Czech Republic
14:40 – 14:50	Application of Extracellular Vesicles associated with Adeno-Associated Virus in transgenesis	Petr Nickl, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
14:50 – 15:10	Discussion with speakers	
SESSION 8 — CLOSING		
15:10 – 15:20	Closing remarks	Radislav Sedlacek, Institute of Molecular Genetics, Czech Academy of Sciences, Czech Republic
POSTER SESSION 2 — ONLINE		
15:20 – 17:00		

Ophir David Klein, MD, PhD

"Developmental cues as a foundation for therapies"

University of California, San Francisco, USA

Ophir Klein serves as the inaugural executive director of Cedars-Sinai Guerin Children's and the David and Meredith Kaplan Distinguished Chair in Children's Health. He is also Adjunct Professor of Orofacial Sciences and Pediatrics at the University of California, San Francisco (UCSF), where he was previously the Larry L. Hillblom Distinguished Professor in Craniofacial Anomalies and the Charles J. Epstein Professor of Human Genetics. Until 2022, he served as Director of the Institute for Human Genetics, Chief of the Division of Medical Genetics, Chair of the Division of Craniofacial Anomalies, and Director of the Program in Craniofacial Biology at UCSF. Dr. Klein was educated at the University of California, Berkeley, where he earned a B.A. in Spanish Literature. He subsequently attended Yale University School of Medicine, where he received a Ph.D. in Genetics and an M.D. He then completed residencies at Yale-New Haven Hospital in Pediatrics and at UCSF in Clinical Genetics. Dr. Klein has received several honors, including a New Innovator Award from the NIH and the E. Mead Johnson Award from the Society for Pediatric Research. Dr. Klein was elected to the American Society for Clinical Investigation, the American Association of Physicians and the National Academy of Medicine, and he is a Fellow of the American Association for the Advancement of Science. Dr. Klein's research focuses on understanding how organs form in the embryo and how they regenerate in the adult, with a particular emphasis on the processes underlying craniofacial and dental development and renewal as well as understanding how stem cells in the intestinal epithelium enable renewal and regeneration.

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Len Pennacchio, Ph.D.

"In Vivo Studies of Human Genome Function"

Lawrence Berkeley National Laboratory, University of California, USA

Dr. Len Pennacchio is a Senior Scientist at the Lawrence Berkeley Laboratory (LBL), Deputy Director of the DOE Joint Genome Institute, and Adjunct Professor at the University of California Berkeley. He received his PhD in Genetics from Stanford University in 1998 under Rick Myers and then served as a DOE Alexander Hollaender Distinguished Fellow at LBL under Eddy Rubin. He has authored over 170 publications and received the Presidential Early Career Award for Scientists and Engineers (PECASE) from the White House for his contributions to the Human Genome Project and understanding mammalian gene regulation in vivo. Dr. Pennacchio has an extensive background in mammalian genetics and genomics as well as with DNA sequencing technologies and their application to address outstanding issues in both the biomedical, energy, and environment sectors. He serves in numerous advisory roles such as NHGRI's National Council, NHGRI's Genome Sequencing Program, the Centre for Genomic Research at the University of Liverpool, as a permanent member of NIH's GCAT Study Section. He also is an Organizer and Co-Chair of three separate annual Advances in Genome Biology & Technology (AGBT) meetings as well as a "Systems Biology of Gene Regulation and Genome Editing" meeting hosted by Cold Spring Harbor Asia. Currently his research is heavily focused on understanding the spectrum of DNA mutations that contribute to human disease through in vivo functional studies.

KEYNOTE LECTURES

- Thursday, 15 September 2022 (10:20 – 11:00) **Ophir Klein,** University of California, San Francisco, USA
  Developmental cues as a foundation for therapies
- Friday, 16 September 2022 (11:20 – 12:00) **Len Pennacchio,** Lawrence Berkeley National Laboratory, USA
  In Vivo Studies of Human Genome Function

SESSION 2 – RARE DISEASES & MODELS I

Thursday 15 September 2022 (11:00 – 12:20)

- 11:00 – 11:20 **Tomas Stopka,** First Faculty of Medicine, Charles University, Czech Republic
  Diamond-Blackfan anaemia model to understand the molecular pathology of the disease development
- 11:20 – 11:40 **Murim Choi,** Seoul National University College of Medicine, Korea
  Efl1 mutant mice recapitulate Shwachman-Diamond syndrome
- 11:40 – 12:00 **Seppo Ylä-Herttuala,** University of Eastern Finland, Finland
  Models and preclinical gene therapy program in familial hypercholesterolemia

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Efl1 mutant mice recapitulate Shwachman-Diamond syndrome

- **Murim Choi [1]**

1. Department of Biomedical Sciences, Seoul National University College of Medicine

E-mail of the presenting author: murimchoi@snu.ac.kr

In many cases, mouse models successfully represent human diseases. Shwachman-Diamond syndrome (SDS; OMIM: #260400) is caused by variants in SBDS (Shwachman-Bodian-Diamond syndrome gene), which encodes a protein that plays an important role in ribosome assembly. Recent reports suggest that recessive variants in EFL1 are also responsible for SDS. However, the precise genetic mechanism that leads to EFL1-induced SDS remains incompletely understood. Here we present three unrelated Korean SDS patients that carry biallelic pathogenic variants in EFL1 with biased allele frequencies, resulting from a bone marrow-specific somatic uniparental disomy (UPD) in chromosome 15. The recombination events generated cells that were homozygous for the relatively milder variant, allowing for the evasion of catastrophic physiological consequences. Still, the milder EFL1 variant was solely able to impair 80S ribosome assembly and induce SDS features in cell line and animal models. Mouse lines carrying null or point mutation were generated and used to compare phenotype that resemble that of SDS. The loss of EFL1 resulted in a pronounced inhibition of terminal oligo-pyrimidine element-containing ribosomal protein transcript 80S assembly. Therefore, we propose a more accurate pathogenesis mechanism of EFL1 dysfunction that eventually leads to aberrant translational control and ribosomopathy.

Models and preclinical gene therapy program for familial hypercholesterolemia

- **Seppo Yla-Herttuala [2]**

1. Seppo Yla-Herttuala
2. A.I.VIrtanen Institute, University of Eastern Finland

E-mail of the presenting author: seppo.ylaherttuala@uef.fi

Familial hypercholesterolemia (FH) is a monogenic disease caused by a mutation in the high-affinity receptor for low density lipoprotein (LDL). FH leads to high levels of LDL and premature coronary-artery disease (CAD) regardless of conventional drug therapy.

We have evaluated efficacy and safety of gene therapy for the treatment of FH by vectors capable of expressing transgenes for long periods of time. After studies in knock-out mice, we have used an animal model of human FH, the Watanabe Heritable Hyperlipidemic Rabbit (WHHL) for intraportal gene transfers of lentiviral (LV) and adeno-associated virus (AAV) vectors carrying the rabbit low-density lipoprotein receptor (rLDLR) under a liver-specific promoter in an attempt to reduce very high LDL levels. Bioreactor-based large-scale methods were used to manufacture both types of vectors at clinical GMP grade.

Gene transfer of rLDLR with LV led to a significant, long-lasting reduction in serum total cholesterol and LDL levels with no significant side-effects. Comparison of LV-rLDLR gene transfer to AAV2-and AAV9 intraportal gene transfers highlighted the efficiency and good safety profile of the LV-rLDLR compared to the AAV vectors. A surprising finding of a significant bile-duct proliferation one year after the AAV2-rLDLR gene transfer was seen and was associated with an increased expression of the matricellular protein Cyr61 in the liver. It appears that the bile-duct proliferation is induced by a specific combination of the vector and the transgene. The finding highlights the need for further long-term studies with AAV vectors to elucidate their efficacy, safety and possible long-term effects in the liver.

In conclusion, the intraportal delivery of LV carrying LDLR reduces total cholesterol levels more efficiently than AAV2 or AAV9 suggesting that it is a feasible approach for the treatment of homozygous human FH.

Reference: Hytönen E, Laurema A, Kankkonen H, Miyanohara A, Kärjä V, Hujo M, Laham-Karam N, Ylä-Herttuala S. Bile-duct proliferation as an unexpected side-effect after AAV2-LDLR gene transfer to rabbit liver. **Sci Rep.** 9: 6934, 2019.

Leinonen HM, Lepola S, Lipponen EM, Heikura T, Koponen T, Parker N, Ylä-Herttuala S, Lesch HP. Benchmarking of Scale-X Bioreactor System in Lentiviral and Adenoviral Vector Production. **Hum Gene Ther**. 31:376-384, 2020.

SESSION 3 — RARE DISEASES & THERAPY DELIVERY

Thursday 15 September 2022 (13:30 – 14:50)

- 13:30 – 13:50 **Heon Yung Gee,** Yonsei University of College of Medicine, Korea
  mTOR inhibitors alleviate proteinopathy resulting from OSBPL2 mutations
- 13:50 – 14:10 **Floraine Samain,** Flash Therapeutics, France
  The same lentiviral platform for efficient DNA or RNA delivery
- 14:10 – 14:30 **Saara Laitinen,** Finnish Red Cross Blood Service, Finland
  Exosomes for CNS therapy

mTOR inhibitors alleviate proteinopathy resulting from OSBPL2 mutations

- **Heon Yung Gee [1]**

1. Department of Pharmacology, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul 03722, Republic of Korea

E-mail of the presenting author: hygee@yuhs.ac

Intracellular accumulation of mutant proteins causes proteinopathies, which lack targeted therapies. Autosomal dominant hearing loss (DFNA67) is caused by frameshift mutations in OSBPL2. Here, we show that DFNA67 is a toxic proteinopathy. Mutant OSBPL2 accumulated intracellularly and transgenic mice overexpressing it exhibited hearing loss, but Osbpl2 knockout mice did not. Mutant OSBPL2 bound to autophagy proteins; the accumulation of mutant OSBPL2 led to defective endolysosomal homeostasis and impaired autophagy. Rapamycin decreased the accumulation of mutant OSBPL2 and partially rescued the hearing loss phenotype in mice. Our findings implicate dysfunctional autophagy by mutant proteins in DFNA67 and recommend rapamycin for DFNA67.

SESSION 4 — SHORT AND TECHNOLOGY TALKS

Thursday 15 September 2022 (16:00 – 17:05)

- 16:00 – 16:15 **Thomas Penning,** Instech Laboratories
  Stress reduction & catheter patency during infusion & blood sampling in rodents
- 16:15 – 16:30 **Vojtech Ledvina,** East Port Life Sciences
  HaloTag and NanoBRET – Two Fusion Tag-based Technologies for Cellular Protein Analysis
- 16:30 – 16:45 **Pavel Krist,** Carl Zeiss spol. s r.o.
  Modern microscopy – high resolution, sensitivity, speed and easy to use
- 16:45 – 16:55 **Sara Bragado Alonso,** IsoPlexis, Germany
- 16:55 – 17:05 **Selena Larkin,** RareCyte Inc., United States
  Spatial Biology: multiplexed protein imaging and quantitation to understand and combat disease

Stress Reduction & Catheter Patency During Infusion & Blood Sampling in Rodents

- **Thomas Penning [1]**

1. Director of Sales Europe at Instech Laboratories, Inc.
2. Foundation Member of the European Academy of Laboratory Animal Surgery (EALAS)

E-mail of the presenting author: tpenning@instechlabs.com

Stress induction is a common issue when drugs have to be injected and results need to be monitored by analyzing blood concentrations. Not only an animal welfare issue, stress can have a direct impact on study results and lead to false conclusions.

This talk will cover blood sampling and infusion practices in rodents. The advantages and disadvantages of vessel catheterization as one possibility of stress reduction will be discussed when repeated blood sampling, intermittent or continuous infusion are study requirements. The talk will also address the surgical implementation of Vascular Access Buttons™ and their role in increasing catheter patency.

INSTECH

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Modern microscopy - high resolution, sensitivity, speed and easy to use

- **Pavel Krist [1]**

1. ZEISS Research Microscopy Solutions

E-mail of the presenting author: pavel.krist@zeiss.com

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Spatial Biology: multiplexed protein imaging and quantitation to understand and combat disease

- **Selena Larkin [1]**

1. RareCyte Inc.

E-mail of the presenting author: slarkin@rarecyte.com

Multiplexed immunofluorescent microscopy affords the study of cell architecture and state, disease markers, potential drug targets and host immune response.

We will describe how quantitative, subcellular measurement of up to 17 protein biomarkers across multiple whole-slide samples per day has been used to optimize biomarker panels for large-scale translational and clinical studies. Examples will include healthy tissue and a range of solid tumors and immune microenvironments.

