Behavioural abnormalities in DMSXL mice, a model of Myotonic Dystrophy type 1

Presenting Author: Silvia Mandillo - *Institute of Biochemistry and Cell Biology - IBBC, National Research Council - CNR, 00015 Monterotondo (Rome), Italy;CNR-EMMA-INFRAFRONTIER-IMPC, 00015 Monterotondo (Rome), Italy*
Authors:
- Elisabetta Golin - *Institute of Biochemistry and Cell Biology - IBBC, National Research Council - CNR, 00015 Monterotondo (Rome), Italy;CNR-EMMA-INFRAFRONTIER-IMPC, 00015 Monterotondo (Rome), Italy*
- Tiziana Orsin - *Institute of Biochemistry and Cell Biology - IBBC, National Research Council - CNR, 00015 Monterotondo (Rome), Italy;CNR-EMMA-INFRAFRONTIER-IMPC, 00015 Monterotondo (Rome), Italy*
- Mara Rigamon - *Tecniplast, 21020 Buguggiate (VA), Ital*

Myotonic dystrophy type 1 (DM1) is a dominantly inherited neuromuscular disease caused by the abnormal expansion of CTG-repeats in the 3'-untranslated region of the DMPK gene, characterized by multisystemic symptoms including muscle weakness, myotonia, cardio-respiratory problems, hypersomnia, cognitive dysfunction and behavioural abnormalities. DMSXL mice carry a mutated human DMPK transgene resulting in >1000 CTG-repeats. They exhibit a pathologic neuromuscular phenotype and also synaptic dysfunction resulting in neurological and behavioural deficits similar to those observed in patients. To further explore additional phenotypes of this DM1 mouse model we developed a comprehensive battery of tests that included the evaluation of several brain functions: exploratory and motor activity, fine motor skills, neuromuscular strength, anxiety, working memory, fear learning, sensorimotor processing, spatial memory, home-cage rest behaviour. Additionally, skeletal and cranio-facial morphology as well as body composition and bone mineral density were assessed using DEXA and micro-CT. Male and female DMSXL mice tested between 7 and 20 weeks of age showed, compared to wild-type littermates: lower body weight, reduced grip strength and running wheel activity and increased anxiety, consistently with previously published data. Interestingly, they also presented some new phenotypes remarkably similar to the characteristic features of the human disease: skeletal and cranio-facial dysmorphology with teeth misalignment, abnormalities in sensorimotor processing and rest-related disturbances during the active phase. These phenotypes confirm the reliability of DMSXL mice to model clinical features of DM1 and open new opportunities to verify the efficacy of novel therapies based on CRISPR/Cas9-mediated gene editing strategies currently under study in our group.

Capturing the unpredictability of CRISPR/Cas9 genome editing outcome in animal models

Presenting Author: Lydia Teboul - *The Mary Lyon Centre at MRC Harwell, Harwell Campus, Didcot, Oxfordshire, OX11 0RD, UK*

Authors:

- Alasdair Allan - *The Mary Lyon Centre at MRC Harwell, Harwell Campus, Didcot, Oxfordshire, OX11 0RD, UK*
- Rosie Bunton-Stasyshyn - *The Mary Lyon Centre at MRC Harwell, Harwell Campus, Didcot, Oxfordshire, OX11 0RD, UK*
- Adam Caulder - *The Mary Lyon Centre at MRC Harwell, Harwell Campus, Didcot, Oxfordshire, OX11 0RD, UK*

Genetically engineered mouse models are invaluable tools to investigate gene and genome function and elucidate disease mechanisms in a complex in vivo context. The revolution of CRISPR/Cas9 (and other programmable, targeted nucleases) has vastly increased the accessibility of genome engineering. Across the scale, from small, subtle and seamless modifications to large complex multifunctional alleles, nuclease mediated engineering introduces novel challenges for genome validation compared to traditional stem cell based, or random integration transgenesis. We must not only identify and scrutinise the allele that we want, but also exclude unexpected mutations and off-target effects, all within the complex genetic architecture of mosaic founders and the heterogeneous lineages of their offspring. Our group has developed thorough and efficient validation strategies, combining a variety of methodologies to confirm precision engineering at the targeted locus and, importantly, screen out animals with undesired mutations including off-target editing, random integrations, chromosomal rearrangements and large deletions. Efficient identification of undesired mutations early in the process reduces the number of mice required to establish a properly validated mouse line. We will highlight the wide array of unintended mutations that targeted nucleases can produce. We will describe how we identify these unintended mutations and thoroughly validate correctly engineered alleles by applying methodologies including Sanger sequencing, long-read Nanopore sequencing and digital droplet PCR along with traditional PCR techniques

Characterization of Champ1 loss-of-function in knockout mice using a combination of discovery and targeted phenotyping approaches

Presenting Author: Louise Lanoue - *Mouse Biology Program, University of California, Davis, California*
Authors:
- Kristin Grimsrud - *Mouse Biology Program, University of California, Davis, California;Department of Pathology and Laboratory Medicine, School of Medicine, University of California, Davis, California*
- Brandon Willis - *Mouse Biology Program, University of California, Davis, California*
- Lynette Bowe - *Mouse Biology Program, University of California, Davis, California*

CHAMP1-related neurodevelopmental disorder is a rare genetic syndrome associated with multiple genetic variants and characterized by behavioral and intellectual disabilities, developmental delay, dysmorphologies, and medical comorbidities. The CHAMP1 gene encodes a zinc-finger protein that functions as a regulator of chromosome segregation during mitosis. To decipher potential genetic causes of this disease, we recapitulated in C57BL/6N mice a predicted loss-of-function two base pair deletion (c.542_543 del CT) of the CHAMP1 gene identified in a 2-year-old male patient with developmental delays. The homozygous Champ1 mutation caused prenatal embryo lethality. At necropsy, 15.5 gestational day embryos exhibited gross dysmorphology (open mouth, shortened frontonasal prominence, and capillary effusion). MicroCT imaging identified additional abnormalities, including heart anomalies and thyroid and spleen hypoplasia. Heterozygous mice survived to adulthood. 5 week old males had dysmorphic facial features tremors, and were smaller than their age and sex -matched wild type sibling controls. Phenotype discovery screening in the IMPC early adult pipeline revealed disturbances in metabolic (clinical chemistry, hematology) and sensory (eye exam) functions, and markedly reduced ambulation (open field) and weight gain. To further characterize the pathophysiological impacts and causative nature of this patient-specific genetic mutation, we further analyzed heterozygous mice for indications of developmental delay using a targeted phenotyping approach. Champ1 mutants exhibited several neurobehavioral anomalies (startle response, marble burying, social novelty, and object recognition) and higher frequencies of eye abnormalities compared to controls. Microcephaly and ventricular dilation were shown by brain MR imaging and confirmed by histopathology. This study demonstrates that strategic use of broad, discovery-based and disease-focused targeted screening can yield useful data that recapitulates the clinical presentation of patients and uncovers additional previously unrecognized and/or unreported phenotypes. Importantly, the data generated from this phenotyping support the disease-causing nature of this particular patient-specific genetic variant, and will be useful for additional studies to inform on the genetically-dependent pathophysiological mechanisms underlining CHAMP1-related neurodevelopmental disorder.