# Advancing Personalised Medicine with Animal Models

## INFRAFRONTIER / IMPC Stakeholder Meeting

November 14th -16th 2017
Athens, Royal Olympic Hotel

INFRAFRONTIER
mouse disease models

IMPC
International Mouse Phenotyping Consortium

"ALEXANDER FLEMING"
Biomedical Sciences Research Center

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# Welcome & Meeting Objectives

**INFRAFRONTIER / IMPC Stakeholder Meeting**
**Advancing Personalised Medicine with Animal Models**
**Athens, Royal Olympic Hotel, November 14th-16th 2017**

The first Stakeholder Meeting of INFRAFRONTIER, the European Research Infrastructure for phenotyping and archiving of model mammalian genomes, will be jointly organized with the International Mouse Phenotyping Consortium (IMPC, www.mousephenotype.org). Focus of this meeting is on 'Advancing Personalised Medicine with Animal Models'. The meeting is open to a wide range of INFRAFRONTIER stakeholders including Personalised Medicine Initiatives, Rare Disease networks, Funders, Regulators and the INFRAFRONTIER user community to discuss advances in CRISPR/Cas9 technology to model human conditions.

**The Stakeholder Meeting will be structured into 3 main sessions**

1. Advancing Personalised Medicine with Animal Models (Nov 14th)
2. International Mouse Phenotyping Consortium – Open Annual Meeting (Nov 15th)
3. Responsible Research (Nov 16th)

**Meeting aims are to:**

- Raising awareness of INFRAFRONTIER / IMPC platforms among current Personalised Medicine initiatives, funders and policy makers
- Share advances in CRISPR/Cas9 technology to model human conditions
- Present use cases for the utility of animal models for identifying targets for precision therapies
- Strengthen interactions with Personalised Medicine initiatives and Rare Disease consortia

## Organizers

**INFRAFRONTIER - https://www.infrafrontier.eu**
INFRAFRONTIER is the European Research Infrastructure for phenotyping and archiving of model mammalian genomes. The INFRAFRONTIER Research Infrastructure provides access to first-class tools and data for biomedical research, and thereby contributes to improving the understanding of gene function in human health and disease using the mouse model. The core services of INFRAFRONTIER comprise the systemic phenotyping of mouse mutants in the participating mouse clinics, and the archiving and distribution of mouse mutant lines by the European Mouse Mutant Archive (EMMA). In addition, INFRAFRONTIER provides specialised services such as the generation of germ-free mice, and training in state of the art cryopreservation and phenotyping technologies.

**BSRC "Alexander Fleming" - https://www.fleming.gr/**
FLEMING is a partner of INFRAFRONTIER, coordinator of the local node INFRAFRONTIER-GR/ Phenotypos and hosting the Stakeholder meeting. BSRC Alexander Fleming performs cutting-edge research, aiming to understand molecular mechanisms of complex biological processes in health and disease. It is also committed to contributing to innovation in medicine, by developing novel therapeutic and diagnostic methods, focusing on immunity and inflammation, cancer, and neurodegenerative diseases. BSRC Alexander Fleming is a non-profit organization operating under the auspices of the Greek General Secretariat for Research & Technology and is supported by the Greek government and by national, European and international grants.

**International Mouse Phenotyping Consortium (IMPC) -www.mousephenotype.org**
The IMPC addresses one of the grand challenges for biology and biomedical science in the 21st century – to determine the function of all the genes in the human genome and their role in disease. The goal of the IMPC is to develop a comprehensive catalogue of mammalian gene function. The IMPC aims to generate a null mutation for every protein-coding gene in the mouse genome, to acquire broad-based phenotype data for each mutation, and to disseminate the mutant resource and phenotype data to the scientific community. Ultimately the IMPC programme will provide information on the function of all genes and genetic networks and a powerful dataset that will underpin fundamental new insights into the genetic bases for disease.

**Financial support is provided by the INFRAFRONTIER2020 and IPAD-MD projects**
INFRAFRONTIER2020 received funding from European Union's Horizon 2020 research and innovation program under Grant Agreement number 730879
IPAD-MD has received funding from European Union's Horizon 2020 research and innovation programme under Grant Agreement number 653961

# Program

| TUESDAY, NOV 14TH, 08:00-19:00, ROYAL OLYMPIC HOTEL, OLYMPIA HALL | |
|---|---|
| **08:00 – 08:45** | **Meeting Registration** |
| **08:45 – 09:00** | **Meeting opening and objectives**<br>Martin Hrabě de Angelis & Steve Brown |
| **09:00 – 09:20** | **Opening lecture**<br>Martin Hrabě de Angelis, Helmholtz Zentrum München - INFRAFRONTIER Research Infrastructure |
| **09:20 – 09:50** | **Keynote lecture**<br>George Kollias, BSRC Fleming - Contribution of disease modelling to precision medicine initiatives |
| **09:50 – 10:00** | **The Greek INFRAFRONTIER Research Infrastructure**<br>Dimitris Kontoyiannis, BSRC Fleming - INFRAFRONTIER-GR / Phenotypos |
| | **Modelling human conditions using genome editing approaches**<br>Chair: Wolfgang Wurst, Helmholtz Zentrum München |
| **10:00 – 10:20** | Jason Heaney, Baylor College of Medicine - Modelling human disease variants in murine ortholog(s) with CRISPR/Cas9 |
| **10:20 – 10:40** | Radislav Sedlacek, Czech Centre for Phenogenomics - KLK5 and KLK7 Ablation Fully Rescues Lethality of Netherton Syndrome-Like Phenotype |
| **10:40 – 11:10** | **Coffee break - Foyer Olympia Hall** |
| **11:10 – 11:30** | Soren Warming, Genentech - In-depth analysis of CRISPR off-targets in genetically engineered rodents |
| **11:30 – 11:50** | Tomoji Mashimo, Osaka University - Efficient generation of conditional knockout mice by CLICK |
| **11:50 – 12:10** | Channabasavaiah Gurumurthy, University of Nebraska - Easi Crispr for conditional and insertional alleles |
| **12:10 – 12:30** | Yann Herault, PHENOMIN-ICS - In vivo chromosomal engineering in rodents to analyse structural variants through Crismere |
| **12:30 – 12:45** | Nadia Rosenthal, JAX: Outlook- Of mice and CRISPR |
| **12:45 – 13:45** | **Lunch break - IOANNIS Roof Garden Restaurant** |
| | **Contribution of (large-scale) mouse resources to Personalised Medicine**<br>Chair: John Seavitt, Baylor College of Medicine |
| **13:45 – 14:05** | Steve Brown, MRC Harwell - Relevance of an encyclopedia of mammalian gene function for precision medicine initiatives |
| **14:05 – 14:25** | Damian Smedley, GenomicsEngland - Use of phenotype data to obtain novel insights into disease causes and mechanisms |
| **14:25 – 14:45** | Rob Burgess, JAX Center for Precision Genetics - Developing personalized gene therapy approaches |

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|---|---|
| **14:45 – 15:05** | Cat Lutz, JAX Rare and Orphan Disease Center - Generation of an allelic series using CRISPR/Cas9 to study familial ALS |
| **15:05 – 15:25** | Jos Jonkers, Netherlands Cancer Institute - CRISPR/Cas9-based mouse models of breast cancer |
| | **Contribution of EU Research Infrastructures and other large EU initiatives to Personalised Medicine**<br>Chair: Radislav Sedlacek, CCP |
| **15:25 – 15:45** | Enzo Medico, University of Torino - EurOPDX Consortium: PDX models as an emerging way to personalized medicine in translational cancer research |
| **15:45 – 16:05** | Andreas Roos, University of Newcastle - RD-Connect: Data sharing and analysis for rare disease research |
| **16:05 – 16:25** | Jerry Lanfear, ELIXIR - CORBEL and contributions of the EU health related infrastructures to Personalised Medicine in Europe |
| **16:25 – 16:45** | **Coffee break - Foyer Olympia Hall** |
| | **Policy, regulatory and funder perspectives on contribution of (large-scale) mouse resources to Personalised Medicine**<br>Chair: Yann Herault, PHENOMIN-ICS |
| **16:45 – 17:05** | Marisa Papaluca, European Medicines Agency (EMA) - Personalised Medicine Regulatory Issues |
| **17:05 – 17:25** | Colin Fletcher, NIH - IMPC program integration |
| **17:25 – 17:45** | Monika Frenzel, International Consortium for Personalised Medicine IC-PerMed - Translation of basic research results into clinical research and beyond |
| **17:45 – 18:00** | Paul Lasko, International Rare Diseases Research Consortium IRDiRC - Future of rare diseases research 2017-2027 |
| **18:00 – 19:00** | **Panel discussion**<br>Chair: Kent Lloyd, UC Davis<br><br>**Aligning the development of (large scale) mouse resources with Personalised Medicine initiatives**<br><br>**Panellists:**<br>• Yann Herault - INFRAFRONTIER / PHENOMIN-ICS<br>• John Seavitt - IMPC<br>• Monika Frenzel - IC-PerMed<br>• Paul Lasko - IRDiRC<br>• Damian Smedley - GenomicsEngland |
| **19:00** | **Closing remarks**<br>Martin Hrabé de Angelis |
| **19:00 – 20:00** | **Networking reception, Foyer - Olympia Hall** |