- **Whole-slide** imaging of FFPE and frozen tissue sections on standard glass slides
- Simultaneous acquisition of **quantitative data** for **12+ markers at subcellular resolution**
- Rapid, **single-round staining and imaging** using standard IHC protocols
- **Flexible panel design** with commercially available antibodies and labeling kits
- Brightfield imaging, enabling **same-section H&E**

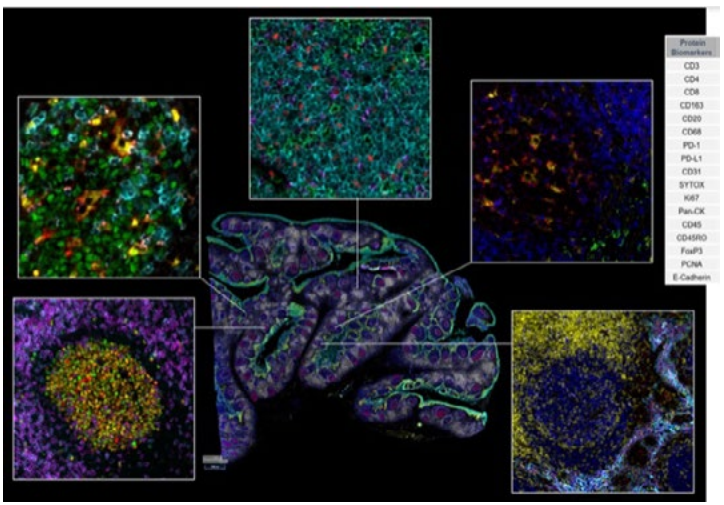

Fig. 1: 17-plex, whole-slide tonsil section. 0.5 µm resolution @ 70 mins / cm2 across all channels

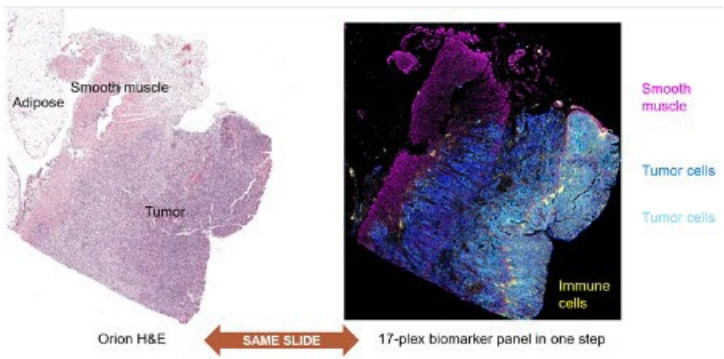

Fig. 2: Same-slide, subcellular resolution immunofluorescent and H&E staining supports machine-learning approaches to enhanced digital pathology

SESSION 5 — RARE DISEASES & MODELS II

Friday 16 September 2022 (9:30 – 10:50)

- 9:30 – 9:50 **Damian Smedley,** Queen Mary University of London, United Kingdom
  Phenotype-based diagnostics and discovery in rare disease
- 9:50 – 10:10 **Reetta Hinttala,** University of Oulu, Finland
  Geno-phenotype discoveries of rare diseases among the Finns
- 10:10 – 10:30 **Stanislav Kmoch,** First Faculty of Medicine, Charles University, Czech Republic
  Finding the genetic causes of chronic kidney disease

Phenotype-based diagnostics and discovery in rare disease

- **Damian Smedley [1]**

1. William Harvey Research Institute, Queen Mary University of London

E-mail of the presenting author: d.smedley@qmul.ac.uk

Professor Damian Smedley leads a Computational Genomics team at Queen Mary University London where his research focusses on the use of phenotype data to obtain novel insights into disease causes and mechanisms. His team is involved in translational aspects for a number of projects such as the International Mouse Phenotyping Consortium (IMPC). In collaboration with other members of the Monarch Initiative he has developed tools that utilise phenotype comparisons for candidate gene prioritisation, particularly for whole genome sequence interpretation of rare disease patients as in the Exomiser software suite. Prof. Smedley served as Director of Genomic Interpretation at Genomics England from 2016-2018 and has led the analysis of the impact of the 100,000 Genomes Project pilot on rare disease diagnosis in healthcare. In this presentation he will present the key findings from all of these projects.

Geno-phenotype discoveries of rare diseases among the Finns

- **Reetta Hinttala [1,2]**

1. Pediatric Neurology, PEDEGO research unit, Faculty of Medicine, University of Oulu, Finland
2. Transgenic and Tissue Phenotyping core facility, Biocenter Oulu, University of Oulu, Finland

E-mail of the presenting author: reetta.hinttala@oulu.fi

Isolated populations have been highly valuable for the discovery and characterization of rare monogenic diseases and their causative genetic variants over the years. Several genetic drift events in the history of Finland have led to an enrichment of certain variants, some of them causative for the Finnish Disease Heritage (FDH). FDH is a group of nearly forty diseases with symptoms ranging from adult-onset mildly disabling, to embryonically lethal. Intellectual disability, visual defects, congenital malformations, bone disorders, hearing loss, metabolic disturbances, epileptic or deteriorating neurological diseases and blood disorders are represented in FDH.

Distinct subpopulations in Finland have maintained the unique repertoire of variants facilitating continuous discovery of novel genotype-phenotype associations. In my talk, I will describe the recent updates on FDH and introduce neurological disorders, which we have initially discovered from paediatric patients in Northern Finland and are now diagnosed worldwide. These include two childhood-onset neurological and multiorgan diseases, which were named according to the typical manifestations and organ-specific findings of the diseases, i.e. FINCA disease (fibrosis, neurodegeneration and cerebral angiomatosis) (MIM# 618278) and HIDEA syndrome (hypotonia, hypoventilation, intellectual disability, dysautonomia, epilepsy, and eye abnormalities) (MIM# 618493). Modifications of the causative genes in cell and animal models have evidently and comprehensively improved our knowledge on these proteins and molecular mechanisms involved in human physiology and pathology. Detailed information on molecular mechanisms behind these diseases will bring along broader understanding of the pathways affected, therefore also aiding understanding of common disease pathologies.

Finding the genetic causes of chronic kidney disease

- **Martina Živná [1], Anthony Bleyer [1,2], Stanislav Kmoch [1,2]**

1. Dept. of Paediatrics and Inherited Metabolic Disorders First Faculty of Medicine, Charles University and General University Hospital in Prague
2. Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, NC, USA

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

Chronic kidney disease (CKD) is complex, clinically heterogeneous disease affecting ~10% population. Origin and course of CKD is determined by genetic and environmental factors. Genetic factors account for 40-75%. Genetic component, genes and their variants, have been increasingly identified, either in families with monogenic forms of CKD or in genome-wide association studies in CKD cohorts. Subsequently, the contribution of rare and common genetic variants in these genes to kidney disease is studied in patients with monogenic CKD and in the general population. At present, ~650 monogenic forms of CKD are known. They are responsible for ~70% of pediatric and ~20% of adult cases. With exception of mutations in PKD1, PKD2, COL4A3-5, UMOD and MUC1 genes, their representation in patient populations is heterogeneous and individually rare. Successful diagnostics and research depends on the motivation, active approach and cooperation of physicians, patients and specialized laboratories. Exome sequencing is basic tool for CKD diagnostics. Depending on the clinical selection it may identify the genetic cause in ~30% of the cases. In unsolved cases we perform whole-genome sequencing and analyze non-coding mutations. A special task presents the analysis of repetitive and homologous regions of the human genome and interpretation of their variability. To determine the causality of most of these variants, we must perform a targeted analysis of body fluids and tissues of patients, or suitable cell and animal models prepared by methods of cell reprogramming or targeted changes in the genome. Consequently, we focus on clinical, molecular and pathophysiologic characterization of individual genetic defects, identification of disease biomarkers, development of specific methods for postnatal, prenatal and preconception diagnosis and identification of potential therapeutic targets and protocols for specific therapeutic approaches.

SESSION 6 – RARE DISEASES & NON-CODING ELEMENTS

Friday 16 September 2022 (11:20 – 13:10)

- 11:20 – 12:00 **Len Pennacchio,** Lawrence Berkeley National Laboratory, USA
  In Vivo Studies of Human Genome Function
- 12:00 – 12:30 **Nadav Ahituv,** University of California, San Francisco, USA
  Functional characterization and therapeutic targeting of gene regulatory elements
- 12:30 – 12:50 **Joris van Arensbergen,** Annogen B.V., Netherlands
  The application of SuRE, a massive parallel reporter assay, for the identification of gene regulatory elements and their functional sequence variants

The application of SuRE, a massive parallel reporter assay, for the identification of gene regulatory elements and their functional sequence variants

- **Joris van Arensbergen [1]**

1. Annogen, Amsterdam Science Park, www.annogen.bio

E-mail of the presenting author: joris@annogen.bio

Gene regulatory elements (GREs) are critical determinants of gene expression and mutations in GREs are thought to be major contributors to human traits and diseases. Therefore, a better functional understanding of GREs could contribute to novel therapies and diagnostics. In addition GREs are of interest to the field of gene- and cell therapy because of their potential to regulate therapeutic gene expression.

Annogen is a spin-off of the Netherlands Cancer Institute focused on the commercial application of the SuRE massive parallel reporter assay. I will give an overview of how we use the screening system to identify and optimize GREs for therapeutic gene expression. Also I will briefly describe how we apply the platform for the identification of functional non-coding sequence variants.

SESSION 7 – SHORT TALKS SELECTED FROM POSTER PRESENTATIONS

Friday 16 September 2022 (14:10 – 15:10)

- 14:10 – 14:20 **Pilar Cacheiro,** William Harvey Research Institute, Queen Mary University of London, UK
  An update on disease models from the IMPC: Can we identify which features impact the ability of mouse knockouts to recapitulate human phenotypes?

- 14:20 – 14:30 **Chiara Peres,** Institute of Biochemistry and Cell Biology, Italian National Research Council, Italy
  An antibody gene transfer approach for therapeutic treatment of rare genodermatoses in a mouse model of KID syndrome

- 14:30 – 14:40 **Jan Krivanek,** Faculty of Medicine, Masaryk University, Czech Republic
  Modelling of a rare mutation in the GALNT3 gene found in patient with teeth and bone defects using genetically engineered mice

- 14:40 – 14:50 **Petr Nickl,** Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
  Application of Extracellular Vesicles associated with Adeno-Associated Virus in transgenesis

An update on disease models from the IMPC: Can we identify which features impact the ability of mouse knockouts to recapitulate human phenotypes?

- **Pilar Cacheiro [1], Hamed Haseli Mashhadi [2], Damian Smedley [1]**

1. William Harvey Research Institute, Queen Mary University of London
2. European Bioinformatics Institute, European Molecular Biology Laboratory

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The data generated through the International Mouse Phenotyping Consortium (IMPC) pipeline is integrated with different human disease resources to perform cross-species phenotype comparisons. The use of standardised vocabularies allows automated phenotype similarity calculations, performed by the PhenoDigm algorithm. Each mammalian phenotype ontology (MP) term is mappped with the corresponding human phenotype ontology (HPO) term, and an overall score is computed, providing a measure of the phenotypic similarity between phenotypes observed in the knockout mice and human disorders.

In the latest IMPC data release (DR17, July 2022), 2,223 mouse genes with a human orthologue associated with a Mendelian disorder have both MP and HPO encoded phenotypes available to perform these computations. For 1,155 (52%) we obtained a PhenoDigm match, reflecting how the IMPC model is able to recapitulate, at least to some extent, the phenotypes observed in humans. For the remaining 1,068 genes (48%) no phenotypic similarity between the mouse knockout and the human disease could be captured by the algorithm, although for some of these IMPC lines phenotyping is still ongoing.

This automated evaluation allows a binary classification of mouse model-human disease pairs into class I if we obtain a PhenoDigm match and class II otherwise. Several attributes of the mouse models and screening pipeline (e.g. life stage, zygosity, viability, traits and measurements evaluated) and disease properties (e.g. disease category, pleiotropy, type of variation) can be used to model the effect on the outcome. Among the preliminary findings from a descriptive analysis, we observed variable matching rates based on disease type, ranging from 41% for dermatological disorders to 75%+ for endocrine disorders. A significantly higher percentage of matches was found for viable genes compared to lethal genes (57% vs 49%). By using this approach, we aim to identify which features impact the ability of IMPC models to recapitulate the patients' phenotypes

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An antibody gene transfer approach for therapeutic treatment of rare genodermatoses in a mouse model of KID syndrome

- **Chiara Peres [1], Caterina Sellitto [2], Chiara Nardin [1], Sabrina Putti [1], Tiziana Orsini [1], Adriana Vitiello [3], Arianna Calistri [3], Ferdinando Scavizzi [1], Marcello Raspa [1], Francesco Zonta [4], Guang Yang [4], Thomas White [2], Fabio Mammano [1,5]**

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Mutations in connexin genes expressed in epidermal keratinocytes cause a variety of rare genodermatoses, ascribed to keratinocyte proliferation and/or differentiation defects that range in severity from increases in skin thickness, to life-threatening and fatal barrier break down. In particular, some pathological connexin 26 (Cx26) variants generate "leaky" or abnormally active hemichannels (HCs) that are causally linked to keratitis-ichthyosisdeafness (KID) syndrome, a devastating disease for which there is no cure[1]. We previously showed that submicromolar concentrations of a human-derived monoclonal antibody (mAb), named abEC1.1, inhibit KID-related leaky Cx26 HCs in vitro[2].

Here, we performed abEC1.1 antibody gene transfer experiments in vivo based on a recombinant AAV vector[3] (AAVmAb) in a well characterized mouse model of KID syndrome[4]. We determined that a single systemic administration of the AAVmAb significantly reduces the visible manifestation of epidermal pathology for up to 4 weeks.

We next performed imaging of DAPI uptake by multiphoton intravital microscopy[5] and showed that treatment acted blocking aberrant activity of mutant HCs in the epidermis in vivo. Finally, we examined treatment effects ex vivo and we showed that AAVmAb recovered a normal epidermal thickness and keratinocytes size. It also caused partial recovery of the expression pattern of epidermal keratins and reduction of proliferation and apoptosis markers.

These results show the important role played by increased HC activity in the skin pathology associated with KID syndrome and the clinical potential of anti-HC mAbs gene transfer approach to treat this disorder. Inhibition of HC activity is an ideal therapeutic target in KID syndrome and genetic delivery of mAbs targeted against mutant HCs paves the way to develop innovative therapeutic approaches to treat connexin-related human genodermatoses.

1. Avshalumova L. et al. 2014.
2. Xu L. et al. 2017.
3. Patel A. et al 2020
4. Mese G. et al 2011
5. Nardin C. et al 2021

Modelling of a rare mutation in the GALNT3 gene found in patient with teeth and bone defects using genetically engineered mice

- **Jan Krivanek [1], Marcel Schuller [2], Zuzana Marincak Vrankova [3,4,5], Hana Palova [6], Ondrej Slaby [6], Radka Chaloupkova [7,8], Petr Nickl [9], Petr Kasparek [9], Jan Prochazka [9], Radislav Sedlacek [9], Petra Borilova Linhartova [3,4]**

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Treatment of rare inherited diseases remains to be a challenging task. The young female patient was diagnosed with chronic recurrent multifocal osteomyelitis in childhood. During an early puberty, a panoramic X-ray of the jaws revealed numerous defects in the anatomy of the teeth, which included significant root shortening. In addition, her blood tests repeatedly showed abnormalities in phosphorus and vitamin D levels. However, no clear association was found to link the diagnoses. To identify the possible causative agent of these diseases, whole-exome sequencing of her DNA was recently performed. The patient was found to be a carrier of an extremely rare allele of the gene for pPolypeptide N-Acetylgalactosaminyltransferase 3 (GALNT3) as a recessive homozygote. Mutation in this gene was in a few clinical case studies associated with hyperphosphatemic familial tumoral calcinosis and hyperphosphatemic hyperostosis syndrome. Modelling of the protein structure showed that the identified point mutation in this conserved sequence causes conformational changes in the protein with a presumed change in its function. In order to study the identified inherited disease, a genetically modified mouse model carrying the same point mutation was created. Studying this mouse model will not only provide the opportunity to explore all phenotypic manifestations but more importantly, to test new therapies.

Application of Extracellular Vesicles associated with Adeno-Associated Virus in transgenesis

- **Petr Nickl [1], Maria Barbiera [2], Jacopo Zini [2], Tereza Nickl [1], Irena Jenickova [1], Jana Kopkanova [1], Marjo Yliperttula [2], Radislav Sedlacek [1]**

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Adeno-associated viruses (AAVs) are small, non-enveloped viruses with an ssDNA genome of ~5kb. AAVs are characterized by their relative safety, long-term expression, and persistence in the nucleus in a form of episome. When a recombinant AAV (rAAV) particle enters the cell, the viral genome is processed into a double-stranded circular episome. These episomes can form concatemer and remain in the nucleus as a large molecule. In the context of transgenics, specific AAV serotypes can serve as ssDNA HDR donor template. AAV particles are able to penetrate zona pellucida, cellular membrane, and reach the pronucleus without significant negative impact on the viability of the embryo. Subsequent electroporation of Cas9/gRNA RNPs allows for the introduction of double-stranded break and induction homology-directed repair mediated by the AAV-originated ssDNA template. The application of this principle has been described by Chen et al. 2019 and named CRISPR-READI. We introduce a new method of acquiring rAAVs by co-isolation with extracellular vesicles (EV) and application of this compound particle in transgenic practice. Our approach not only simplifies rAAV production, but also gives rise to a new delivery vector with unique features.

POSTER SESSION

Poster session 1 (on-site) - Thursday 15 September 2022 (14:50 – 16:00)

Poster session 2 (on-line) - Friday 16 September 2022 (15:20 – 17:00)

A) RESEARCH POSTER PRESENTATIONS

- **Poster 1** – **Goretti Aranaz-Novaliches:** Regulation of Amelogenin by Non-canonical poly A polymerase Tent5a is essential for Enamel formation
- **Poster 2** – **Felipe Castro Nepomuceno:** Bone Mineral Density and Tissue Mineral Density Automated Calculation Using Deep Learning
- **Poster 3** – **Roldan De Guia:** Inter-Laboratory Comparability and Robustness of Clinical Blood Chemistry Reference Intervals at The International Mouse Phenotyping Consortium
- **Poster 4** - **Klevinda Fili:** Functional assessment of mice carrying a de novo missense GRIN2B mutation associated with Autism Spectrum Disorder (ASD): an approach to studying rare diseases
- **Poster 5** – **Veronika Iatsiuk:** Regulatory role of Cul4a in the colorectal cancer progression
- **Poster 6** – **Irena Jenickova:** Adeno-Associated Virus Vectors Enable Efficient Gene Conversion in Mouse Embryos
- **Poster 7** – **Lukas Kucera:** A mixture of innate cryoprotectants is key for freeze tolerance and cryopreservation of a drosophilid fly larva
- **Poster 8** – **Carlos Eduardo Madureira Trufen:** Systems Biology-driven framework to analyze phenotyping data: a first endeavor
- **Poster 9** – **Blanka Mrazkova:** Trabd2b sustains the balance between Wnt and Shh signaling gradients during mouse head and brain development
- **Poster 10** – **Tereza Nickl:** CRL4-mediated ubiquitination regulates neural crest cells development
- **Poster 11** – **Betul Ogan:** Role of FAM83H in immune system homeostasis
- **Poster 12** – **Michaela Prochazkova:** Proper function of Cullin-RING ubiquitin ligase complexes is substantial for tooth morphogenesis
- **Poster 13** – **Miles Raishbrook:** The significance of Fam84b in retinal homeostasis
- **Poster 14** – **Jolana Tureckova:** Looping morphogenesis of embryonic gut is orchestrated by Atf2-dependent mesentery differentiation
- **Poster 15** – **Karel Chalupsky:** Effect of vegan diet on plasma lipidome in Czech and Italian population
- **Poster 16** – **Karel Chalupsky:** Female Gunn rats show a distinct lipid phenotype related to UGT1A1 mutation
- **Poster 17** – **Karel Chalupsky:** Novel human Constitutive Androstane receptor (CAR) ligand MI-883 for the treatment of hypercholesterolemia
- **Poster 18** – **Petra Kralova Viziova:** Analysis and validation of 5-Azacytidine resistance model in CDX model
- **Poster 19** – **Frantisek Spoutil:** Revealing hidden function of enamel matrix proteins through their evolutionary history

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(PO-1) Regulation of Amelogenin by Non-canonical poly A polymerase Tent5a is essential for Enamel formation

- **Goretti Aranaz-Novaliches [1], Frantisek Spoutil [1,2], Olga Gewartowska [3], Jan Prochazka [1,2], Radislav Sedlacek [1,2]**

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The formation of enamel, amelogenesis, is orchestrated by ameloblast that secrete enamel matrix proteins (EMPs) such as Amelogenin (Amelx) and Ameloblastin (Ambn) that help deposit hydroxyapatite crystals. In this study, we used a Tent5a knock-out (KO) mouse model to show that Tent5a non-canonical poly-A polymerase is crucial for EMPs synthesis, secretion and enamel mineralization. Non-canonical poly A polymerases belong to the Terminal nucleotidyl transferases (TENTs) superfamily and their role is to protect the mRNA from degradation, confer stability, and promote translation of the mRNA. Previously, TENT5A loss of function mutations has been found in patients with osteogenesis imperfecta.

Micro-computed tomography revealed that Tent5a deficient mice model exhibits teeth hypomineralization, thinner enamel layer and disrupted enamel patterning. Using nanopore direct mRNA sequencing, we have identified that Tent5a polyadenilates Amelx and other secreted proteins mRNA to increase their expression during amelogenesis. Tent5a is localized in the cytoplasm and also in the endoplasmic reticulum where it regulates Amelogenin synthesis. Furthermore, the self-assembly into the extracellular organic matrix was impaired in Tent5a KO mice and is essential to direct hydroxyapatite deposition in enamel formation.

Here we report that cytoplasmic polyadenylation of secreted proteins by Tent5a is necessary for biomineralization of teeth.

(PO-2) Bone Mineral Density and Tissue Mineral Density Automated Calculation Using Deep Learning

- **Felipe Castro Nepomuceno [1], Julia Potip [1], Vendula Novosadová [1], Radislav Sedláček [1]**

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Machine learning (ML) approaches have been on the uprise for the past decades, for its capability of solving problems faster. One of the branches of ML is called Deep Learning (DL). With it it is possible to analyze and interpret large amounts of data faster and easier. By providing a set of training data to the algorithm, it can learn to extract features from the data, by doing transformations to the data, and fit them to specific labels or categories. In this work, we used Deep Neural Networks (NN), a type of DL, to automatize the procedure of measuring and calculating the bone mineral density (BMD) and tissue mineral density (TMD) in mice. The data used in the process is a set of images of X-ray scan for each mouse in the cohort. Using data from previously calculated cohorts as a training and validation set, the models for the trained NN were created. The process of the automation was split into 4 steps: identify the phantoms, separate the head from the rest of the body, remove the holder from the images, and mask the phantoms and body to calculate the BMD and TMD. After these steps, the coefficients of the phantoms and the body are calculated and it allows for the calculation of the BMD and TMD values. The automation of this pipeline speeds up the process (as it was previously done manually mice by mice) and it frees up the time for other tasks.

(PO-3) Inter-Laboratory Comparability and Robustness of Clinical Blood Chemistry Reference Intervals at The International Mouse Phenotyping Consortium

- **Roldan De Guia [1,3], Sharon Cheng [2,3], Karel Chalupsky [1,3], The International Mouse Phenotyping Consortium (IMPC) [3], Piia Keskivali-Bond [2,3], Radislav Sedlacek [1,3], Jan Rozman [1,3]**

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Clinical chemistry has the primary purpose of performing analytical procedures for the quantification of different ions, enzymes, biomolecules, and other serological components of a biological material. Standardized assays are essential for patient screening, diagnosis of diseases, and clinical management. Reference intervals (RIs) or decision limits are being used by clinical laboratories after a streamlined, established procedure following international guidelines set by the International Federation of Clinical Chemistry (IFCC) or Clinical and Laboratory Standards Institute (CLSI). This study aims to define and establish RIs in the different IMPC research centres by implementing guidelines similar to IFCC/CLSI. Furthermore, we analyzed variability and robustness of different clinical chemistry parameters across 4 IMPC centres as part of external quality assessment (EQA) and post-analytical, RI validation.

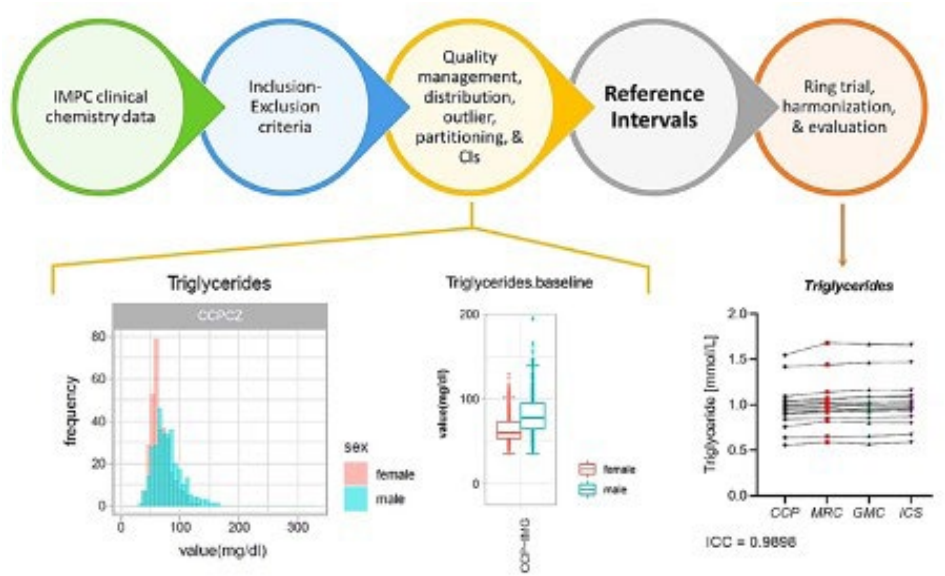

Fig.: Simplified schematic for the calculation of reference intervals from IMPC clinical chemistry data: The procedure follows the IFCC/CLSI guidelines. CCP Triglyceride is shown as an example for frequency distribution, outlier elimination, sex partitioning, and ring trial assessment.

(PO-4) Functional assessment of mice carrying a de novo missense GRIN2B mutation associated with Autism Spectrum Disorder (ASD): an approach to studying rare diseases

- **Klevinda Fili [1], Miriam Candelas Serra [1], Agnieszka Kubik Zahorodna [2], Viktor Kuchtiak [1], Tereza Smejkalova [1], Ladislav Vyklický [1]**

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Based on recent epidemiologic data, nearly a sixth of the world's population suffers from a neurological disorder, and one in 100 children worldwide has Autism Spectrum Disorder (ASD). Sequencing data for neurological and psychiatric patients indicate numerous mutations in genes encoding N-methyl-D-aspartate receptor (NMDAR) subunits. NMDA receptors are glutamate-gated ion channels that mediate signaling at most excitatory synapses in the nervous system.
We have created and evaluated a transgenic mouse carrying a missense mutation (L825V) in the Grin2b gene, coding for the GluN2B NMDAR subunit, associated with Autism Spectrum Disorder (ASD). To characterize the impact of this mutation, we used a combination of methods, including patch-clamp recording, as well as immunochemical methods and behavioral tests.

Using the recombinant wild-type and mutated subunits, we assessed the NMDAR channel open probability (Po). The Po of the wild-type diheteromeric receptors was determined to be 9.9 ± 1.0%, the Po of the triheteromeric receptors with a mutation in one GluN2B subunit was decreased to 4.9 ± 0.8% and in diheteromeric receptors with a mutation in both subunits to 1.0 ± 0.1%. Subsequently, we used primary hippocampal neurons prepared from WT and Grin2bWT/L825V mice to characterize the surface expression and the density of whole-cell and synaptic currents mediated by the NMDAR. The whole-cell NMDAR current densities, but not AMPAR current densities, were reduced in neurons prepared from Grin2bWT/L825V compared to WT mice. Interestingly, the sensitivity to ifenprodil, a GluN2B antagonist, was decreased in neurons from heterozygous mice. At synapses, the deactivation rate of NMDAR was significantly accelerated in Grin2bWT/L825V compared to WT, and the peak current density was not changed. The immunochemical analysis showed that the GluN2BL825V surface expression in the soma and spines was not altered. The behavioral tests indicated differences in certain cognitive tasks between WT and Grin2bWT/L825V mice.

The Grin2bWT/L825V mice provide a relevant model of ASD, that we plan to use in subsequent experiments to rectify the deficits through genetic and pharmacological treatments.

(PO-5) Regulatory role of Cul4a in the colorectal cancer progression

- **Veronika Iatsiuk [1], Jolana Tureckova [1], Petra Baranova [2], Kerstin Huebner [3], Frantisek Spoutil [1], Vendula Novosadova [1], Carlos Eduardo Madureira Trufen [1], Regine Schneider-Stock [3], Radislav Sedlacek [1], Jan Prochazka [1]**

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Colorectal cancer (CRC) is a second leading cause of cancer-associated mortality. Among others, cancer development is associated with alteration in ubiquitination process. Aberrant expression of the Cul4a gene is a cause of many tumor types, including CRC. Thereby, in this study we aim to identify the role of CRL4 in the alteration of the regulatory pathways, resulting in the gastro-intestinal homeostasis disorders and tumor expansion.

Our current research showed that Cul4aKO on ApcMin/+ colorectal carcinoma background leads to the tumor development in the distal colon, what is only occasionally observed for ApcMin/+. Further studies revealed that ApcMin/+Cul4aKO mice have prolonged lifespan then regular ApcMin/+. We suggested that this phenomenon could be associated with changes in tumor invasiveness. Followed molecular studies leads us to the suggestion that observed phenotype could be a cause of Cul4a influence on the intracellular trafficking of Smad3 transcriptional factor via negative regulation of Huwe1 Ub-ligase. So, our results show that Cul4a is a critical regulatory element in intestine homeostasis in the context of CRC progression.

(PO-6) Adeno-Associated Virus Vectors Enable Efficient Gene Conversion in Mouse Embryos

- **Irena Jenickova [1], Petr Nickl [1], Jana Kopkanova [1], Sandra Horejsova [1], Csilla Michalikova [1], Elena Vikhrova [1], Cyril Barinka [2], Radislav Sedlacek [1]**

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Site-specific recombinase technology is a powerful tool with wide applications including mouse transgenesis. Here, the gene conversion in 1-cell embryo simplifies the procedure of generation of completely converted mouse, and compared to the traditional breeding, reduces the animal consumption. We have shown earlier that the Cre and Dre recombinases can be electroporated into the mouse zygotes as proteins and mediate the conversion with a high efficiency. However, the procedure has not been applied for flippase (Flp) and Vika recombinase due to inefficient in vitro protein synthesis of these recombinases. Therefore, we have been investigating a new technology based on adeno-associated viruses (AAV). The Flp, Vika, Cre, and Dre recombinases were produced as AAV vectors and applied on mouse embryos of the Rosa26-VFRL-EGFP reporter line (MuX reporter strain), carrying a transgenic cassette for Cre/Dre/Flp/Vika -dependent expression of GFP protein. The AAV-based electroporation-less technique works with the high efficiency, comparable to the Cre/Dre protein electroporation. In addition, our approach can be used for direct conversion of EUCOMM gene trap allele tm1a to tm1c allele using AAV Flp vector. We demonstrate versatility of adeno-associated virus vectors and their application in transgenic practice.