# Program

| WEDNESDAY, NOV 15TH, 08:30-19:00, ROYAL OLYMPIC HOTEL, OLYMPIA HALL | |
|---|---|
| **08:30 – 08:45** | **Opening remarks – Steve Brown** |
| | **IMPC mouse production**<br>Chairs: Lauryl Nutter, Steve Murray |
| **08:45 – 09:05** | Overview of production and tech dev - Lauryl Nutter |
| **09:05 – 09:25** | Design QA and allele & mouse line QC pipelines -Lauryl Nutter |
| **09:25 – 09:45** | Mouse line QC – what about off-target mutagenesis? - Kevin Peterson |
| **09:45 – 10:15** | Community impact – distribution & collaboration - Josh Wood & Steve Murray |
| **10:15 – 10:45** | **Coffee break - Foyer Olympia Hall** |
| | **IMPC phenotyping and data analysis**<br>Chairs: Annie Mallon, Damian Smedley, Ann Flenniken and John Seavitt |
| **10:45 – 11:10** | Overview of the data in the database (from embryo to late onset) – Luis Santos |
| | **Summaries and discussions of phenotyping pipelines and platforms** |
| **11:10 – 11:25** | Late adult pipeline - Sara Wells, Hamed Haselimashhadi |
| **11:25 – 11:40** | Immunophenotyping - Lauryl Nutter |
| **11:40 – 11:50** | Pain assessment - Elissa Chesler |
| **11:50 – 12:05** | Metabolomics - Art Beaudet |
| **12:05 – 12:25** | Combined proteomics and metabolomics analysis - Dave Schibli, Oliver Fiehn |
| | **Lightning updates from other working groups** |
| **12:25 – 12:30** | Behaviour and sensory WG - Ann Flenniken |
| **12:30 – 12:35** | Morphology WG In Life - Heather Cater |
| **12:35 – 12:40** | Morphology WG End of Life - Colin McKerlie |
| **12:40 – 12:45** | Cardiac WG - Cory Reynolds |
| **12:45 – 12:50** | Metabolism WG - Jan Rozman |
| **12:50 – 12:55** | Embryo WG - Steve Murray |
| **13:00 – 14:00** | **Lunch break - IOANNIS Roof Garden Restaurant** |
| | **IMPC phenotyping and data analysis continued**<br>**Summaries of analyses, including papers in preparation** |
| **14:00 – 14:10** | Embryo image analysis - Henrik Westerberg |
| **14:10 – 14:20** | Multivariate analyses - Habib Ganjgahi |
| **14:20 – 14:30** | Behaviour paper - Elissa Chessler |
| **14:30 – 14:40** | Bone paper - Anna Swan |

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|---|---|
| **14:40 – 14:50** | Metabolomics paper - John Seavitt |
| **14:50 – 15:00** | Subviable paper - Hugh Morgan |
| **15:00 – 15:10** | Cardiovascular paper - Corey Reynolds |
| **15:10 – 15:20** | Conservation paper - Violeta Munoz-Fuentes |
| **15:20 – 15:50** | **Coffee break - Foyer Olympia Hall** |
| | **Data and programme integration** |
| **15:50 – 16:10** | Summary and discussion of data integration opportunities: from MARRVEL to MONARCH - Damian Smedley |
| **16:10 – 16:30** | IMPC and CMG: Formalizing partnership in advancing human mendelian genomics - Jennifer Posey, BCM |
| | **IMPC outreach and dissemination**<br>Chair: Terry Meehan |
| **16:30 – 16:45** | Update on IMPC Portal - Terry Meehan |
| **16:45 – 17:00** | IMPC website redesign - James Sudlow/Duncan Sneddon |
| **17:00 – 17:10** | Update on outreach - Amy Johnson |
| **17:10 – 17:30** | Update on publications and other metrics analyses - Sheethal Jose, Colin Fletcher |
| | **Closing remarks – Steve Brown** |
| **17:30** | **Panel of Scientific Consultants – private session (conference 4)** |
| **18:30** | **Feedback to IMPC Steering Committee (conference 4)** |
| **17:30 – 19:00** | **Poster session and networking reception, Foyer - Olympia Hall** |

# Program

| THURSDAY, NOV 16TH, 08:30-12:00, ROYAL OLYMPIC HOTEL, OLYMPIA HALL | |
|---|---|
| | **Responsible research - Contribution of large-scale mammalian resources to animal welfare and reproducibility**<br>Chair: Lluis Montoliu, Spanish National Research Council (CSIC) |
| **08:30 – 08:50** | Lluis Montoliu, CSIC-CNB - Fostering responsible research with genome editing technologies: a European perspective |
| **08:50 – 09:10** | Andy Greenfield, MRC Harwell - Editing mammalian genomes: ethical considerations |
| **09:10 – 09:30** | Martin Fray, MRC Harwell - Sharing mutations: Biobanks are still required in the post-CRISPR/Cas9 era |
| **09:30 – 09:50** | Kirsty Reid, EFPIA - Industry initiatives and contributions towards developing better disease models |
| **09:50 – 10:20** | **Keynote lecture**<br>Malcolm Macleod, University of Edinburgh - Data quality and reproducibility in preclinical research |
| **10:20 – 10:45** | **Coffee break - Foyer Olympia Hall** |
| **10:45 – 12:00** | **Stakeholder presentations**<br>Chair: Jesus Ruberte, Autonomous University of Barcelona<br><br>• **Raffaele Teperino, Helmholtz Zentrum München**<br>Multigenerational metabolic control: the role of Polycomb<br>• **Anniina Hiltunen, University of Oulu**<br>Mouse models in the study of FINLA disease and NHLRC2 gene<br>• **Amy Findlay, University of Edinburgh**<br>Characterisation of Retinal Degeneration in Idh3a Mutant Mice<br>• **Bruno Pereira, University of Porto, Institute for Research and Innovation in Health - IPATIMUP**<br>RNA-binding protein MEX3A role on intestinal stem cell homeostasis and implications for cancer<br>• **Binnaz Yalcin, IGBMC Strasbourg**<br>Large-scale neuroanatomical screen uncovers novel genes involved in brain morphogenesis<br>• **Maria Sakkou, BSRC Alexander Fleming**<br>A common mesenchymal-cell specific role for TNFR2 in the development of polyarthritis and comorbid heart valve stenosis |
| **12:00** | **Meeting Summary / Wrap-Up**<br>Radislav Sedlacek & Dimitris Kontoyiannis |
| **12:00 – 13:00** | **Lunch - IOANNIS Roof Garden Restaurant** |

INFRAFRONTIER mouse disease models

IMPC International Mouse Phenotyping Consortium

"ALEXANDER FLEMING" Biomedical Sciences Research Center

**Royal Olympic Athens**
http://www.royalolympic.com/

**Location**
http://www.royalolympic.com/athens_center_hotel

**How to get there**
From Athens International Airport "Eleftherios Venizelos"

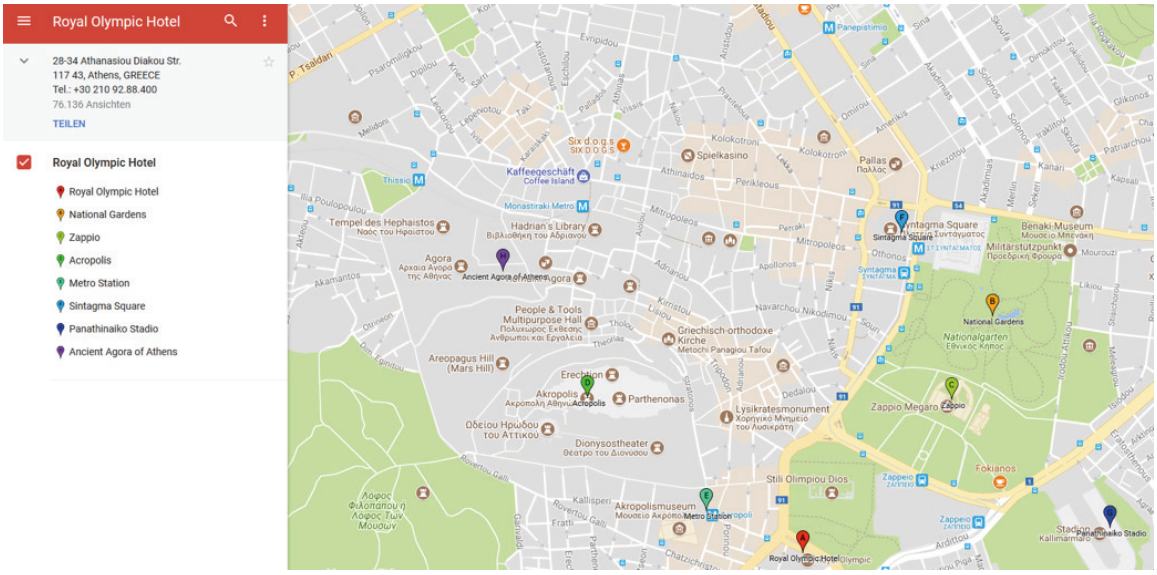
**By METRO / UNDERGROUND**
You will board the Metro from the Airport's Station and get off at Syntagma Station. At Syntagma Station you switch lines in the direction of Elliniko and get off at the first Station, the "Acropolis Station". From Acropolis Station the Hotel is 100m away, walking along Ath. Diakou Street. Cost one way is 10€.

**By TAXI**
Royal Olympic Hotel can arrange transportation from the Airport to the Hotel and vice versa for the dates you wish, with 1 a/c Mercedes taxi and an English speaking driver, upon arrival.

- Cost one way, between 06:00 - 23:00 is 60€.
- Cost one way, between 23:00 - 06:00 is 70€.

**Alternative TAXI options to and from the airport**
http://www.aia.gr/traveler/access-and-transportation/taxi-and-limousine/

**Public transport**
http://www.aia.gr/traveler/access-and-transportation/transportation/public-transportation
http://www.athensairportbus.com/en/metro/airportticket.html



# List of Participants

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| Elissa | Chesler | The Jackson Laboratory | USA |
| Jacqueline | White | The Jackson Laboratory | USA |
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| Anita | Bandrowski | UCSD/SciCrunch | USA |
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# Abstracts: Stakeholder presentations

- Raffaele Teperino, Helmholtz Zentrum München - Multigenerational metabolic control: the role of Polycomb
- Anniina Hiltunen, University of Oulu - Mouse models in the study of FINLA disease and NHLRC2 gene
- Amy Findlay, University of Edinburgh - Characterisation of Retinal Degeneration in Idh3a Mutant Mice
- Bruno Pereira, University of Porto, Institute for Research and Innovation in Health - IPATIMUP - RNA-binding protein MEX3A role on intestinal stem cell homeostasis and implications for cancer
- Binnaz Yalcin, IGBMC Strasbourg - Large-scale neuroanatomical screen uncovers novel genes involved in brain morphogenesis
- Maria Sakkou, BSRC Alexander Fleming

**Multigenerational metabolic control: The role of Polycomb**

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Diabetes and Obesity are complex diseases with multifactorial aetiologies, involving both genetic and environmental components. While Genome Wide Association Studies (GWAS) have established a genetic framework for our current understanding of diabetes and obesity, little is known about the contributions of additional regulatory layers, especially epigenetics.