(PO-7) A mixture of innate cryoprotectants is key for freeze tolerance and cryopreservation of a drosophilid fly larva

- **Lukas Kucera [1], Martin Moos [2], Tomas Stetina [2], Jaroslava Korbelova [2], Petr Vodrazka [2], Lauren Des Marteaux [2], Robert Grgac [2,3], Petr Hula [2,3], Jan Rozsypal [2], Milos Faltus [4], Petr Simek [2], Radislav Sedlacek [1], Vladimir Kostal [2]**

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Insects that naturally tolerate internal freezing produce complex mixtures of multiple cryoprotectants (CPs). Better knowledge on composition of these mixtures, and on the mechanisms of individual CP interactions, could inspire development of laboratory CP formulations optimized for cryopreservation of cells and other biological material. Here, we identify and quantify (using high resolution mass spectrometry) a range of putative CPs in larval tissues of a subarctic fly, Chymomyza costata, which survives long-term cryopreservation in liquid nitrogen. The CPs proline, trehalose, glutamine, asparagine, glycine betaine, glycerophosphoethanolamine, glycerophosphocholine and sarcosine accumulate in hemolymph in a ratio of 313:108:55:26:6:4:2.9:0.5 mmol l-1. Using calorimetry, we show that artificial mixtures, mimicking the concentrations of major CPs in hemolymph of freeze-tolerant larvae, suppress the melting point of water and significantly reduce the ice fraction. We demonstrate in a bioassay that mixtures of CPs administered through the diet act synergistically rather than additively to enable cryopreservation of otherwise freeze-sensitive larvae. Using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), we show that during slow extracellular freezing trehalose becomes concentrated in partially dehydrated hemolymph where it stimulates transition to the amorphous glass phase. In contrast, proline moves to the boundary between extracellular ice and dehydrated hemolymph and tissues where it probably forms a layer of dense viscoelastic liquid. We propose that amorphous glass and viscoelastic liquids may protect macromolecules and cells from thermomechanical shocks associated with freezing and transfer into and out of liquid nitrogen.

(PO-8) Systems Biology-driven framework to analyze phenotyping data: a first endeavor

- **Carlos Eduardo Madureira Trufen [1], Vendula Novosadova [1]**

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The International Mouse Phenotyping Consortium (IMPC) and Mouse Genome Informatics (MGI) websites collect information from wild-type and transgene mice with knocked-out genes and provide phenotyping data. Although those data are freely available, to our knowledge, no Systems Biology-driven framework has been set to develop insight into the function of every gene. We are looking for genes that yield craniofacial abnormalities. From these genes, we performed over-representation analysis with datasets such as KEGG, Reactome, and Gene Ontology. The following steps will be to identify the main signaling pathways related to this phenotype and look for genes with functions not yet characterized.

(PO-9) Trabd2b sustains the balance between Wnt and Shh signaling gradients during mouse head and brain development

- **Blanka Mrazkova [1], Veronika Gresakova [1], Tereza Nickl [1], Ivana Bukova [2], Frantisek Spoutil [2], Paul Trainor [3], Jan Prochazka [1,2], Radislav Sedlacek [1,2]**

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Wnt signaling pathways are crucial for proper cell differentiation and proliferation during both embryonic development and adult tissue maintenance. Thus, they are tightly regulated by plethora of interacting proteins on multiple levels. One of such potential Wnt-regulating proteins is Trabd2, a transmembrane manganese-dependent metalloproteinase, previously described to cleave some of the Wnt proteins. However, the mechanism of its action has been described only in vitro. To examine its biologic functions in vivo, we generated Trabd2-deficient mouse model using CRISPR/Cas9 technology. The Trabd2b-deficient mice display pathological effects on head and brain development whereas the level of damage differs between individual embryos. Some of the embryos remind of WT embryos, while others have hydrocephaly, and some are exencephalic lacking developed facial area. This suggests a wide range of Trabd2 penetrance. To identify the connection between Trabd2 and Wnt regulatory networks, we used Wnt signaling challenged model of nucleoredoxin (Nxn) knockout mice (Nxn acts as a negative regulator of Wnt signaling) crossed with the Trabd2 mutants. Similarly to Trabd2 mutants, Nxn heterozygous embryos show wide range of phenotypes whereas the most pathological one is without developed skull, facial parts and eyes, with non-distinguishable brain sections. Additionally, Trabd2b mutants lack Shh expression in floor plate suggesting that Trabd2b regulates Shh signaling pathway as well. Observed phenotypes result from defects in neural tube closure during early embryonic development which requires proper balance of Wnt and Shh signaling activities, important for molecular definition of brain development field, neural tube closure and later cranial neural crest cells migration, and normal patterning. In conclusion, we propose that Trabd2 metalloproteinase is a novel Wnt and Shh signaling regulator sustaining the balance between these two signaling gradients during mammalian development.

(PO-10) CRL4-mediated ubiquitination regulates neural crest cells development

- **Tereza Nickl [1], Blanka Mrazkova [1], Petr Nickl [2], Michaela Prochazkova [1], Jan Prochazka [1], Radislav Sedlacek [1,2]**

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Cullin-4 (CUL4a/CUL4b) is a scaffolding protein of CRL4 complex, an E3 ubiquitin ligase involved in ubiquitination and mostly subsequent degradation of target proteins regulating various biological processes, such as genome stability control, cell cycle regulation or development orchestration. CRL4 complex achieves high functional flexibility by interaction of the adaptor protein DDB1 with various substrate receptors (DCAFs) in order to assemble into specific temporal-spatially regulated protein degradation complex. The understanding of how the combinatorial potential of CRL4 complex assembly results in substrate specificity is essential for revealing the molecular function of Cul4 under physiological and pathological conditions.

We focus on the function of CRL4 complex during mammalian craniofacial morphogenesis. Accurate migration of neural crest cells (NCCs) is critical for precise development of forming of cranial bones, cartilages, connective tissues and muscles of the head, as well as nerves. We confirmed the colocalization of Cul4 expression with NCCs using LacZ staining. We crossed the Wnt1Cre driver and Ddb1 cKO mouse lineages to assess the role of CRL4 in head mesenchyme originating from the NCCs. We observed that DDB1 loss in neural crest originated cells causes severe malformation of embryonic orofacial structures with particular dysmorphology of facial processes. Mass spectrometry analysis of pulled-down proteins interacting with DDB1 suggests involvement of DNA damage machinery and CRL4-mediated ubiquitination in regulation of NCC migration.

To capture the truly interacting proteins at the physiological condition, in vivo approach to determine protein-protein interactions in live cells is thus the only way. What we would like to accomplish in our work is the preparation of a system based in BioID2, a smaller biotin ligase from Aquifex aeolicus, that would allow to study protein complexes directly on a mouse model generated by a knock-in of this tag. Combination of AAV technology and CRISPR/Cas9 mediated homologous recombination in zygotes, CRISPR-READI, is used to create these strains.

(PO-11) Role of FAM83H in immune system homeostasis

- **Betul Ogan [1]**

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Fam83h is a keratin-associated protein, expressed mainly in epithelial cells and has been suggested to be responsible for intracellular transport, regulation of cytoskeletal networks, and enamel formation. Defects in *FAM83H* are a cause of amelogenesis imperfecta type 3 (AI3), a soft enamel disease. Interestingly, two AI patients from one family in the Czech Republic with confirmed *FAM83H* mutation developed juvenile rheumatoid arthritis. To understand the role of Fam83h in immune system homeostasis, we have generated Fam83h mutant mice (*Fam83htg/tg*) with an 88aa N-terminal deletion.

Fam83htg/tg animals exhibit decreased body size, sparse and scruffy coat, scaly skin, weakness, and hypoactivity. While we have not observed any obvious dentin-related phenotype, *Fam83htg/tg* pups show severe swelling of their forepaws accompanied by severe bone deformation at as early as 3 weeks of age. The majority of the *Fam83htg/tg* animals present increased neutrophil as well as G-CSF and inflammatory cytokine levels in their peripheral blood. We have shown that *Fam83h* is expressed in immune organs and bone marrow (BM), where the source of its expression is in stromal cells. As a result, *Fam83htg/tg* mice show decreased thymus size and altered T cell selection and BM hematopoiesis. These findings will contribute to the unraveling of the role of *Fam83h* in the development of arthritic lesions and, in general, regulation of the immune system.

(PO-12) Proper function of Cullin-RING ubiquitin ligase complexes is substantial for tooth morphogenesis

- **Michaela Prochazkova [1], Ivana Bukova [1], Radislav Sedlacek [1], Jan Prochazka [1]**

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The main role of the Cullin-RING ubiquitin ligase (CRL) complexes is the ubiquitination of substrate proteins. Altered expression of genes from CRL family has been associated with many different types of invasive cancer diseases. Even though it has been shown that many cancerogenic processes are based on misused developmental molecular programs, the role of CRLs in these processes is largely unknown with exception of the connection between CRL4 and the teratogenic potential of thalidomide during prenatal limb outgrowt via regulation of Fgf8 (Ito et al., 2010). Notably, Fgf8 expressing population is essential also for development of tooth primordia (Prochazka et al., 2015).

We have mapped the expression pattern of distinct CRL genes and revealed that CRL3 and CRL4 genes are the most specifically expressed within developing tooth primordia. Using explant tissue culture approach we uncovered that inhibition of CRL complexes severely disrupts embryonic tooth development – the migratory epithelial cells fail to form the molar primordia.

The function of CRL3 can be specifically inhibited by small molecule DI591 and this inhibition impairs proper invagination of odontogenic epithelium during tooth bud formation. Regarding CRL4, we have identified COP1 as putative substrate binding protein within this complex in the developing mandible via coIP followed by mass-spectrometry analysis in the mandibular protein lysates. In order to evaluate the role of Cop1 and CRL4 as such in odontogenic tissue in vivo we crossed Cop1 flox and Ddb1 flox with Fgf8CreER mouse line. Phenotypization of these models revealed that conditional deletion of CRL4 genes severaly affects size and morphology of molar primordia and future teeth. We also examined the ETS trasncription factors, downstream effectors of Fgf pathway, as plausible targets of CRL4/COP1 complex during odontogenesis.

Our results uncovered that Cullin-RING ubiquitin ligase complexes are essential for proper behavior of orofacial epithelium during tooth formation and morphogenesis.

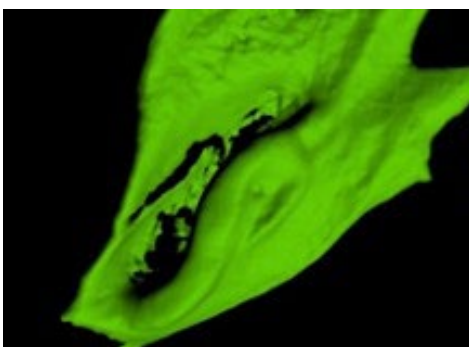

Fig: 3D reconstruction of Ddb1 cKO molar tooth germ at E14.5.

(PO-13) The significance of Fam84b in retinal homeostasis

- **Miles Raishbrook [1], Marcela Palkova [1], Jan Prochazka [1], Radislav Sedlacek [1]**

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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, as well as signs of choroidal neovascularisation (CNV) at older ages. Fam84b was recently reported to play a role in endosomal trafficking of cell surface receptors. Here we present evidence from a preliminary co-localisation study indicating that Fam84b becomes associated with early endosomes under epidermal growth factor (EGF) stimulation. The focus of this ongoing project is to elucidate the molecular function of Fam84b within the context of retinal homeostasis.

(PO-14) Looping morphogenesis of embryonic gut is orchestrated by Atf2-dependent mesentery differentiation

- **Jolana Turečková [1], Blanka Mrázková [1], Veronika Iatsiuk [1], Marie Munawar Cheema [1], Tereza Michalčíková [1], Petra Kompaníková [2], Kerstin Huebner [4], František Malinka [1], Vendula Novosadová [1], Vítězslav Bryja [2,3], Radislav Sedláček [1], Regine Schneider-Stock [4], Jan Procházka [1]**

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Activating transcription factor 2 (Atf2) is a promiscuous gene involved in many physiological and pathological processes described in adult. To delineate its role during embryonic development, we generated Atf2-deficient mouse model which displays aberrant intestinal looping morphology. We showed that rather than the small intestine, the dorsal mesentery's development is affected by Atf2-deficiency. As a consequence, the small intestine undergoes a randomized folding process, which is under normal conditions precisely defined and evolutionarily highly conserved for individual species. Additionally, the transcriptomic analyses revealed the Atf2 regulation of this process via non-canonical/PCP signaling pathway whose changed expression modulates the mesenteric smooth muscle cells fate. Our work brings new findings on the process of gut looping morphogenesis in mammals and suggests the molecular pathways responsible for its regulation.

(PO-15) Effect of vegan diet on plasma lipidome in Czech and Italian population

- **Klára Dohnalová [1,2] , Kryštof Klíma [1], Nikola Ďásková [2,3] , Magdaléna Procházková [4] , Marina Heniková [4], Eliška Selinger [4], Jan Gojda [4], Monika Cahová [5], Sonia Tarallo [6], Alessio Naccarati [6], Giulio Ferrero [7], Tilman Kuhn [9,10] , Rikard Landberg [8], Radislav Sedláček [1], Karel Chalupský [1]**

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Veganism is nowadays a popular lifestyle choice. People decide to exclude meat and other animal products from their diet mainly for ethical and environmental reasons as well as various health benefits. One of them is a lower risk of cardiovascular diseases related to lipid metabolism. However, mechanisms of the protective effects remain widely elusive. A vegan diet is more abundant in unsaturated fatty acids, which have known beneficial effects on cardiovascular health, and is a rich source of dietary fibre and various bioactive compounds. It also contains less saturated fatty acids, which are associated with many diseases. We investigated plasma lipidome of healthy vegans and comparable omnivorous controls from the Czech Republic (VG = 56, CO = 33) and Italy (VG = 40, CO = 38) to see whether the health benefit could be associated with distinct lipid profiles and if these show geographical specifics. Age, height and BMI were preselected parameters comparable between the groups. We found similar lipid profiles in both Czech and Italian cohorts. A major difference between vegans and omnivore controls was an increase of unsaturation in triglycerides, especially an increase of triglycerides containing fatty acid 18:3, linolenic acid. These changes probably reflect the different intake of fatty acids between the groups. Differences were also sex-independent and apply to both cohorts to a similar extent. On the other hand, DHA, a highly unsaturated anti-inflammatory fatty acid found primarily in animal tissue, was lower in vegan plasma. Overall, our findings support the positive effect of a vegan diet on lipid composition in relation to cardiovascular health.

(PO-16) Female Gunn rats show a distinct lipid phenotype related to UGT1A1 mutation

- **Klára Dohnalová [1,2], Kryštof Klíma [1],Dagmar Zudová [1], Roldan de Guia [1], Libor Vítek [2], Radislav Sedláček [1], Karel Chalupský [1]**

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The enzyme UGT1A1 from the family of UDP-glucuronosyltransferases is a key enzyme responsible for conjugation of bilirubin. It enables the elimination of this toxic compound from the body, thereby limits its toxicity. Gunn rats having a mutation in UGT1A1 are thus exhibiting major hyperbilirubinemia. We discovered that female Gunn rats have also reduced total cholesterol and lipoprotein levels in comparison with control rats, indicating changes in lipid metabolism. Therefore, we run RP-UHPLC lipidomic analysis of plasma and liver tissue homogenates of female Gunn rats and controls to investigate if there are any changes in lipid content that could provide an explanation for this female phenotype. The most prominent changes in mutants were an increase of acylcarnitines in the liver and a reduction of triglyceride content in the liver. In plasma, we found a decrease of all measured lipid classes with the exception of shorter and more saturated phospholipids. These results, especially the increase of acylcarnitines, are suggesting changes in energy metabolism, particularly in beta-oxidation. Overall, our results show that hyperbilirubinemia in female Gunn rats is associated with changes in lipid profile in both liver and plasma. We provide evidence of a connection between bilirubin and lipid metabolism and the sex-dependent phenomenon caused by UGT1A1 mutation.

(PO-17) Novel human Constitutive Androstane receptor (CAR) ligand MI-883 for the treatment of hypercholesterolemia

- **Karel Chalupský [1], Klara Dohnalová [1,2], Ivana Mejdrová [3], Jan Dušek [4], Kryštof Škach [3], Josef Skoda [4], Tomas Smutny [4], Petr Pávek [4], Radim Nencka [3]**

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The constitutive androstane receptor (CAR, NR1I3) is a ligand-activated transcription factor belonging to the nuclear receptor subfamily NR1I and is denoted as NR1I3. CAR is a nuclear receptor involved in both xenobiotic clearance and endobiotic metabolism and homeostasis. Emerging evidence strongly suggests that the receptor also regulates important genes controlling cholesterol and bile acids homeostasis as well as glucose and lipid metabolism. CAR is dominantly expressed in parenchymal (hepatocyte) cells of the liver. It was shown that CAR activation prevents the increased hepatic and serum cholesterol levels caused by a diet containing 1% cholesterol in mice after TCBOPOB treatment due to increased bile acid metabolism and excretion, upregulated removal of LDL, even with increased cholesterol synthesis (Rezen et al. 2009). In another study, authors showed that CAR activation in hypercholesterolemic models (Ldlr-/- and ApoE-/-) significantly decreases total and hepatic cholesterol accumulation. CAR activation has been also shown to increase fecal excretion of muricholic acid (MCAs), connected with increased BAs synthesis, sulfo-conjugation, and excretion into bile, but decreased BAs reabsorption Sberna et al. 2001).

In addition, it has been proposed in several independent animal studies that CAR activation may improve glucose homeostasis and insulin sensibility in the treatment of type 2 diabetes (Dong et al., 2009; Gao et al., 2009). CAR activation also inhibits the expression of lipogenic genes in mice what might be a promising therapeutic intervention in the treatment of obesity, steatosis, of hypercholesterolemia (Dong et al., 2009; Gao and Xie, 2012; Jiang and Xie, 2013; Molnar et al., 2013). Together with antiapoptotic effect, activation of CAR with mouse ligand TCPOBOP has been proposed to ameliorate steatohepatitis in a methionine diet-induced model of NASH (Baskin-Bey et al. 2007). CAR activators have been also proposed as potential therapy in cholestatic diseases or liver regeneration (Tschuor et al., 2016). Currently known human CAR ligands such as CITCO have limited selectivity and activates also Pregnane X receptor (PXR) of the same subfamily, which exerts unfavorable effects on glycemia and liver steatosis. We discovered the compound MI-883 that directly activates human CAR in nanomolar concentrations and regulate CAR target genes in 3D spheroids of primary human hepatocytes, humanized CAR mice or 3D hepatocytes, but that it is not an agonist of PXR receptor or other nuclear receptors tested. The compound MI-883 has suitable pharmacokinetic properties and stability in liver microsomes and S9 fraction. MI-883 compound significantly decreases cholesterol and LDL plasma levels, suppresses lipogenesis genes in the liver, increases insulin sensitivity and reduce body weight. The effect of MI-883 on cholesterol plasma levels is mainly mediated by increased elimination of bile acid conjugates from the liver into bile and due to decreased reabsorption from the gut.

(PO-18) Analysis and validation of 5-Azacytidine resistance model in CDX model

- **Petra Kralova Viziova [4], Lubomir Minarik [1,2], Kristyna Pimkova [1], Juraj Kokavec [1], Frederic Vellieux [1], Vojtech Kulvait [1], Karina Savvulidi Vargova [3], Radislav Sedlacek [4], Zuzana Zemanova [5] and Tomas Stopka [1,2]**

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The mechanisms by which myelodysplastic syndrome (MDS) cells resist the effects of hypomethylating agents (HMA) are currently the subject of intensive research. A better understanding of how MDS cells bypass a progressive decrease in efficacy of HMA and progress to acute myeloid leukemia (AML) requires the development of new cellular models. We utilized MDS/AML cell lines and developed a model of AZA (Azacitidine) resistance whose stability was validated by an orthotopic intra femoral transplantation into female NSG-S mice. Proliferation of cells has been monitored weekly with luminescence using Spectral Lago X instruments Imaging, images were processed with Aura Imaging Software. AZA-R evolution was monitored using whole exome sequencing (WES), cytogenetics, and RNAseq. By integrated analysis of expression and mutations, we observed deregulated phosphatidylinositol 3 kinase (PI3K)/AKT signaling, RAF/MEK/ERK signaling, SRC signaling, and nuclear processes involving HDAC and BRD4. We further showed that these pathways can be modulated by specific inhibitors that significantly block the proliferation of AZA-R cells but are unable to increase their sensitivity to AZA. Our data reveal a set of molecular mechanisms of the AZA-R phenotype, which can be used for broadening therapeutic options at progressing states during AZA therapy. In the matter of increasing sensitivity of AZA-R cells to AZA, we observed aberrant changes in cellular redox homeostasis that can be targeted using specific inhibitors involved in ROS production. Engraftment of AZA-R cells into NSG-S mice resulted in significantly shorter overall survival and lower bioluminescent signal in comparison with AZA-S cells. Our work provides new insights into the mechanisms of AZA resistance phenotype and targetable pathways that that can be further explored in mouse models in vivo in order to find new therapeutic strategies.

(PO-19) Revealing hidden function of enamel matrix proteins through their evolutionary history

- **Frantisek Spoutil [1], Goretti Aranaz-Novaliches [1], Michaela Prochazkova [1], Tomas Wald [2], Vendula Novosadova [1], Petr Kasparek [1], Radim Osicka [3], Janne E. Reseland [4], Staale P. Lyngstadaas [4], Hanna Tiainen [4], Kristyna Bousova [5], Jiri Vondrasek [5], Radislav Sedlacek [1] & Jan Prochazka [1]**

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To create intrigue microstructure of calcium crystallites of tooth enamel, ameloblasts secrete highly specialized enamel matrix proteins (EMPs), which built up scaffold for later mineralization. All EMPs started to diverge from a common ancestral gene somewhere around the Pre-Cambrium border, long before true enamel appeared in the fossil record. Also their conservancy in toothless species (whales, platypuses, armadillos etc.) suggests that non-canonical functions from the early times of their evolution are still under selection.

To elucidate this hypothesis, we carried out an unbiased, comprehensive phenotyping and employed data from the International Mouse Phenotyping Consortium to show functional pleiotropy of amelgenin, ameloblastin, amelotin, and enamelin genes in mouse mutants. All these mutant lines were affected in multiple physiological systems.

Evolutionary conserved motifs and functional pleiotropy support the hypothesis of role of EMPs as general physiological regulators in the metabolism of calcium. We could thus illustrate how their non-canonical function can still effect the fitness of modern species by an example of influence of amelogenin and ameloblastin on the bone physiology of mutant mice.

We show, that EMPs are excellent model, which can help us to understand, how evolution works on the protein level.

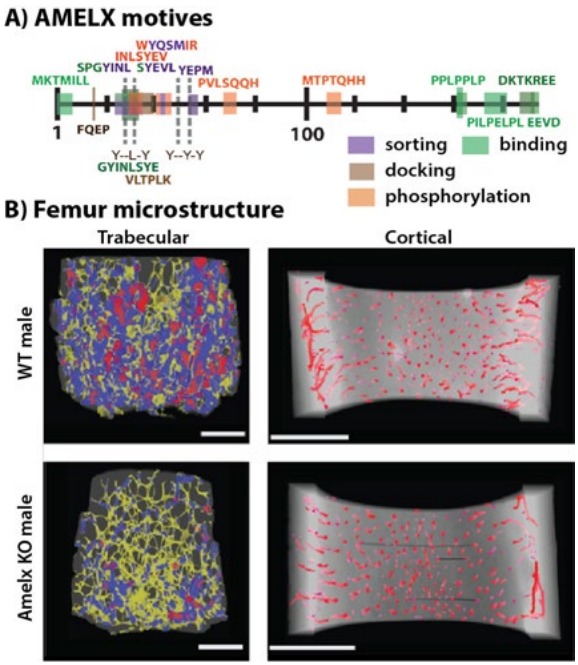

Fig.: A) Scheme of functional motives distribution on the amelogenin protein chain with their ameloacid structure in mice. Polymeration motives (YLY and YYY) crucial for amelogenesis highlighted. Types of mitoves differ in color: violette for sorting, brown for docking, orange for phosphorylation, and green for binding. All motives were probably present already in a common ancestor of a mouse and a human at least, but some of them were dected already in Latimeria. B) Comparison of trabecular (left) and cortical (right) bone microstructure between WT (top) and Amelx KO (bottom) males. Bar = 0.5 mm

B) INFRASTRUCTURE POSTER PRESENTATIONS

- **(PO-20)** Biochemistry and Haematology Unit (CCP, Phenotyping Module)
- **(PO-21)** Bioimaging & Embryology Unit (CCP, Phenotyping Module)
- **(PO-22)** Cardiovascular Unit (CCP, Phenotyping Module)
- **(PO-23)** Hearing & Electrophysiology Unit (CCP, Phenotyping Module)
- **(PO-24)** Histopathology Unit (CCP, Phenotyping Module)
- **(PO-25)** Immunology Unit (CCP, Phenotyping Module)
- **(PO-26)** Metabolism Unit (CCP, Phenotyping Module)
- **(PO-27)** Metabolomics Unit (CCP, Phenotyping Module)
- **(PO-28)** Neurobiology & Behaviour Unit (CCP, Phenotyping Module)
- **(PO-29)** PDX & Cancer Models Unit (CCP, Phenotyping Module)
- **(PO-30)** Vision Unit (CCP, Phenotyping Module)
- **(PO-31)** Bioinformatician Unit (CCP, Phenotyping Module)
- **(PO-32)** Transgenic and Archiving Module (CCP)
- **(PO-33)** Preclinical testing at the Czech Centre for Phenogenomics

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(PO-20) Biochemistry and Haematology Unit (CCP, Phenotyping Module)

- **Roldan Medina De Guia [1], Mariya Glushchenko [1], Eva Štefancová [1], Francisco Machancoses Hernández [1], Jan Prochazka [1], Jan Rozman [1], Radislav Sedlacek [1]**

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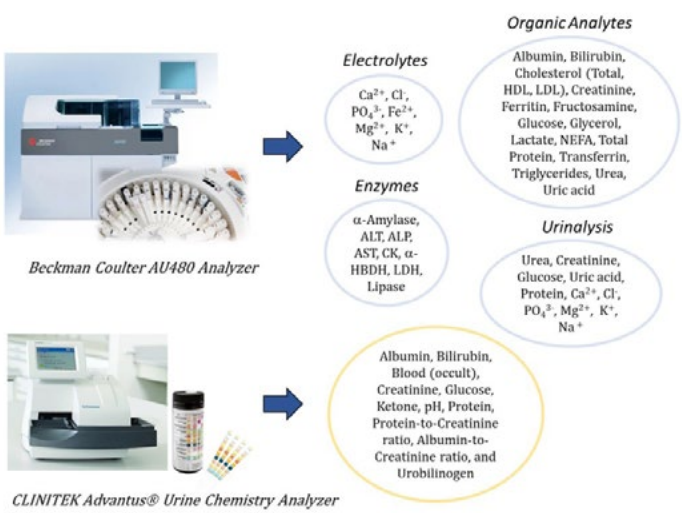
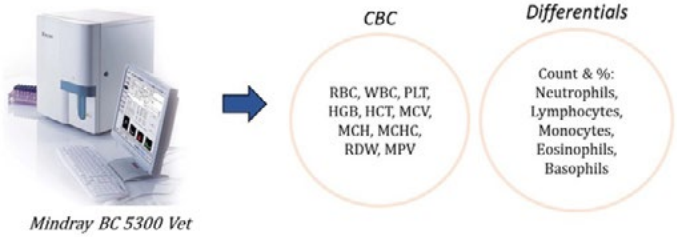
Clinical Chemistry (the study of the chemical composition of the blood plasma), haematology (the study of the blood cellular components), and urinalysis (analysis of chemical and cellular composition of urine) are integral part of clinical pathology which provides a quantifiable way to assess animal 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/.