Polycomb and Trithorax Group (PcG/TrxG) proteins were first identified in Drosophila for their roles in silencing homeotic genes and are now recognized as constituting a chromatin-based transcriptional regulatory system with key roles in multicellular development, stem cell biology and cancer. Perhaps the most striking feature of PcG proteins is their ability to effect cellular memory, that is, to transmit silenced gene expression states through mitosis. ChIP-seq profiling have highlighted that PcG targets show a very strong bias for genes controlling development and cell fate decisions. Interestingly, Gene Ontology analyses have also revealed Cellular Metabolism as one of the top GO terms for PcG protein-bound regions.

Using mouse genetics coupled to in-depth phenotyping and state-of-the-art genomics, we have shown that PcG controls metabolism in mouse. Here, we will present our most recent data on the role of PcG in controlling metabolism across multiple generations.

**Mouse models in the study of FINLA disease and NHLRC2 gene**

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Our group has discovered a novel FINLA disease which causes FIbrosis, Neurodegeneration and Leptomeningeal Angiomatosis (Uusimaa et al. under review). Whole exome sequencing of three pediatric patients suffering from this fatal cerebropulmonary disease revealed a mutation in the NHLRC2 gene. The function of NHL repeat containing protein 2 is currently unknown and it has not been linked to any other human disease previously.

To study the function of Nhlrc2 in vivo, heterozygous C57BL/6N-A$^{tm1Brd}$ Nhlrc2$^{tm1a(KOMP)Wtsi}$/Wtsi mice were obtained from Infrafrontier-EMMA repository (strain number EM:10219). These mice carry the knock out (KO) first allele with a LacZ reporter tagged insertion. Homozygous Nhlrc2 KO pups were not born suggesting embryonic lethality. We proceeded to analyze embryonic day (E) 10.5 embryos and subsequently E2.5 morulae. No homozygotes were detected which leads us to conclude that Nhlrc2 has critical importance in early development in mouse. Heterozygotes were completely healthy and X-gal staining showed widespread Nhlrc2 expression throughout the body at E 14.5 in these mice.

To circumvent the early lethality, we have crossed the Nhlrc2 KO mouse line with a flippase expressing mouse to gain a conditional mouse line. We are currently selecting and testing an appropriate Cre mouse line. In the meantime we are using in and ex utero electroporation (IUE and EUE) methods to obtain data on the role of Nhlrc2 on the developing brain. In IUE and EUE the neuronal progenitor cells in the ventricular zone of the conditional Cre-inducible KO embryos are transfected with pGAC-Cre-IRES-GFP plasmid. The same method can also be used for over-expression studies by using expression plasmid containing the Nhlrc2 transgene. IUE preserves the viability of the embryo, since only a small population of cells is affected. Phenotype of the transfected neurons can be studied in dissociated neuronal cell culture, organotypic slice culture and in vivo. GFP-expression of the transfected cells enables time laps imaging and fluorescence-activated cell sorting of the cells for further analyses.

Recently, we have generated a point mutant mouse line carrying exactly the same mutation than in the FINLA patients to study the mechanism behind the disease. The point mutant mouse was generated by microinjection of preassembled crRNA/tracrRNA/Cas9 protein complex together with single-stranded oligodeoxynucleotide (ssODN) as a HDR-template to introduce the point mutation, into mouse zygotes. Two promising founder mice were obtained and they are currently

bred to verify the correct insertion of the ssODN and germline transmission.

The complete loss of Nhlrc2 causes early preimplantation lethality in mice. Traditional mouse models in combination with flippase and Cre recombinase systems allow us to bypass the early lethality and to study the tissue specific function of the protein. However, this takes time and resources. Meanwhile, IUE and EUE provide us with a fast and versatile tool for studying the effects of the loss of Nhlrc2 on neurogenesis already in the conditional mouse. To further study the pathomechanism behind the FINLA disease we have utilized the CRIPS/Cas9 system to generate a mouse model with the same point mutation seen in the patients.

**Characterisation of Retinal Degeneration in Idh3a Mutant Mice**

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Isocitrate dehydrogenase (IDH) is an enzyme required for the production of α-ketoglutarate from isocitrate. IDH3 generates the NADH used in the mitochondria for ATP production, and is a tetramer made up of two α, a β and a γ subunit. Loss of function and missense mutations in both IDH3A and IDH3B have previously been implicated in families exhibiting retinal degeneration. A child homozygous for an IDH3A mutation has also been described with severe neurological disease, including retinal degeneration. Using mouse models we have investigated the role of IDH3 in retinal disease and mitochondrial function.

We identified mice with late-onset retinal degeneration in a screen of ageing mice carrying ENU-induced mutations. Low-resolution mapping followed by whole genome sequencing found a missense mutation in the α-subunit gene, Idh3a (E229K). Mice homozygous for this mutation, exhibit signs of retinal stress, indicated by GFAP staining, as early as 3 months and photoreceptor degeneration from 7 months, but, as with the human IDH3A mutations, no other tissues appear to be affected. We used CRISPR/Cas9 technology to produce a knockout of Idh3a and find that homozygous mice do not survive past early embryogenesis. $Idh3a^{E229.K/-}$ compound heterozygous mutants exhibit a more severe retinal degeneration when compared to $Idh3a^{E229K/E229K}$, showing severely deteriorated ERG responses by 2 months, accompanied by photoreceptor loss and GFAP staining. Analysis of mitochondrial function, using Agilent Seahorse technology, on MEF mutant cell lines highlighted a reduction in both mitochondrial maximal respiration and reserve capacity levels in both $Idh3a^{E229K/E229K}$ and $Idh3a^{E229K/-}$ cells.

It has been previously reported that the retina operates with a limited mitochondrial reserve capacity and we suggest that this, in combination with the reduced reserve capacity in mutants, explains the degenerative phenotype observed

**RNA-binding protein MEX3A role on intestinal stem cell homeostasis and implications for cancer**

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**INTRODUCTION:** Stem cells play a fundamental role in replacing multiple cell types throughout life in nearly all adult tissues, but their exclusive and powerful ability for self-renewal is also replicated in cancer. We have previously shown that the human RNA-binding protein MEX3A regulates intestinal differentiation, cell polarity and stemness associated with gastrointestinal carcinogenesis. By in situ hybridization (ISH) we determined that Mex3a mRNA expression is accumulated at the base of the crypts of the small intestine and colon. Our aims are to determine if MEX3A protein constitutes a novel intestinal stem cell (ISC) marker with functional relevance for intestinal development and disease.

**MATERIAL AND METHODS:** To track MEX3A major biological functions, we are characterizing the very first knockout mouse model harboring a Mex3a targeted deletion established under the framework of the European INFRAFRONTIER-I3 call. These mice are currently being interbred with an Lgr5-EGFP knock-in strain in which EGFP fluorescence is observed in ISCs of the small intestine. In parallel, we are establishing ex vivo cultures of murine intestinal organoids by isolating intestinal crypts from mice with different genotypes. This system allows long term maintenance of crypt-villus axis functional equivalents and their interrogation concerning alterations in relevant signaling pathways.

**RESULTS AND DISCUSSION:** Homozygous mutant mice present progressive weight loss, lethargy, and dehydration signs between postnatal day (P)14 and P25. Histological analysis of the Mex3a-/- mice intestine revealed crypt atrophy, with a strong decrease in Paneth cell number and a reduction in proliferating cells. At the villi level, we noticed that Mex3a-/- mutant mice retained suckling-type enterocytes at a time when these had already been replaced by adult enterocytes in controls. This feature was confirmed by electron microscopy, which demonstrated that Mex3a-/- enterocytes had smaller lysosomal vacuoles with multi-lamellar structures often in the form of whorls of concentric rings. These presumably defective cells might compromise nutrient uptake, causing the observed weight loss and death by malnutrition. All these morphological

alterations could be explained by delayed epithelial cell turnover, driven by abnormal ISC dynamics. Strikingly, ISH for Olfm4, a standard ISC marker, showed a significant reduction in ISCs number. Notwithstanding, the remaining Mex3a-/- ISCs can still originate “mini-guts”, but these are reminiscent of fetal spheroids, suggesting that MEX3A might be determinant for the adult stem cell fate. To further validate the results, we crossed our Mex3a+/- mice with an Lgr5+/EGFP knock-in mouse, in which GFP is under the transcriptional control of the endogenous Lgr5 promoter, acting as a reporter of LGR5+ cells. Interestingly, already 15% of our Mex3a+/-;Lgr5+/EGFP heterozygous compound mice displayed postnatal lethality with a similar intestinal phenotype as described above, another strong indication that MEX3A and LGR5 act upon the same cellular context.

**CONCLUSION:** Together, these results clearly demonstrate that MEX3A is determinant for the maintenance of LGR5+ ISCs with an impact in intestinal homeostasis and possibly its regenerative potential.

**Large-scale neuroanatomical screen uncovers novel genes involved in brain morphogenesis**

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**Abstract**
To understand the genetic basis of mammalian brain development, we analyzed neuroanatomical parameters in 1,316 IMPC mouse mutant lines. We identified 158 genes whose disruptions yielded NeuroAnatomical Phenotypes (NAP), mostly affecting the commissures and the ventricles. These mutations perturb the overall equilibrium of the brain architecture. Groups of functionally similar NAP genes participate in shared neural pathways involving the cell cycle, the cytoskeleton and importantly, the synapse, displaying distinct fetal and postnatal expression dynamics. Overlap analysis with human intellectual disability genes accompanied by brain malformations showed corresponding defects in 71% of mouse NAP genes. Human orthologues of NAP genes appear to be negatively selected but 81% have not yet been associated with human cognitive disorders, constituting a new pool of candidate genes testable against human brain diseases. Our resource provides the first comprehensive map of mouse NAP genes and predicts that at least 3,000 genes could be involved in brain morphogenesis.