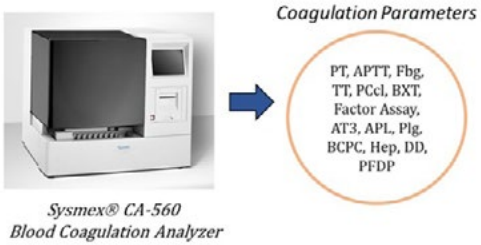
Instrumentation and Technologies:



CLINICAL CHEMISTRY PLATFORMS:
The Beckman Coulter AU480 Clinical Chemistry Analyzer (top) and the Siemens CLINITEK Advantus® Urine Chemistry Analyzer (bottom). Electrolytes, enzymes, and organic analytes can be measured as part of a clinical chemistry panel. Available panels include: liver, kidney, pancreas, inflammation, lipid, cardiac & muscle, anemia, bone and IMPC. The CLINITEK Advantus® utilizes reflectance spectrophotometry to semi-quantitatively analyze urine test strips.



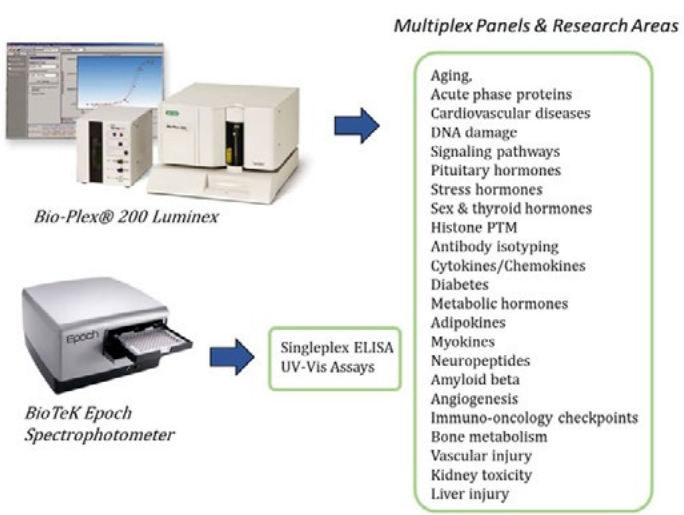
Mindray BC 5300 Vet



Sysmex® CA-560 Blood Coagulation Analyzer

HAEMATOLOGY PLATFORMS:

The Mindray BC-5300 Vet for measurement of veterinary complete blood count and WBC differentials (top). The Siemens Sysmex® CA-560 Automated Blood Coagulation Analyzer for measuring different blood coagulation parameters via coagulation, chromogenic, or immunoassay methodology (bottom).



Bio-Plex® 200 Luminex

BioTeK Epoch Spectrophotometer

IMMUNOASSAY PLATFORMS:

Singleplex ELISA assays or multiplexing using spectrophotometer (bottom) or the Bio-Plex® 200 Luminex (top). Samples that can be analyzed include serum/plasma, lavages, urine, milk, culture media, and cell/tissue culture supernatants.

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(PO-21) Bioimaging & Embryology Unit (CCP, Phenotyping Module)

- **Jan Prochazka [1], Frantisek Spoutil [1], Michaela Prochazkova [1], Ivana Bukova [1], Tereza Nickl [1], Veronika Martinkova [1], Natalie Polakova [1], Sylvie Dlugosova [1], Barbora Kinska [1], Jan Rozman [1], Radislav Sedlacek [1]**

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Our main goal, as a part of the International Mouse Phenotyping Consorcium, is to describe phenotype effect of genes in mouse model from the perspective of adult skeletal morphology and anatomy of embryos as they are important areas, where mutation can be manifested.

Massive usage of µCTs in both areas, which provides detailed 3D information and allows additional analysis, lead to fusion of previously independent units. Our set of three SkyScan µCTs (Bruker) supported with other devices, especially two 2D in-vivo bioluminescence-fluorescence systems (Xtreme, Bruker and Lago X, Spectral Instruments Imaging) gives us an opportunity to provide a large number of analysis from biomedical research to zoology and beyond.

We are able to scan in-vivo up to the resolution of 9 µm/vx or superfast whole-body scans of mice in 1 minute at the resolution of 50 µm/vx. In the case of ex-vivo scans, we are able to go up to 0.5 µm/vx. The wide range of possibilities enables to analyze morphology of the whole skeleton as well as detailed microarchitecture of trabecular bone and its mineralization, 3D and 4D body composition with fat deposition, progression of bone arthrosis, lung function, digitalization for 3D geometric morphometry and tumorigenesis. We tested also other samples, e.g. fossils.

We may apply phase retrieval enhancing contrast between different phases e.g. dentine and enamel. Contrast-Enhanced CT is used mainly in embryo imaging, but also for imaging of soft tissue of adults (e.g. guts, eye) and other samples (e.g. insects). We use Lugol solution, PTA or PMA as contrast agents. We are able to test new, nanoparticle-based contrast agents and their usage in blood stream contrast, e.g. for detection of tumor development.

To sum up, µCT and other modalities of our unit are powerful tool in modern biology and biomedicine, especially if connected with machine learning approach, and we will be more than happy to help you to visualize and to analyze your samples whatever they are.

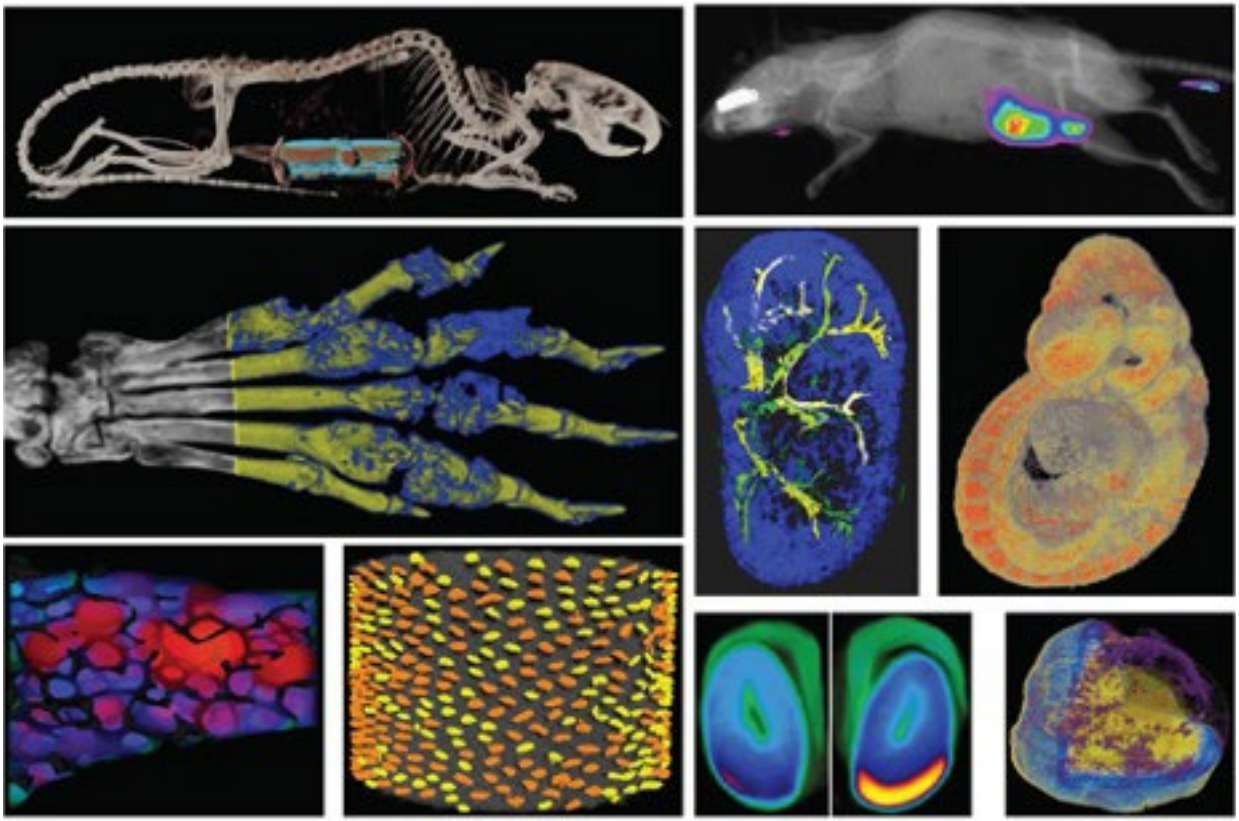

Fig.: Examples of outputs from Bioimaging and Embryology unit.

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(PO-22) Cardiovascular Unit (CCP, Phenotyping Module)

- **Zuzana Nichtova [1], Jiri Lindovsky [1], Petr Macek [1], Sara Brilhante Viegas Dias [1], Jan Majernik [1], Jan Prochazka [1], Jan Rozman [1], Radislav Sedlacek [1]**

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

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The heart and cardiovascular system represent a unique system without which the life is impossible.

Cardiovascular unit at CCP, IMG provides to the investigators the services which help to access cardiovascular phenotypes in rodent models (mice and rats). We use multiple non-invasive in vivo techniques which monitor morphological and physiological parameters of the heart and cardiovascular system. One of the best developed technique is an echocardiography, which uses an ultrasound imaging to visualize the cardiovascular structures and to evaluate cardiac function using by a small probe which sends out high-frequency sound waves and receives echoes. Except of the visualizing of the heart and vessels, the echocardiography enables to analyze how blood flows through them or to evaluate the pumping function of the chambers of the heart. The cardiovascular unit is able to visualize fetal, pup or adult cardiovascular system; the unit provides an option for 4D visualization of the heart, or strain analysis. The electrical activity of the heart can be monitored by ECG (electrocardiography) in awake or anesthetized rodents. The complete picture of the function of cardiovascular system can be achieved by blood pressure measurement. The cardiovascular unit has established protocols for challenging of the cardiovascular system by physiological overload using by treadmill (graded maximal exercise test or progressive maximal exercise test to assess mouse cardio-metabolic phenotypes) or pathologically by pharmacologically induced overload (e.g. by catecholamines). The Cardiovascular unit provides also services of general sonography, such as gravidity check, blood flow e. g. in eyes or tumors, measurement of tumor size with vascularization (%), imaging of different abdominal, pelvic, other anatomic structures. Well established is also IGI – image (ultrasound) guided injection, which allows to carefully inject the micro- or nanoliter volumes (e.g. virus or drug) into specific structure/localization in the organ (fetal, pup, adult). Phenotyping of the cardiovascular system is important for understanding of function of the genes or mechanism of diseases, and plays an important role in development of a therapeutic approaches which can improve the quality of human lives or/and their survival.

(PO-23) Hearing & Electrophysiology Unit (CCP, Phenotyping Module)

- **Jiri Lindovsky [1], Jan Majernik [1], Jan Prochazka [1], Jan Rozman [1], Radislav Sedlacek [1]**

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

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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
- In development: 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

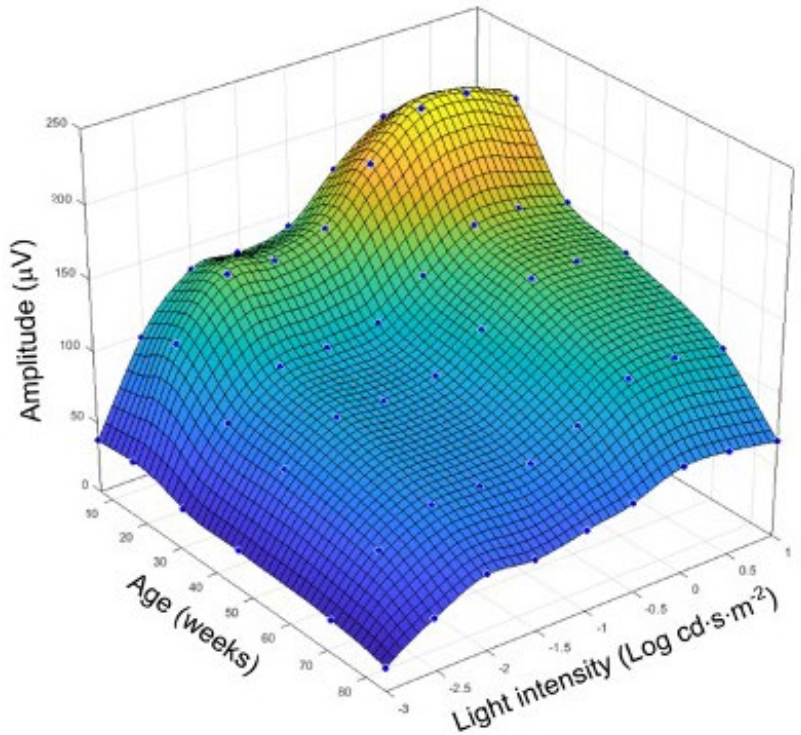

Fig.: Amplitude of ERG b-Wave in Young and Aged Mice.

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(PO-24) Histopathology Unit (CCP, Phenotyping Module)

- **Dagmar Zudova [1]**

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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 immunochemistry. 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 conjuction 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.

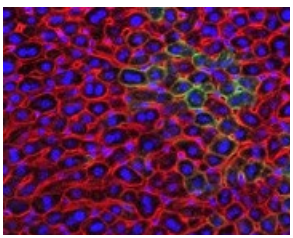
Fig: Liver- Cre

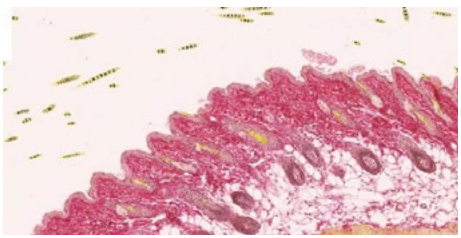
Fig: Skin- Picro Sirius Red

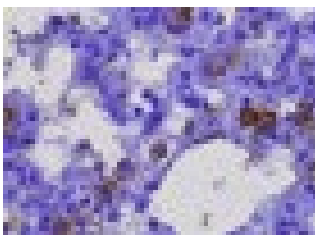
Fig: Lungs - IHC, macrophages

(PO-25) Immunology Unit (CCP, Phenotyping Module)

- **Jana Balounova [1]**

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

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Head: Jana Balounová
Team: Laura Jane Dowling, Michaela Šímová, Carlos Eduardo Madureira Trufen, Kamila Křížová, Kristína Vičíková
Contact: jana.balounova@img.cas.cz

As an integral part of the terminal screen, immunophenotyping involves characterization of particular 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. According to these guidelines, we utilize two panels (IMPC Panel A & Panel B) to discriminate various populations of lymphoid and myeloid cells in the mouse spleen or other tissues (peripheral blood, lymph nodes, thymus, bone marrow, peritoneal lavage, intestine). We have developed 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 software and statistically evaluated. In collaboration with our partner, Accela, we have recently launched the Isolight system enabling for functional multi-omic chip-based measurement of functional assays at a single cell level. Furthermore, the Immunology Unit is equipped with gentleMACS tissue dissociator (Miltenyi Biotec) for tissue dissociation, 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).