**A common mesenchymal-cell specific role for TNFR2 in the development of polyarthritis and comorbid heart valve stenosis**

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Mesenchymal TNF signaling is etiopathogenic for inflammatory diseases such as Rheumatoid arthritis (RA) and spondyloarthritis (SpA). The role of TNFR1 in arthritis has been well documented; however, mesenchymal TNFR2 functions are unknown. Our objective is to investigate the mesenchymal-specific role of TNFR2 in the TnfΔARE mouse model of polyarthritis and spondyloarthritis (SpA), and of the described heart valve stenosis comorbidity by cell specific, Col-VI mediated, gene targeting. Interestingly, we find that mesenchymal TNFR2 signaling is detrimental for both arthritis and valve stenosis pathologies, however systemic TNFR2 appears to be protective since its complete deletion leads to severe deterioration of both phenotypes. We identify that isolated mesenchymal cells from joints (SFs) and aortic heart valves (VICs) lacking TNFR2, fail to acquire pathogenic “activated phenotypes” and display increased expression of anti-inflammatory cytokines associated with decreased AKT signaling. Comparative RNAseq experiments showed that the majority of the deregulated pathways in TnfΔARE mesenchymal-origin SFs and VICs, including proliferation, inflammation, migration and disease-specific genes, are regulated by TNFR2, thus in its absence they are maintained in a quiescent non-pathogenic state. Moreover, we demonstrate that arthritis and co-existent valve stenosis share common cellular mechanisms converging at the mesenchymal TNF/TNFR2 dependent activation. Our data indicate a pleiotropy of TNFR2 functions, with mesenchymal TNFR2 driving cell activation and arthritis/ valve stenosis pathogenesis only in the presence of systemic TNFR2, whereas systemic non-mesenchymal TNFR2 overcomes this function providing protective signals and containing both pathologies.

**1) Individual zygote electroporation of Cas9 RNA-guided nuclease (RGN) for efficient generation of exdels in mice**

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Delivery of charged molecules across a plasma membrane by application of an electric field, a process known as electroporation, has been demonstrated. Traditionally, electroporation is used to facilitate delivery of molecules into thousands or even millions of cells ("bulk"-electroporation, or bEP). These cells are placed between two large electrodes and electric fields are applied in a series of pulses. The measure of success of bEP is a balance between cell survival and efficiency of material delivery. To increase survival and delivery efficiency, we collaborated with Ravata and worked to validate their technology, the Ravata Individual Zygote Electroporator (RIZE), which enables electroporation of single zygotes in parallel. To assess the effectiveness of this new technology under high-throughput conditions, we compared the efficiency of delivering Cas9 RNA-guided nuclease (RGN) into mouse zygotes using bEP vs RIZE in our KOMP2 production pipeline. We tested increasing durations of electric fields compared to our current protocol (18ms across 6 pulses) using bEP. For 8 unique gene targets, at a total duration of 60ms with the same electric field as bEP, we found that zygote recovery, litter rate, and litter size were not significantly different using bEP or RIZE. However, the proportion of pups with genotype-confirmed exdels was higher using RIZE (19% of liveborn offspring) compared to bEP (11% of liveborn offspring). Further, using RIZE we were able to subject zygotes to the same electric field as bEP for 90ms (5X longer than our current protocol) without negatively impacting viability, litter rate, or litter size. Under these conditions, the proportion of exdels was twice that using RIZE (32%) compared to using bEP (15%). Similarly, the number of lines that generated a mutant founder on the first attempt (n=14) was significantly higher using RIZE (93%) compared to bEP (58%). These studies indicate that RIZE is a highly efficient technology for genome editing using RGN in mouse zygotes.

**2) Efficient genome-editing of rodent models using CRISPR-Cas9 by high throughput zygote electroporation**

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The emergence of CRISPR-based endonuclease technology represents a paradigm shift in our approach to generating animal models that precisely recapitulate human disease. Several methods for employing CRISPR-Cas9 with varying levels of success have been developed. We hypothesize that optimizing the preparation and delivery of CRISPR-Cas9 will increase the efficiency of generating mutant rodent models. To test this hypothesis, we compared the effects of continuous strand (single guide, sgRNA) vs two separate strands (paired guide, pgRNA), Cas9 mRNA vs Cas9 protein, microinjection vs electroporation, and mixed embryo vs single gene embryo transfer to generate ExDels (exon deletion mutants) and SNPs (single nucleotide polymorphisms). Our ex vivo results demonstrated that Cas9 nuclease preparation and electroporation had the largest impact on the generation of ExDels. To compare gRNA and Cas9 preparations, we tested 12 randomly selected genes and found that for microinjection pgRNA with Cas9 protein was superior to other component combinations. Further, we found that electroporation was significantly more efficient for generating ExDel mutants and SNPs via oligo insertion (ssODN). We then compared Cas9 protein by electroporation at both 8uM and 16uM to edit 63 and 33 genes, respectively, and found that 16uM protein was 1.6 fold more efficient than 8uM. We did not observe a significant variation in the survival rate, litter rate, litter size, or mutant rate between multiplex vs single gene embryo transfer. Although there was no difference in the ExDel success rate (ExDels per live pup born) by electroporation of 16uM Cas9 with pgRNA (31% of 187 live pups for 23 genes) vs sgRNA (28% of 79 live pups for 10 genes), both methods were significantly better than microinjection of Cas9 mRNA with sgRNA (11% of 2,008 live pups for 226 genes, a nearly 3 fold difference. In addition, genome editing by zygote electroporation of CRISPR components reduces animal use and production costs by more than 3,500 mice annually (>40%). For these reasons, we have implemented zygote electroporation as the primary strategy for producing ExDel mutants for phenotyping in our KOMP2 project.

**3) Humanized mouse models advance precision medicine: Generating disease phenotype models in mice by CRISPR/Cas9 zygote electroporation and microinjection**

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Clinically relevant animal models are a vital resource to interrogate disease mechanisms in patients. Genome editing technologies using CRISPR/Cas9 allow for editing of endogenous genes and insertion of human coding regions into animal models with which to mimic human phenotypes and bridge the gap between benchtop research and clinical outcomes. We have used CRISPR/Cas9 to generate several mouse models that mimic human disease and specific patient phenotypes. For example, in our KOMP2 pipeline, we have generated an exon deletion (ExDel) mutation in the sorting nexin 10 gene (Snx10) in mice which resulted in bone fragility and failure of tooth eruption that is similar to the osteoporosis seen in humans with SNX10 mutations (OMIM 614780). Other examples in genome-edited mice include ExDel mutations for the androglobin gene (Adgb) causing congenital hydrocephalous and for hedgehog acyltransferase-like gene (Hhatl) causing muscular skeletal deficits. Humanization experiments using CRISPR/Cas9 and single strand DNA oligonucleotide (ssDNA) to elicit genome editing via HDR targeting other heritable alleles (e.g., Alzheimers Disease) are underway. Precision models produced by a combination of genome editing and allelic humanization using CRISPR/Cas9 serve as clinically valuable animal models to confirm disease causation, understand disease pathophysiology, and use as surrogates for development and testing treatment strategies in individual patients.

**4) Generation of a patient-specific rodent model of desminopathy using CRISPR/Cas9 electroporation of rat zygotes**

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Using patient-derived genetic and molecular profiles to develop a patient-specific animal model is a key step in the implementation of precision medicine. We used CRISPR/Cas9 technology to recapitulate in rats patient-specific genomic variants to create a model of Desmin-Related Myofibrillar Myopathy (DRM). DRM, or desminopathy, is a heritable myopathic disease caused by mutation in the gene Desmin (Des) that encodes for a protein that regulates muscle structure and function. In humans, mutations in Des result in contraction-induced skeletal muscle injury, idiopathic cardiomyopathy, arrhythmogenic right ventricular cardiomyophathy (ARVC) and premature death. Because rats are often better models of human neuromuscular disease than mice, we set out to humanize the Des gene with bi-locus heritable SNVs (single nucleotide variants) of nucleotides AG with CC at position 1036-1037 in exon 6 that were identified in a proband and family members with DRM to recapitulate the desminopathy phenotype. In parallel experiments, electroporation and microinjection was used to introduce Cas9 ribonucleoprotein (RNP) and single strand DNA oligonucleotide (ssDNA) encoding the human genomic sequence with these two SNVs into zygotes of wildtype Sprague-Dawley rats. Homology directed repair (HDR) resulted in a humanized (h)Des male rat, which transmitted the allele through the germline to 5 offspring (2 males, 3 females). Phenotypic characterization of the hDes rat is pending. In conclusion, we successfully reproduced the specific familial Desmin mutation by CRISPR/Cas9 genome editing using zygote electroporation in rats. Additionally, we demonstrated the ability to establish a line of Desmin rats using ssODN assisted HDR by electroporation and subsequent breeding.

**5) Genome editing in Rats: generation of a target point mutation by Pronuclear or Cytoplasmic Injection or by Electroporation of CRISPR/Cas9**

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CRISPR/Cas9 system has become a powerful and efficient tool to edit genomes. It can be applied on biomedicine for the development of new gene therapy approaches, and in biotechnology for the generation of new genetically modified organisms. It allows to produce knockout and knockin models avoiding need of embryonic stem cells (ESC) for gene targeting by homologous recombination and opening the possibility of genome edition for all animal species.

Rats are physiologically, genetically and morphologically closer to humans, have a well-characterized and rich behavioral display for neurological studies, and have larger size than mice. But the difficulty of working with rat ESC has limited the generation of genetically modified rat models. CRISPR system has overcome these troubles and allows genome editing by direct injection of one cell embryos.

Here we show and compare the results obtained in the generation of a rat model carrying a point mutation by pronuclear or cytoplasmic microinjection or by electroporation of CRISPR system in one cell embryos.