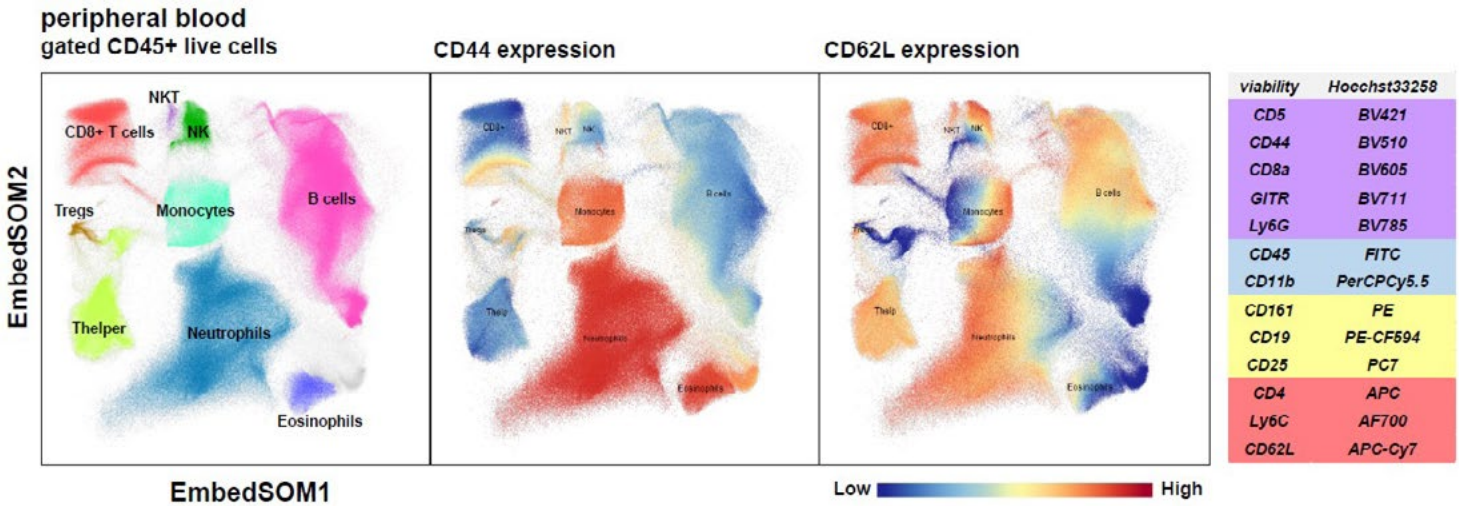
Selected Publications:

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

CRL4-DCAF12 Ubiquitin Ligase Controls MOV10 RNA Helicase during Spermatogenesis and T Cell Activation.
T. Lidak, N. Baloghova, V. Korinek, R. Sedlacek, J. Balounova, P. Kasparek, L. Cermak.
Int J Mol Sci. 2021 May 20;22(10): 5394.
doi: 10.3390/ijms22105394.

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-26) Metabolism Unit (CCP, Phenotyping Module)

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

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

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Rodent models, especially genetically engineered mouse models, are important for discovering gene functions involved in energy metabolism and glucose homeostasis. For first-line phenotyping, we perform intraperitoneal glucose tolerance test, non-invasive body composition, and indirect calorimetry as a starting point for further in-depth and hypothesis-driven studies. Environmental chambers with controlled light:dark regimes, humidity, and temperature allow us to perform cold challenges, thermoneutral studies, or changes in light-dark rhythms while acquiring indirect calorimetric data in mice or rats. Moreover, we could assess the effect of feeding a specific diet (e.g. a high-fat diet) on overall metabolism.

To study glucose metabolism in more detail, we implemented several tests: basal and maximal blood insulin concentrations can be determined during glucose tolerance testing; to assess insulin sensitivity, we perform an insulin tolerance test. Finally, hepatic gluconeogenesis can be checked with a pyruvate tolerance test. These complementary methodologies help explain possible defects in glucose metabolism caused by genetic modification or specific treatments. Another newly integrated method is telemetry of physiological parameters, such as body temperature at two locations of the body, or real-time measurement of blood sugar levels. These parameters can be measured in home-caged mice or in combination with indirect calorimetry.

The combination of telemetry with indirect calorimetry opens up a wide range of new possibilities for monitoring metabolic functions in real-time and under ad libitum or challenge conditions or, during experiments involving specific treatments with minimum human intervention.

We perform non-invasive body composition analysis based on TD-NMR technology, which provides a fast and precise method to determine lean and fat mass, and free fluids in mice and rats.

The advantages of not having to anesthetize the animals and the very fast analysis allow repeated measurements of body composition in time series over time. Like all units at CCP, our metabolism services benefit from integration with other units of the center, enabling the systemic and comprehensive characterization of experimental rodent models.

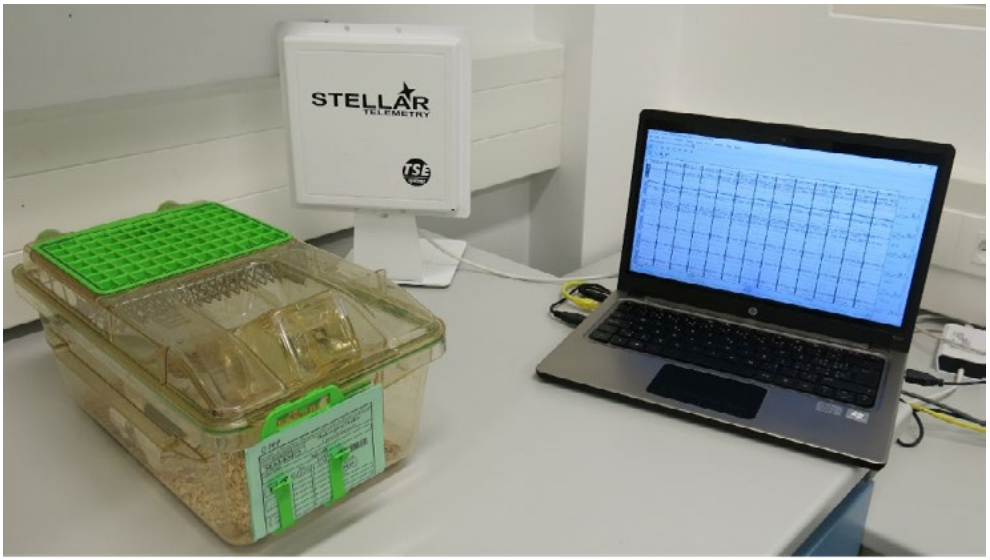

Fig. 1: Telemetry experiment using the Stellar antenna. In this particular experiment, we monitor two temperatures at the same time, located in the intraperitoneal cavity and interscapular brown adipose tissue.

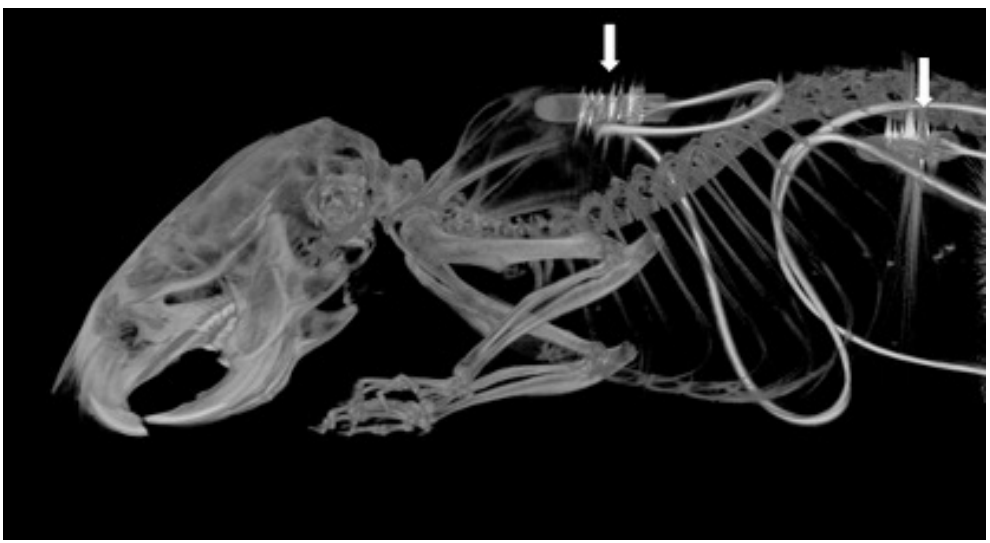

Fig. 2: Micro-computer tomography image, showing the location of the two temperature sensors (i.p and BAT) and their wires leading to the implanted transmitter (not visible in the image).

(PO-27) Metabolomics Unit (CCP, Phenotyping Module)

- **Klára Dohnalová, Kryštof Klíma, Lukáš Kučera, Evgeniya Biryukova, Karel Chalupsky, Jan Prochazka, Jan Rozman, Radislav Sedlacek**

Metabolism Unit, Phenotyping Module, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the ASCR, 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 disease-relevant 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-28) Neurobiology & Behaviour Unit (CCP, Phenotyping Module)

- **Agnieszka Kubik-Zahorodna [1]**

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

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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.

Neurobiology and Behaviour module off¬ers 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 a¬ffect (Elevated Plus Maze, Forced Swim Test, Tail Suspension Test), cognitive function (Cued and Contextual Conditioning, Context Discrimination, 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.

Oversimplified „impoverished" environments together with stress from human handling may be responsible for substantial heterogeneity in the results of conventional behavioural tests. Social group housing in a large enclosure equipped with multiple gadgets in IntelliCage provides environmental enrichment beyond typically employed protocols. It also eliminates stressful interaction between the animal and the experimenter.

Mice behaviour often relies on intact olfactory system. Last year we added to our tests portfolio functional olfactory test – the habituation/dishabituation task. The test is based on the mice ability to recognise new odours and discriminate between them. The habituation/dishabituation task also requires neither extensive training nor prior food deprivation.

INSTRUMENTATION & TECHNOLOGIES:
IntelliCage by NewBehaviour (TSE Systems) – state of the art equipment designed for automatic, long-term, studying cognitive functions of the rodents in the social groups while avoiding human factor.

DigiGait (Mouse Specifics Inc.) – state of the art equipment for gait analysis in various challenging conditions but unified for each animal.

ANY-maze controlled Fear Conditioning system (Stoelting, Ugo Basile SRL) – applied for standard cued and contextual fear conditioning, but also memory extinction, context discrimination etc.
Viewer for Animal Tracking (Biobserve) – with a variety of mazes covers testing in Barnes Maze, open field, light/dark box, elevated plus maze, novel object recognition, spontaneous alternation, forced swim test, 3-chamber sociability box.

Acoustic Startle Reflex (Med Associates Inc.) – used for sensorimotor gaiting evaluation. Startle reflex can be a subject to study its habituation, sensitization, or fear potentiation.

Tail Suspension Cage (Bioseb) – screening tool for antidepressants or studying depressive-like mouse phenotypes.

Rotarod (TSE Systems) – tests animal balance and coordination or motor learning on the rod.

(PO-29) PDX & Cancer Models Unit (CCP, Phenotyping Module)

- **Petra Kralova Viziova [1], Kristýna Hornová [1], Andrea Hartmanová [1], Ana Rita da Silva Oliveira [1], Jan Procházka [1], Jan Rozman [1], Radislav Sedláček [1]**

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics, 25250, Vestec, Czech Republic

E-mail of the presenting author: petra.kralova-viziova@img.cas.cz

The PDX (patient derived tumor xenograft)/Cancer Models Unit is dedicated to create novel orthotopic mouse/rat PDX and CDX models, to use them in cancer, immunology, and pharmacology research. PDX are models of cancer where the tissue or cells from a patient's tumor are implanted into a highly immunodeficient or humanized mouse. We use the well-suited NSG and NSG-SGM3 mouse strains to accomplish this purpose. The samples for implantation or xenografts are placed orthotopically and then the tumor development and tumor size, as well as longevity, are followed to establish efficiency of treatment phase. Together with other CCP units, the mice can be tested through in vivo approaches (luminescense, fluorescence, CT, ultrasound, blood parameters) to follow tumour and metastasis development and changes in blood profile. Mouse tumor samples can be viably stored in our cryobank and in parallel to evaluations in the CCP units (histopathology, hematology and biochemistry, immunology, bioimaging). Additionaly samples may also be analyzed using uCT, MALDI imaging and/or metabolomics approaches. PDX mouse models represent therefore a promising research platform for personalized medicine including pharmacological and metabolic studies. The main benefit is the possibility of in-house multimodal analysis of the models.The unit is also capable of cell/tissue culturing and freezing, and implantation of telemetric device as well. We have recently developed a highly efficient intrafemoral xenografting for leukemia studies and microinvasive approach for mammary fat pad xenografts. Other surgery modalities include intrasplenic, subcapsular kidney, liver, intramuscular, ovary fat pad, testicular, intracaecal wall, intestinal wall, lung, subcutaneous xenograft etc. We have broad experience with cell culturing and cell line derived xenografts. Our day-to-day monitoring of the animals allows us to collect important information such as healing times, changes in body weight, body condition score, changes on mucous membranes, skin reaction, behavioural tendencies, and prevalent disease symptoms during the study. We commit to a high level of biosafety and sterility, that allows us to proceed with long-term studies lasting up to several months. The unit is equiped with an X-RAD Irraditor, Endoscope Storz and CellVizio.

PARTICIPATION IN PROJECTS:

- Participating as a member in the EuroPDX consortium (https://www.europdx.eu/).
- Research and therapy of myelodysplastic syndrome model with Biocev and 1st Faculty of Medicine (Charles University).
- Genotherapy of mammary and ovarian cancer on orthotopic models with IOCB (Prague).
- Testing of targeted polymeric conjugates for lymphoma therapy with IMG/IMC.
- PDX ALL3 intrafemoral leukemia model and testing of novel drugs with IOCB and The University Hospital (Brno).
- Immuno-oncology preclinical research on one side and dual flank mice model with IOCB.
- Testing of immunotherapeutic drugs in cancer dual flank and mammary fat pad models with IOCB.
- Rare lung cancer PDX models establishment with Biocev and General University Hospital in Prague.
- Developing of pancreatic cancer model with Biocev.
- Implantation of telemetric devices for metabolism studies in CCP.
- Development of precise orthotopic head-neck, ovarian and melanoma cancer models for preclinical testing.
- CAR T therapy testing in cooperation with Biocev and IHBT.