**6) Extending the range of alleles obtained using CRISPR/Cas9 technology in mouse embryos: Generation and validation of mutations**

**Gemma Codner***, Fran Pike, Joffrey Mianne Adam Caulder, Ruairidh King, Rachel Fell, Alasdair Allan, Jorik Loeffler, Skevoulla Christou, Christopher McCabe, MLC Gene Delivery team, Marie Hutchison, Michelle Stewart, Sara Wells, Lydia Teboul

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Mouse models are valuable tools to understand genes functions, genetic diseases and to develop and test new therapeutic treatments in vivo. The ability to introduce tailored modifications within the mouse genome is essential to generate them. The CRISPR/Cas system has brought new perspectives for the generation of mouse models in a more efficient and precise fashion, at reduced price, all within a shorter time scale. Here we report the use of the CRISPR/Cas9 technology at the Mary Lyon Centre, MRC Harwell Institute, to introduce a wide range of modifications within the mouse genome through different methods. We first present our high throughput mouse production pipeline that generates alleles containing indels, tailored deletions or point mutations through direct injection into zygotes. We report the use of the CRISPR/Cas9 technology to engineer and enhance the genetic background of the C57BL/6 N mouse strain by correcting mutations in the Cdh23 and Crb1 genes and show the technology can also generate imperfect alleles. Finally, we present data obtained for enhancing the homologous recombination capacity in one-cell embryos through the use of long single stranded-DNA and the strategy for validation of such mutations.

Developing these methods and tools for genome engineering will enable the generation of a range of increasingly complex alleles in mice.

**7) CRISPR/Cas9 for in vivo gene editing: the hidden face of paradise**

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The efficiency and simplicity of CRISPR/Cas9 technology to make precise changes to the genome of living cells has led to new revolution in genetics.

This approach is now driving innovative applications from basic biology to biotechnology and medicine. To date, the Streptococcus pyogenes Cas9 (SpCas9) has been used broadly to achieve efficient genome editing in a large variety of species and cell types, including human cell lines, bacteria, zebrafish, yeast, mouse, fruit fly, roundworm, rat, common crops, pig, and monkeys.

The molecular mechanisms at the origin of the correction of CRISPR/Cas9 target double-strand breaks are mediated by the cell DNA repair machinery. However, those DNA repair mechanisms are highly complex and poorly amenable to control. As a result, targeted alleles with additional modifications, such as deletions, partial or multiple integrations of the targeting vector, duplications and more drastic rearrangements can be observed. Moreover, mosaicism taking place at preimplantation development stage renders the identification of unwanted genomic modifications at targeted locus very challenging in F0 generation animals. Finally, dsODN and ssODN used for the generation of Kin models can integrate randomly in the genome thus bringing additional complexity to resulting genotypes.

Many articles have emphasized the easy to use nature of the CRISPR/Cas9 system but few have explained the complexity of generated genotypes and the impact of CRISPR/Cas9 double strand breaks and downstream repair mechanism on the cell genome. Here, our aim is to illustrate the complexity of genotypes resulting from application of CRISPR/Cas9 through presentation of in vivo and in vitro gene editing data in mouse and/or rats. The data will be discussed in light of application of CRISPR/Cas9 to in vivo gene editing in mouse and rats and how it can impact safety of genetic therapy in humans.

**8) CRISPR/Cas9 mutant mouse model creation through in vitro fertilization and zygote electroporation**

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CRISPR/Cas9 has been widely adapted for animal model creation, while the standard protocol for CRISPR-based genome editing in mice still relies on microinjection into one-cell stage zygotes. By using electroporation of CRISPR components into zygotes with intact zona pellucida in combination with in vitro fertilization (IVF) technique, we succeeded in introducing non-homologous end joining (NHEJ)-mediated indels, exon deletions, and point mutations in the C57BL/6N mouse genome. We extended our approach to frozen/thawed zygotes which were obtained by administration of inhibin antiserum (IAS) and equine chorionic gonadotropin (eCG, known as IASe superovulation) followed by IVF, resulting in decrease in the number of oocyte donors. Introduction of mutations into mouse zygotes by electroporation can be a convenient and efficient alternative to microinjection for mouse model creation.

**9) Towards an optimized workflow for CRISPR/Cas mediated mouse transgenesis**

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In contrast to conventional embryonic stem cell transgenesis the use of site-directed DNA endonucleases in mouse zygotes has enabled fast generation of mouse models with scarless introduction of specific mutations, allowing to precisely recapitulate the genetic background of human diseases. Due to its simplicity in design, economic advantage and exceptional efficiency, the CRISPR/Cas9 system has become the method of choice for genetic editing in mice and other species. We have established a standardized workflow for cloning-free CRISPR/Cas9-mediated mouse transgenesis in strain C57Bl/6 mice via pronuclear injection in our facility. By now we have successfully generated more than 100 mutant mouse lines with genetic modifications of various complexity ranging from simple knock-outs and introduction of point mutations to multiplex knock-outs and introduction of entire transgenes. Nevertheless, conventional pronuclear injection is not only technically demanding but also invasive and restricted to zygotes with visible pronuclei. To overcome these limitations, we have recently explored electroporation as an alternative route to deliver CRISPR/Cas9 components and DNA repair templates into mouse zygotes. In order to maintain an easily adaptable workflow we employed a standard electroporation system (Bio-Rad Gene Pulser XCellTM) and used intact zygotes to omit delicate zona pellucida weakening.

To determine differences in embryo viability, we compared the competence of C57Bl/6 zygotes to form blastocysts upon electroporation or pronuclear injection. Embryo viability significantly improved. In fact, electroporation had no effect on development and is even comparable to the development of non-treated zygotes, proving that electroporation is less harmful than pronuclear injection. We also investigated the ability to modify zygotes without visible pronuclei via electroporation, whose presence is a requirement for pronuclear injection. Indeed, electroporation resulted in similar ratios of transgenic blastocysts compared to pronuclear injection demonstrating that this technique is less selective regarding the embryonic stage while retaining full efficiency of transgenesis.

Hence, our results highlight electroporation as a promising route for less harmful and selective CRISPR/Cas9 mediated mouse transgenesis and demonstrate its potential to profoundly decrease animal numbers.

**10) CRISPR and the 3Rs - reducing animal numbers in high-throughput mouse production**

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The CRISPR/Cas9 system has revolutionised the way in which researchers study genetics and biological processes. Due its ease in disrupting gene function it is now a major part of the continuing IMPC project to knock out all protein-coding genes in the mouse genome. One of the main attractions of CRISPR is the greatly-reduced time needed to create mutant mouse models, as experiments can be performed directly in mouse zygotes without the need for gene-targeting in ES cells.

By comparing our existing ES cell-based high-throughput mouse production to our new CRISPR pipeline, we show that the germ line transmission rate is much more predictable using CRISPR. This has allowed us to optimise many aspects of our mutant founder production, including PCR screening, embryo transfer numbers and founder mating numbers. F0 mosaics transmit the mutation over a much wider range of percentage mutagenesis than their ESC-chimera counterparts.

We demonstrate that in addition to efficiency gains in cost and time, there is also a significant reduction in animals required to achieve GLT. When applied to the scale of projects such as the IMPC, the benefit from a 3Rs-perpective is enormous.

**11) Generation of new CRISPR-edited mouse models for investigating non-syndromic types of albinism**

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Albinism is a rare human genetic condition characterized by severe visual abnormalities (foveal hypoplasia, abnormal ganglion cells chiasmatic projections, nystagmus, photophobia and iris transillumination) that may be often, but not always, presented with associated skin, hair and eye hypopigmentation, and affects approximately 1:17,000 newborns. Albinism is genetically heterogeneous. Mutations in at least twenty genes are associated with the corresponding types of albinism (Montoliu et al. PCMR 2014; Montoliu and Marks, PCMR 2017). Additional forms are being characterized and studied within the albinism spectrum of cases, such as FHONDA (Montoliu and Kelsh, PCMR 2014) and other gene candidates. Historically, we have been using genetically modified mouse models to investigate OCA1 variants (Giraldo and Montoliu, PCMR 2002; Lavado and Montoliu, Front. Biosci. 2006; Murillo-Cuesta et al., PCMR 2010) associated to mutations in coding and non-coding genomic sequences, using artificial chromosome-type transgenes. More recently, we have been using CRISPR-Cas gene-editing approaches (Seruggia and Montoliu, Transgenic Res. 2014; Mojica and Montoliu, Trends in Microbiology 2016) to functionally assess the relevance of DNA regulatory elements in the tyrosinase gene (Seruggia et al. Nucleic Acids Res. 2015). We are now extending the use of CRISPR technology to generate multiple new mouse mutants carrying patient-specific mutations (avatar mice) in which we will be able to investigate the molecular underlying mechanism causing the phenotypic alterations observed in all these cases of albinism. These CRISPR-edited mice will be instrumental to devise adapted therapeutic approaches by which we could possibly treat or alleviate the visual abnormalities found in this human rare disease.

**12) Comorbid TNF-mediated heart valve disease and chronic polyarthritis share common mesenchymal aetiopathogenesis.**

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**Objectives**
Rheumatoid arthritis (RA) is a chronic condition characterized by prolonged inflammation of the joints leading to bone and cartilage destruction. The key molecular and cellular regulators of RA pathology are the Tumor Necrosis Factor (TNF) and the mesenchymal-origin Synovial Fibroblasts (SFs), respectively. Apart from the joint pathology, RA patients also show higher morbidity rates, mainly due to the development of extraarticular conditions including cardiovascular, gut and skin disease manifestations. The Tg197 arthritis model develops TNF-driven and mesenchymal synovial fibroblasts (SFs)-dependent polyarthritis. Here, we investigate whether this model develops, similarly to human patients, co-morbid heart pathology and we explore cellular and molecular mechanisms linking arthritis to cardiovascular comorbidities.

**Methods**
The established human TNF-transgenic model of arthritis(Tg197) was used to evaluate possible arthritis-related cardiovascular disease. We further studied the role of Valve Interstitial Cells(VICs) in the pathology, using the ColVI-Cre mouse which specifically targets mesenchymal cells. Tg197 ColVI-Cre Tnfr1fl/fl and Tg197 ColVI-Cre Tnfr1cneo/cneo mice were used to explore the role of mesenchymal TNF signaling in the developing heart valve disease. Similarities of pathogenic VICs and SFs were analysed by RNA-sequencing.

**Results**
Tg197 mice develop left-sided heart valve disease, characterized by valvular fibrosis with minimal signs of inflammatory cell infiltration and thickened areas, consisting almost entirely of ColVI-expressing mesenchymal VICs, indicating proliferation of this cell type as a hallmark of the observed phenotype. Development of the pathology results in valve stenosis and left ventricular dysfunction, accompanied by arrhythmic episodes and, occasionally, valvular regurgitation. TNF-dependency of the pathology was indicated by the disease amelioration following pharmacological inhibition or genetic ablation of TNF-signaling. Interestingly, Tg197-derived VICs exhibited an activated phenotype ex vivo, resembling the activation of pathogenic Tg197-derived SFs. A significant functional correlation between these two mesenchymal cells was further supported by RNA-seq analysis, suggesting common pathogenic cellular mechanisms operating in arthritis and cardiovascular comorbidities.