Fig. 1: Endoscope Storz

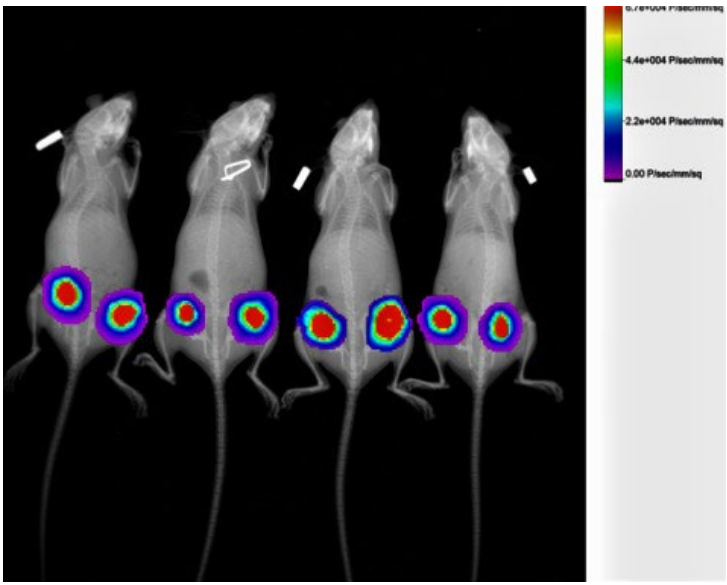

Fig. 2: Mammary fat pad model

Fig 3: Irradiator X RAD 320

Fig. 4: CellVizio Dual band with Microprobes

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(PO-30) Vision Unit (CCP, Phenotyping Module)

- **Marcela Palkova [1], Viktoriia Symkina [1], Jan Procházka [1], Jan Rozman [1], Radislav Sedláček [1]**

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Vision unit is a part of the Czech Centre for Phenogenomic and 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 (Optical coherent tomograph Heidelberg Engineering - OCT) and retinal vascular plexuses (OCT-A Heidelberg Engineering). All procedures are non-invasive, painless and allow long-term studies with repeated examination of eyes.

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 crosssectional 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 compare/assess the physiological relevance of the morphological abnormalities in the retina for the vision and it is described in more detail in the Electrophysiological section.

Besides covering of the routine IMPC workflow, the unit also collaborated on many other research projects related to vision, such as the role of Zfp644 gene in the development of myopia (Szczerkowska et al. 2019). A strong retinal pathology discovered in Fam84b knockout strain initiated a more detailed longitudinal study which has become part of a master thesis and was presented at the conference last year.

To image degenerative processes of the retina for special projects, we have started to use transmission electron microscopy in a close cooperation with Dr. Z. Nichtova PhD. (Head of Cardiovascular unit) and with a kind help of Imaging Methods Core Facility in Biocev.

1. Szczerkowska et al.: Myopia disease mouse models: a missense point mutation (S673G) and a protein-truncating mutation of the Zfp644 mimic human disease phenotype. 2019; Cell & Biosci 9-21.

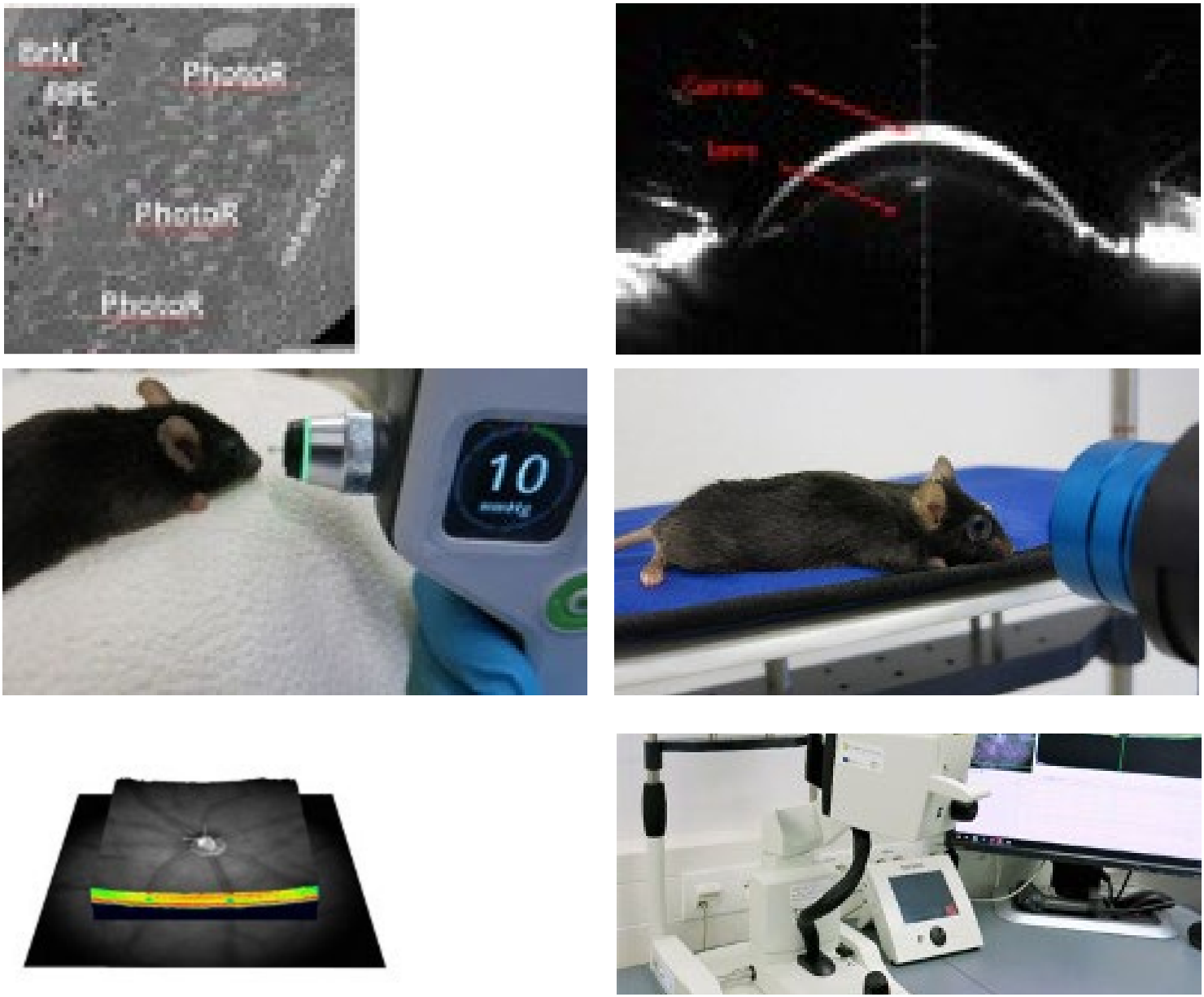

Fig.: Equipment and examples of analyses in Vision Unit

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(PO-31) Bioinformatician Unit (CCP, Phenotyping Module)

- **Vendula Novosadova [1], Carlos Eduardo Madureira Trufen [1], Felipe Castro Nepomuceno [1], Pavlo Bohinskiy [1]**

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

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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 metabolomics 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.

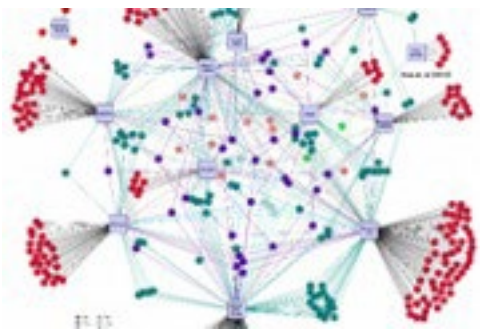

Fig. 1: Sketch of Neural network used for our analysis

Fig. 2: Software used in our team

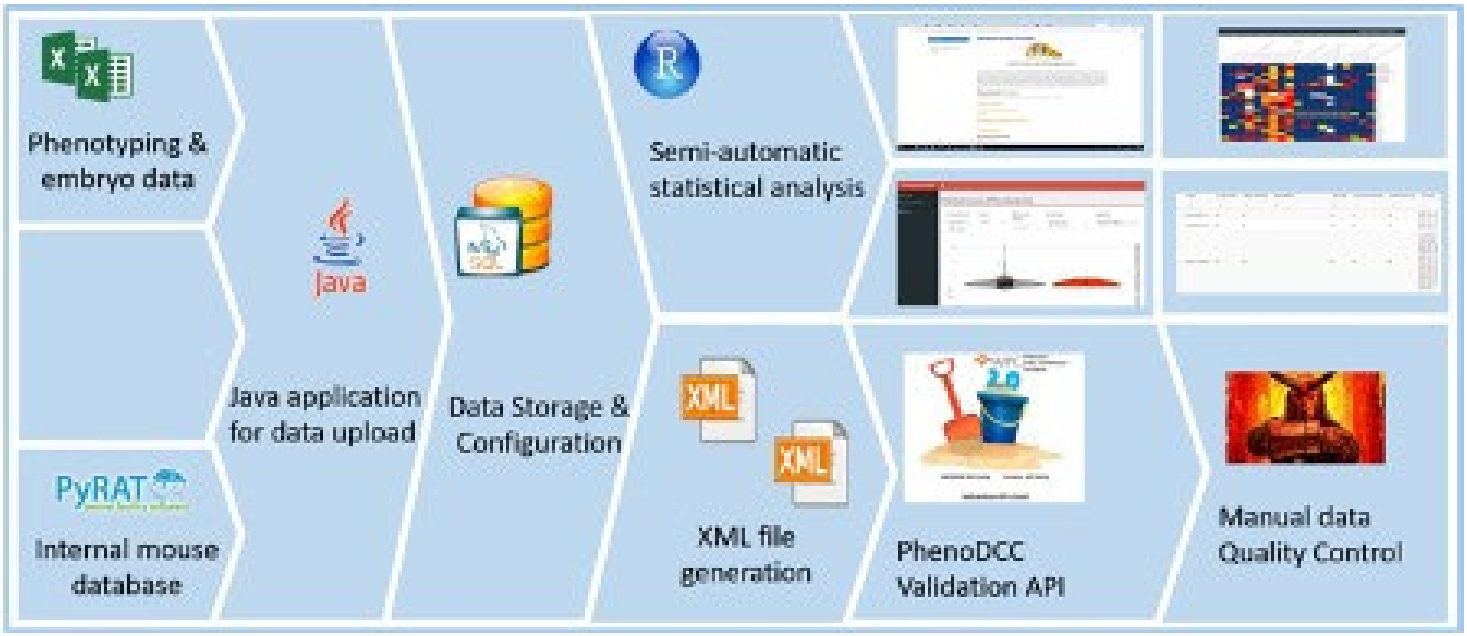

Fig. 3: Pipeline for sendig IMPC data

(PO-32) Transgenic and Archiving Module (CCP)

- **Head of the module: Radislav Sedlacek**
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Transgenic and archiving module is a key part of Czech Centre for Phenogenomics, responsible for generation of novel genetically modified mice and rats using state-of-the-art technologies. TAM consists of subunits for the Genome Engineering & Model Generation and the Genotyping and Breeding, Cryopreservation & EMMA/Infrafrontier Services. Both subunits altogether provide complete service, from the initial gene-targeting design, generation of tools and transgenic rodent models to the genotyping and breeding of desired animal models.

The most commonly used genetic background in CCP is C57Bl/6N, but we are able to generate models on various backgrounds. Vast majority of newly generated mutant rodents are “knock-out” or “knock-in” models based on CRISPR/Cas9 targeting tools and zygote electroporation. Although classical transgenic models generation via pronuclear injection (PNI) of plasmid or BAC DNA is also used. Founder and G1 mice are analyzed to confirm germ line transmission (GLT). The successfully produced mouse/rat lines are cryopreserved (embryo or sperm cryopreservation). Furthermore, we offer mice production with the ES targeting technologies. Routinely we produce models from targeted embryonic stem cells originating from EUCOMM and KOMP repositories. Majority of modifications in these ES cell lines are so called “knockoutfirst”alleles that represent a LoxP-flanked critical exon with LacZ reporter element.

In cooperation with animal facility of CCP we provide consultation, assistance services, and information on the design and use of genetically modified transgenic mice. We also assist in animal rederivation (cleaning of the rodent line), reanimation (creating of the line from frozen embryos or sperms) as well as models import and export using cryopreserves sperm and embryos.

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. We 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.

OUR SERVICE COMPRISES:

- 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
- Embryo and sperm cryopreservation, and reanimation of strains from frozen material
- Ovary transplantation
- Rederivation/ cleaning of mouse/rat strains
- Genotyping service
- Import/ Export arrangements (together with the animal facility module

Instrumentation & technologies

NEPA 21 type II electroporator (NEPAGENE), micromanipulation microscopes Olympus IX83 equipped with TransferMan4r and XYRCOS (Hamilton Thorne) and Leica DMI6000B with FemtoJet4i. Freezing machine Asymptote EP600 (Grant), MicroePore pinpoint cell penetrator (WPI), sperm analyzer Mouse Traxx (Olympus CX41). Automatic capillary electrophoresis QIAxcel Advanced system.

Projects in selected publications:

- Efficient allele conversion in mouse zygotes and primary cells based on electroporation of Cre protein. Irena Jenickova, Petr Kasparek, Silvia Petrezselyova, Jan Elias, Jan Prochazka, Jana Kopkanova, Michal Navratil, Cyril Barinka, Radislav Sedlacek Methods 2021 Jul 191

- KLK5 and KLK7 Ablation Fully Rescues Lethality of Netherton Syndrome-Like Phenotype. Kasparek P, Ileninova Z, Zbodakova O, Kanchev I, Benada O, Chalupsky K, Brattsand M, Beck IM, Sedlacek R. PLoS Genet. 2017 Jan 17

- A viable mouse model for Netherton syndrome based on mosaic inactivation of the Spink5 gene. Kasparek P, Ileninova Z, Haneckova R, Kanchev I, Jenickova I, Sedlacek R. Biol Chem. 2016 Dec 1

- Efficient gene targeting of the Rosa26 locus in mouse zygotes using TALE nucleases. Kasparek P, Krausova M, Haneckova R, Kriz V, Zbodakova O, Korinek V, Sedlacek R. FEBS Lett. 2014 Nov 3

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(PO-33) Preclinical testing at the Czech Centre for Phenogenomics

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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. The progress made in biomedical sciences during recent years brought a dramatic increase in the number of potential biological disease targets but without a visible increase in the translatability of those advances into significant health benefits. The reasons for this are poor hypotheses, irreproducible data, ambiguous preclinical models, statistical errors, insufficient transparency, and lack of data sharing in research.

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 preclinical 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.

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