**Conclusions**
TNF-mediated joint pathologies, commonly associated with comorbid heart halve disease, are efficiently modeled in Tg197 arthritis model and are commonly underlined by mesenchymal cell-specific pathogenic mechanisms.

**13) Preclinical evaluation of polyethylenimine-mediated RNA interference of Polo-Like Kinase 1 gene for ultrasound image-guided treatment of hepatocellular carcinoma**

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Virus-induced chronic hepatitis is a leading cause of hepatocellular carcinoma (HCC) in France and Europe. In the developed Western world, only 10-15% of cases can be attributed to hepatitis B virus (HBV) infection, while chronic hepatitis C appears to be the major risk factor for HCC (up to 70% of cases) in Europe. Indeed, HCC is the second cause of cancer death worldwide, and due to limited treatments options, the 5-year overall survival rate is <20%.

Unsatisfactory therapeutic options are due to several hurdles, such as a poorly understood pathogenesis, heterogeneity of HCC, limited number of targets and small animal models only partially addressing pathogenesis. Our goal within the TheraHCC program was to develop relevant animal models for pathogenesis and preclinical therapeutic studies. In particular, Ultra-Sound (US) image-guided orthotopic injection of human HCC tumor cells in the liver parenchyma has been set-up and validated. In a first series of experiments, US image-guided injection of Huh-7 cells in immunodeficient mice was successfully performed, leading to tumor engraftment in the targeted hepatic lobe with a similar efficacy as after injection under laparotomy.

In a second series of experiments, we evaluated in vivo a therapeutic innovative approach based on image-guided local delivery of Doxorubicine or siRNA targeting the Polo-Like Kinase 1 (PLK1) using a polyethylenimine (PEI)-based nanovector. We were able to show improved efficacy of doxorubicin upon local versus IP administration, strong tumor-growth reduction and a potent antitumor effect of the PLK1 nanovector.

The use of US imaging for intra-hepatic or intra-tumoral injections allows us to refine this model, in agreement with the 3R rules of animal experimentation. These data also provide support to the in vivo applicability of PLK1 nanovector administration for HCC treatment and hints for further developments.

**14) Strengthening the neuromuscular junction as a concept for the treatment of congenital myasthenic syndromes and motor neuropathies with synaptic dysfunction**

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Congenital myasthenic syndromes (CMS) arise from disruption in the development and maintenance of the neuromuscular junction (NMJ), through mutations in the genes encoding its components. Impaired neurotransmission and muscle weakness are characteristics of these disorders; current treatments for these conditions are limited. The Neuronal factor agrin and its downstream pathway are vital in the development and maintenance of the NMJ. Neurotune AG has developed a modified form of agrin (NT-1654) which has been shown to stimulate the NMJ development pathway.

The aim of this project is to test NT-1654 in mouse models of human CMS and motor neuropathies, and provide evidence for its use as a therapeutic compound in humans. We have conducted dose finding studies to identify safe, non-toxic concentrations of the drug in two animal models of CMS: $COLQ^{(-/-)}$, and $Agrin^{(nmf380)}$, and one of hereditary neuropathy: $GARS^{(C201R)}$. Daily subcutaneous injections of either 1mg/kg, 5mg/kg or 10mg/kg of NT1654 or vehicle were administered and animals monitored for signs of adverse reactions and toxic effects. So far all animals have all tolerated the drug well, with no adverse changes being noticed. Full studies administering NT1654 have now been initiated using these three animal models. Preliminary data regarding body weight, grip strength, swallowing ability, and survival will be presented. It is hoped that these investigations will provide the rationale for beginning human trials of NT1654 in patients.

**15) Effect of overexpressing the human G51D alpha synuclein in a novel generation of knock-in rats**

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Parkinson´s disease is a multifactorial disorder characterized by the presence of aberrant aggregate primarily formed by the protein alpha-synuclein.

Rodent model of the disease are classically generated by overexpressing wild-type or mutated human SNCA (gene encoding for alpha-synuclein SNCA) and only reproduce a weak phenotype with a slow progression.

This absence of strong phenotype might be due to the presence of the endogenous snca gene which might interfere with the aggregation of the human protein.

We generated a KI rat model by disrupting the endogenous snca gene by inserting a coding-codon optimized human SNCA cDNA harboring protein modifications reported to promote alpha-synuclein related phenotype (G51D mutation and a truncation at the amino acid 111).

The validation of the model was performed by using immunoblotting and immunohistochemistry and highlighted the presence of the transgene throughout the brain without signs of aggregation.

As the strategy to generate this novel model has been reported to work for different loci, we suggest that the humanization of the SNCA locus needs optimization in order to model elevated expression of the transgene and therefore lead to a pronounced synucleinopathy.

**16) Myeloid Trib1 dependent regulation of tissue specific and systemic phenotypes**

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Chronic inflammation underpins many diseases that have both significant morbidity and mortality worldwide, particularly obesity, diabetes and cardiovascular disease. The Tribbles (TRIB) pseudo-kinase proteins have a key role in controlling both immunity and metabolic inflammation. TRIB1 in particular has been identified in GWAS studies to be associated with plasma lipid traits and as a risk factor for CAD. Studies in mice have demonstrated that hepatic Trib1 expression reduces circulating lipids. Additionally, TRIB1 is a regulator of alternatively activated macrophages. However the direct role of Trib1 in immuno-metabolic control of diseases remains unexplored.

Using specific mouse models that either over-express (Rosa26Trib1.Tg x Lyz2Cre) or knockout (Trib1 fl/fl x Lyz2Cre) Trib1 in myeloid cells only, we have systematically analysed global phenotypic data including plasma lipid profiling, clinical blood chemistry, weight analyses, body composition, gross anatomy and histological evaluation (and tissue banking) of major organ systems of human disease including the liver and adipose tissue. Furthermore we have used these models to investigate the role of Trib1 in atherosclerosis using the same global phenotyping pipeline and extra assessments of atherosclerotic lesions (including size, morphology and stability); in fundamental tissues including the whole aorta and aortic sinus, measurements of which are applicable to human disease.

Additionally we have looked at cell specific changes in these models including profiling the macrophage phenotype in bone marrow derived macrophages (BMDMs), liver Kupffer cells (KCs) and adipose tissue macrophages (ATMs) by immunofluorescence staining and direct isolation of these cells from primary tissues that can be used in in vitro experiments. From our data we have found that Trib1 has a major impact in a cell- and context-specific manner on whole body lipid homeostasis, inflammation and immunity, much more complex than previously thought.

We believe our mouse models provide novel insights into gaining understanding of human disease causes and mechanisms as genomic profiling (transcriptomic data) of human cells (monocyte derived macrophages; MDMs) are highly concordant with the findings we have gained from the mouse models in vivo. We envisage our models can be used to investigate the gene and protein networks that underpin diseases of chronic inflammation and to potentially screen for small molecules to be used in human therapies.

**17) Targeting gene expression in infertile oocytes**

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Infertility affects more and more couples worldwide and these couples need to conceive through assisted reproduction technology (ART). In Finland, one woman out of 6 is infertile. The main pathology leading to infertility, in women, is polycystic ovary syndrome (PCOS) and represent 15% of the patients.

Transcription occurs during oocyte growth and cease when the oocyte reaches the germinal vesicle (GV) stage. At the same time, de novo DNA methylation is established mostly in a CpG context which regulates gene transcription Methylation contributes especially to the control of gene expression of the next generation through the imprinted genes as DNA methylation is a dynamic pattern in the epigenetic regulation. If an epigenetic error occurs on these genes, it may lead to an imprinted disease. Currently, the mechanisms regulating fertility are not fully understood.

The aim of this work is to analyse and identify through transcriptome approach downstream genes from PCOS and control oocytes. A small pilot study was designed and few single-oocytes libraries were generated based on the single cell transcriptome sequencing (scT-Seq, Angermueller et al. 2016, Nat Method) and sequenced. Based on the analysed transcriptomic data, we are planning to design humanised mouse using the Crispr/Cas9 technology.

**18) Phenotypic penetrance of congenital heart defects in DiGeorge Syndrome modulated by retinoic acid in the diet**

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Congenital heart disease (CHD) is defined as structural anomalies of the heart and great vessels that appears during embryogenesis. DiGeorge Syndrome (DGS) is the most common genetic deletion syndrome caused by a microdeletion on chromosome 22 of 3Mba and is often accompanied by developmental defects that include CHD, for which it is a commonly used model to study heart malformations. One of the main characteristics of this human syndrome is phenotypic variability linked to a remarkable homogeneity of the genetic profile, pointing to the existence of genetic and epigenetic modifiers such as environmental factors. Which are exactly these modifiers and, specially, how they act are still poorly defined, but diet could be one of the candidates.

Retinoic acid (RA), the active metabolite of vitamin A, regulates major embryonic growth and an unbalance in its levels during development results in congenital malformations such as CHD. Tbx1 has been described as the major gene responsible for the DGS defects and is directly linked with the associated heart malformations. Interestingly, multiple studies support a mutually repressive regulatory interaction between RA signaling and Tbx1 in the control of neural crest migration and differentiation of the pharyngeal arches and cardiac outflow tract. So, our hypothesis is that RA is a potent modifier that can affect phenotypic penetrance for the CHD trait in the DGS. This could be mediated by changes in expression of candidate genes and/or epigenetic changes as for example methylation.

The aim of this research is to study how environmental factors such as diet can cause CHD and modulate the penetrance of the deletion causing DGS. To do this we used both WT and Df1 female mice, with a hemizygous deletion of 1.2Mb in the 22q11.2-related region equivalent to the critical deletion in humans. We have three different experimental groups depending on their dietary treatment: RA-supplemented, -control and -deficient diet for 10 weeks. Once the dietary treatment is finished both WT and Df1 female mice are mated with Df1 or WT males, respectively. Embryos are collected at gestational day E10.5 and E18.5 and phenotyped. At E10.5 stage the temporary embryonic structures corresponding to the III-IV-VI pharyngeal arches are revised by India ink staining and, at E18.5 stage both great vessels are revised externally and internal cardiac structures are evaluated by hematoxylin-eosin staining. Serum retinol levels are determined in the blood of the pregnant females.

Our results indicate that Df1 embryos of RA-supplemented WT mothers develop significantly more cardiovascular malformations than Df1 embryos of RA-supplemented Df1 mothers (77.8% vs 16.7%). These interesting results suggest that environmental factors can effectively modify the phenotypic expression of CNV syndromes as those exemplified by DGS. In addition it also shows how the interaction of the genotype and the diet of the mother exert a very great influence on the phenotypic penetrance of mutations in the embryo. Currently ongoing gene expression and methylation analysis using methylation-arrays and RNAseq should help clarify the molecular mechanisms of how a Df1 genotype in the mother can protect Df1 embryos when challenged by dietary RA supplementation or restriction.

**19) EurOPDX Consortium: PDX models as an emerging way to personalized medicine in translational cancer research**

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Counteracting high attrition rates in oncology drug development and providing optimal therapeutic management of cancer patients require preclinical models that properly recapitulate the complexity and diversity of human tumours. Patient-derived tumour xenografts (PDXs), established by transplanting tumour fragments into immunodeficient mice, are being widely embraced by the scientific community as preclinical tools for target and biomarker discovery. Started in early 2013, the EurOPDX Consortium now gathers 19 academic institutions throughout Europe and in the US (www.europdx.eu). EurOPDX includes world-renowned experts at the forefront of research in basic, preclinical, translational and clinical oncology across multiple pathologies, and displays a wide range of expertise in technological platforms. In addition, the distributed PDX collection of the consortium consists of more than 1,500 subcutaneous and orthotopic models for over 30 different pathologies.
The primary goal of the Consortium is to increase the visibility of this collection, and share the established PDX models with the academic scientific community to perform collaborative multicentre and multipathology 'xenopatient' trials within molecularly defined tumour subsets, resulting in hypothesis-driven personalized medicine strategies for cancer patients. In addition, our objective is to improve the predictability of preclinical data by (i) improving characterisation of the models, (ii) implementing new PDX models to strengthen the representativeness of the collection, and humanized PDX models, (iii) harmonising working practice, (iv) integrating complementary models (e.g. ex vivo 2D and 3D assays) in innovative drug development pipelines, and (v) leveraging deep molecular and pharmacogenomic profiling data of the collection of models to discover predictive biomarkers, new targets, and new strategies to overcome drug resistance.
Towards these objectives, we were recently granted 5 million euros under the Horizon 2020 programme to build "EDIReX, the EurOPDX Distributed Infrastructure for Research on patient-derived Xenografts", and are currently in Grant Agreement preparation phase with the European Commission. By teaming up with other key academic, technological and SME partners, our goal with EDIReX is to establish a cutting-edge European infrastructure offering Trans-national Access to PDX resources to academic and industrial cancer researchers, including the distribution of cryopreserved samples to third parties, the structured biobanking of user-developed models, and the performance of efficacy studies.
We will provide an overview of the current achievements of the Consortium and the objectives of the EDIReX project starting 2018.

**20) Untargeted Metabolomics in Systematic Phenotyping**

Karel Chalupsky, Ashkan Zareie, Vendula Novosadova;

Czech Centre for Phenogenomics

Blood metabolites reflect total changes in whole organism and combine genetic, regulatory and environmental influences. Since analysis of metabolome is very complex procedure and usually cannot be done in same day when blood samples were collected, a sample storage has to be implemented. Here, we tested three different conditions of sample handling in order to demonstrate the change of results when the same method of analysis is used on the fresh, frozen, refrozen samples. We have analyzed metabolome from six C57BL6N male mice on Agilent 6545 iFunnel Q-TOF using C18 Zorbax column with increasing gradient of 5 % methanol to 100% over 25 minutes with 50 mmol/l NH4F using both polarities. We have found molecular features (mzRT pairs) that are highly expressed among the mice in fresh and frozen samples, but completely lost (or in some cases present but with very low intensity values) among refrozen samples. Our data suggest that a single freezing step and storage of plasma sample in -80 at least for two weeks will not change or reduce number of detected molecular features and therefore the left over plasma after regular IMPC screen can be used for metabolomics.

**21) The 'forced oscillation' technique as semi-high throughput method for the screening of lung function phenotypes**

Benoit Piavaux

Czech Centre for Phenogenomics

Until recently the high throughput method of choice for screening of large number of mice was the unrestrained whole body plethysmography, also within the IMPC consortium. This was an awkward choice as this method has shown to be a bad estimation of the lung function and has many issues which makes it unfit for high-throughput screening. Hence, it was abandoned by the IMPC consortium and no lung function measurements are currently suggested by the consortium.

We have adapted and validated the forced oscillation technique with Scireq's Flexivents to be usable as semi-high throughput (50 animals per day) screening method and have included the forced oscillation technique in our pipeline at 13 weeks of age as an alternative to the previously suggested unrestrained whole body plethysmography. As these measurements are performed in the middle of the adult pipeline, tracheotomy was not an option and we therefore perform intubation instead. As we wanted the method to be fast and with the least possible harm to the mice we do not use any challenge with a bronchoconstrictive agent as this can lead to severe adverse reactions in some mice.

Using this method we obtained hits in our cohorts. To confirm that these were true hits and not just false positives, we analyzed histological sections of the lung of these mice. In all cases we were able to identify phenotypic differences which can explain the differences in lung function.

In summary, we have adapted the forced oscillation technique for lung function phenotyping with Scireq's Flexivent to fit our phenotyping pipeline. Using this method we were able to find lung function hits in our cohorts. Analysis of histological section from these cohorts showed differences which can explain the difference in lung function, thus confirming the validity of the hits.

**22) In-vivo microCT imaging for high through put phenotyping**

Frantisek Spoutil, Nicole Chambers, Jan Prochazka

Czech Centre for Phenogenomics

MicroCT technology provides significant advantages in the analysis of skeleton morphology, bone structure and body composition phenotypes. Our imaging pipeline allows the generation of all data required for further analysis in one scanning event with spatial resolution 35μm voxel size. Semi-automatized software pipeline allows further data processing. First, entire skeleton is separated for morphological evaluation in 3D. Such a phenotyping approach can reveal changes in bone morphology in much higher detail then standard 2D x-ray projections. The same dataset is then used for segmentation analysis of body composition based on differential x-ray absorption of lean and fat tissues. With the advantage of 3D matrice for body composition, we can easily access from same dataset approach also localization of adipose tissue within body, providing more biological relevant information comparing subcutaneous and visceral deposition of adipose tissue. Together with information from fat and lean tissue we are also able to obtain information from mineralized tissues based on alteration of x-ray absorption in bones and teeth. Mineralization defects can be identified rapidly and scanning resolution is sufficient for additional evaluation of individual bones structure to better localize mineralization defects in bones (trabecular, cortical or general mineralization problem). The sensitivity of body composition analysis was cross-correlated with metabolism screen under high fat diet challenge conditions. A pilot experiment provided very promising results stressing the importance of evaluation of distribution of adipose tissue that microCT based body composition analysis allows.

In summary, in-vivo microCT scans provide a valuable source of data for morphology and body composition phenotype analysis, and allow many more detail additional tests from original 3D matrix without the need of repeat of scanning of new cohorts.

**23) A suggested approach to improve AUC evaluation in glucose tolerance testing at the German Muse Clinic (GMC)**

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The intraperitoneal glucose tolerance test (GTT) is a widely used functional test to investigate glucose tolerance. There is no standardized evaluation of GTT results in mice but typically, the comparison of areas under the glucose curve (AUC) is included to assess if whole body glucose uptake is impaired. Usually, researchers apply a simple trapezoidal rule to determine AUCs above zero levels. To account for large differences in basal blood glucose levels affecting AUC, one could calculate the AUC only above basal glucose levels instead of total AUC. These approaches are associated with several problems. Differences in AUC values calculated as area above zero levels do not necessarily indicate that glucose clearance is impaired but could just be due to different starting points/basal glucose levels. On the other hand, AUC above basal glucose level only may be confounded if the final blood concentration is lower than basal glucose level at the start of the test thereby erroneously diminishing AUC values. In addition, using AUC values for the whole test duration may result in the failure to detect interesting differences in glucose excursions, such as a very strong early increase in blood levels followed by accelerated clearance or if the glucose peak occurs later than usual. Last but not least, in hyperglycemic mice after glucose injection single values are above the measurement range of the instrument used often restricted to 600 mg/dL. Due to missing values, it is not possible to calculate accurate AUCs at all. We evaluated the suggested approaches in analyzing AUC levels by calculating AUC values above zero level for glucose curves “normalized” towards a common T0-level, replacing AUC-values exceeding the upper measurement limit by the upper limit and calculating not only total AUC but also AUC for the first 30 minutes and the remaining 90 minutes of the test.

**24) Large-scale analysis of mouse energy metabolism in the International Mouse Phenotyping Consortium (IMPC)**

**Jan Rozman**[1,3], Martin Kistler[1], Monja Willershäuser[2], The IMPC Consortium, Helmut Fuchs[1], Valerie Gailus-Durner[1], Martin Klingenspor[2], and Martin Hrabě de Angelis[1,3]

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[3] German Centre for Diabetes Research (DZD)

Mouse models for obesity have significantly contributed to the understanding of the genetic, molecular, and neuroendocrine mechanisms involved in energy balance regulation. To investigate the genetic causes promoting positive energy balance, we need a deeper understanding of the proximate physiological mechanisms. This requires the measurement of energy absorption, storage and expenditure, and suitable procedures to adjust for differences in body size and body composition refining the statistical analysis. Large-scale studies on energy balance regulation in genetic mouse models, such as the indirect calorimetry test from the early onset phenotyping pipeline in IMPC, apply these procedures with the aim to discover new genes involved in metabolic regulation that could be targets for the correction of excess energy storage in white adipose tissue.

We analyzed energy balance parameters acquired in the Metabolic Screen of the German Mouse Clinic during the IMPC pilot program EUMODIC. Genes linked to metabolic regulation were identified by comparing mutant and wildtype mouse lines after adjustment for differences in body mass and body composition using regression models to evaluate residual variation in energy expenditure. We now plan to evaluate the applicability of this approach using the current IMPC data release with calorimetry data from more than 2000 mutant lines.

**25) Statistical tools for analysis of large flow cytometry data sets**

**Yi-Li Cho***, Irina Treise*, Irina Fuchs*, Gregor Miller*,
Helmut Fuchs*, Valérie Gailus-Durner*, Martin Hrabě de Angelis*

*Institute of Experimental Genetics Helmholtz Zentrum München, Germany

The invention of flow cytometry facilitates basic research and clinical practice to its advantage of simultaneous multiparametric analysis. Therefore, the application of flow cytometry technology in the International Mouse Phenotyping Consortium (IMPC) enables the collection of more than 100 parameter data sets including a vast diversity of innate and adaptive immune cell components. The classical way to analyze such large data sets is quite time-consuming and subjective. Moreover, much information may be neglect when processing such complex data. For these reasons, new approaches are in high demand in order to simplify the analysis and make an unbiased evaluation. Here, we proposed a statistical tool to perform a deviation measure of overlap parameters from interest data sets to the variance of metadata from wild-type group. The pooled deviation can then be employed in estimating the magnitude of genotype-related effect on immune system and further identifying a clinically relevant gene.

**26) Rodent Little Brother: A Home Cage Analysis system for group housed mice**

R. Bains1, D. Sneddon1, **H. Cater**1, P. Nolan1 and S. Wells1

1MRC Harwell, Harwell Science and Innovation Centre, UK OX11 0RD

The Home Cage Analysis (HCA) system was developed in collaboration with Actual Analytics, Edinburgh, as part of the CRACK-IT initiative funded by the NC3Rs. The HCA system enables continuous assessment of multiple biologically-relevant phenotypes over long periods on individuals, as well as social interactions within the group. Most recently we have been using this system to monitor behaviours over more than one Light:Dark cycle.

Here we present some of our most recent findings:

1. Activity data from three inbred strains captured over a standard 12 hour Light:Dark cycle: Statistically significant strain differences were seen in the total activity as well as indication of anticipatory activity seen prior to lights off
2. Use of trainable machine learning algorithms to automatically analyse specific behaviours over extended periods of time: early detection of phenotypes in progressively degenerative GA models by monitoring voluntary climbing behaviour
3. Enriching the phenotype data sets with behaviours observed during dark phase: detection of phenotypes expressed exclusively in the active phase
4. Social interactions: pairwise separation of each individual with a cage informs welfare as well as socially isolate type behavioural phenotypes
5. Informed welfare decisions: including post-surgery care and new substance dosing

In addition to all of the above we have also observed a number of novel behaviours and are beginning to develop a list of home cage behaviour ontologies with the aim to refine mouse behaviour phenotypes. Used as a complement to the conventional out-of-cage phenotyping batteries, the system has the potential to greatly enrich the phenotype datasets in mouse strains and mutants.

**27) Optimization of the tail suspension test in mice**

F. Riet[1], A. Litt[2], B. Petit-Demoulière[1], S. Taubert[2], C. Bam'hamed[1], C. Mittelhaeuser[1], H. Méziane[1], **P. Reilly**[1], T. Sorg[1], Y. Hérault[1,2]

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The tail suspension test is examined for models of depression to measure the time for a mouse to submit immobile to an uncomfortable position. A shorter duration of struggling against the uncomfortable circumstance of tail-suspension is indicative of depression-associated hopelessness. The current standard for automated measurement of immobility relies on the change in force exerted through the tail to a strain gauge. This becomes challenging for comparison between animals of different weights, as is commonly the case for genetically modified strains. Smaller animals would need greater relative movement to register an effect on the strain gauge. To solve this, we have devised a motion-sensing strategy to automate the tail-suspension measurements. In this study we compare each of the automated systems to the gold-standard, human-observation of immobility. We find that, whereas the strain gauge measurement functions well with large mice, it is poor with respect to smaller mice. By contrast, our innovation of motion sensing works well with mice of all weights.

**28) PHENOPY: home-cage behavioral protocols across multiple timescales in mice**

Edoardo Balzani, Matteo Falappa, Fuat Balci, Valter Tucci

Istituto Italiano di Tecnologia, Genoa

Behavioral phenotyping constitutes a major bottleneck in mouse genetics; classical and lengthy behavioral protocols are not suitable for large-scale phenotyping and they are vulnerable to unaccountable sources of variance leading to inconsistent results between centers. Consequently, the annotation of behavioral phenotypes within the extensive archives of mouse lines available nowadays is nearly stalled. In order to overcome this impasse, we developed a home-cage approach that radically increases the throughput of data gathering from individual animals and facilitates data mining by offering new opportunities for multimodal data comparisons. Using a simple work-for-food testing strategy that is implemented in home-cages, we present here as an example a test protocol for timed behaviors, however, the approach is suitable for a wide range of domains (e.g., attention, decision-making, memory). The spontaneous behavioral activity of mice in performing the behavioral task for 24 hr a day for several days, allows the concomitant monitoring of the circadian rhythms, providing an integrated assessment of circadian profile of different behaviors. We provide a Python-based open source analytical platform (Phenopy) that is aimed to support researchers in behavioral and biological sciences. Phenopy has been designed as a user-friendly tool that is accessible to the scientists with no programming background. To complement this tool, we will use a cloud-like system that can serve as a repository for new Python-coded scripts that can be automatically imported in the user-friendly interface. Our home-cage approach aims to implement a behavioral testing system that is suitable for large-scale studies in different laboratories. By adopting open source computational tools we provide a platform to design new behavioral protocols in home-cage and this new concept brings with it the potential to facilitate data sharing across different laboratories.

INFRAFRONTIER
mouse disease models

# New innovative technology in rodent body temperature telemetry workshop

## *Focus on brown fat metabolism*

**23rd - 24th January 2018**

***This workshop will be held at***

***The Czech Centre for Phenogenomics, Czech Republic***

## Guest speakers

**Prof. Dr. Martin Klingenspor**
*TU Munich*

**Dr. Marcel Scheideler**
*Helmholtz Zentrum München*


***Includes hands on surgical training***

Contact info@infrafrontier.eu for more information

Czech Centre for Phenogenomics

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

TSE systems

## Preliminary Programme

### Tuesday 23rd January 2018

| | |
|---|---|
| 09:00 – 09:30 | Arrival and registration |
| 09:30 – 10:00 | Welcome and Introduction to CCP & workshop |
| | *Radislav Sedlacek (CCP/IMG)* |
| 10:00 – 10:50 | Lecture – A novel endocrine gut - brown fat - brain axis controls meal induced thermogenesis and food intake |
| | *Prof. Dr. Martin Klingenspor (TU Munich)* |
| 10:50 – 11:40 | Lecture – Overview of Stellar technology focus on temperature probes |
| | *Harm J Knot (TSE systems)* |
| **11:40 – 12:40** | **LUNCH** |
| 12:40 – 13:00 | Introduction to implantation surgery |
| | *René Remie (RRSSC, Almere)* |
| 13:00 – 14:30 | Live demonstration – surgical implantation of temperature probes in mice |
| | *René Remie (RRSSC, Almere)* |
| 14:30 – 15:20 | Lecture – Tracking metabolite changes using Q-TOF LC/MS |
| | *TBC* |
| **15:20 – 15:50** | **COFFEE BREAK** |
| 15:50 – 16:40 | Lecture – Molecular characterization of adipocyte browning – a microRNA case study |
| | *Dr. Marcel Scheideler (Helmholtz Zentrum München)* |
| 16:40 – 17:30 | Lecture – How thermogenesis affects whole body energy metabolism |
| | *Dr. Jan Rozman (Helmholtz Zentrum München)* |
| 17:30 – 18:00 | Final discussion, Q & A |
| **19:30 –** | **COURSE DINNER** |

### Wednesday 24th January 2018

*For pre-registered participants*

| | |
|---|---|
| 09:30 – 12:00 | Practical training for implantation surgery |


Contact info@infrafrontier.eu for more information

## INTERNATIONAL TRAINING COURSES 2017-2018

### In vivo CRISPR-Cas9 genome editing

March 14th-15th, 2018 - Strasbourg, France
November 08th-09th, 2018 - Strasbourg, France

This training aims to provide a general framework to get scientists started using CRISPR-Cas9 for *in vivo* gene editing in rodents: principles, rodent's models, PRO and CONS, achievement, challenge. Technical insight are discussed based on practical cases, in house results and bibliographic analysis. The interactive discussion groups allow attendees to expose and discuss with experts their own scientific issues and give crucial skill to progress in their projects.

Objectives
Learn more about gene editing and how it works
Hear about current advances on many technical aspects
Optimize the RNA guide design to the genotyping analysis (bioinformatics workshop)
Highlight crucial issue in your own scientific project.

### Primary phenotyping of mouse embryos

March 27th-29th, 2018 - Strasbourg, France

This training is aimed at providing a theoretical and practical background knowledge destined for researchers and engineers that are willing to acquire primary expertise in mouse development. The topics will cover dissection of post implant embryos, histology, whole mount Lac Z staining and confocal imaging of whole embryos.

Objectives
Acquire primary expertise in mouse development.
Be able to design primary phenotyping experiment on mouse embryos.
Learn about all the primary phenotyping analysis on mouse embryos.
Dissect of post implant embryos.
Determine the window of lethality in utero.
Value embryos viability at neonatal step.
Observe embryos by echography.
Image embryos by MicroCT.

**All information for detailed program and registration is available online**
**http://www.phenomin.fr/training-courses/**

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# IMPC

International Mouse Phenotyping Consortium

ARE **YOU** INTERESTED IN A **PARTICULAR GENE**?

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The IMPC is creating knockout mouse lines for every single protein-coding gene in the mouse genome, and characterising them through standardised, quality-controlled phenotyping tests.

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# OUR PRINCIPLES

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**INFRAFRONTIER / IMPC Stakeholder Meeting**
November 14th - 16th 2017 | Athens, Royal Olympic Hotel