Methods: A subset of 20 adults (10 males, 10 females) from PHENODM1 have been scanned using 3.0T MRI scanner and bilateral lower limb axial T1-weighted and T2-weighted STIR images have been analysed. All pelvic girdle, thigh and lower leg muscles were scored bilaterally according to the Mercuri scale on axial T1-weighted sequences.
In addition to the OMMYD-2 measures described we compared our findings to quantitative strength assessments in the ankle dorsiflexors and plantiflexors, knee extensors and hip flexors.
The average of the quadriceps and ankle dorsiflexors Mercuri scores were considered for the non-parametric correlations against muscle strength. Kruskal-Wallis Test was utilised to compare the different mean values between the stratified sample.
Results: The mean age at MRI was 41.2±9.9 years with a mean disease duration of 22.2±10.5 years. On T1-weighted images the most severely affected muscles were: gastrocnemius medialis (Mercuri median score: 3), soleus (2b), peroneus longus (1), tibialis anterior (1), flexor digitorum (1), vastus (1), sartorius (1) and biceps femoris (1). STIR abnormalities were detected in 9 patients, mainly in gastrocnemius medialis and soleus. Significant correlation values were identified between sartorius (MRI score) and hip flexors strength and between ankle dorsiflexors strength and MRI score for dorsiflexors and plantiflexors. Tibialis anterioris' Mercuri score gave indications of discrimination between patient performance on functional outcomes.
Conclusions: This study provides the foundation for MRI assessments as non-invasive biomarker for DM1. Future work on larger cohorts and longitudinal assessments will be required for validation.

D28

Generation of a mouse model of FSHD to reveal the DUX4 expression profile and dynamics

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is characterized by a descending, often asymmetric, skeletal muscle atrophy. The genetic basis of the disease is linked to DNA hypomethylation of *D4Z4* macrosatellite repeats on chromosome 4q35. This happens due to either contractions of the D4Z4 repeat array (FSHD1) or mutations of chromatin modifiers (SMCHD1, DNMT3B) in FSHD2, causing aberrant expression of the DUX4 retrogene mapped in each D4Z4 unit. Toxic DUX4 protein is produced from the distal D4Z4 if a 3' polyA addition site (pLAM region), which stabilizes the mRNA. DUX4 expression is considered the primary cause of FSHD, but its toxicity is a major issue in the development of animal models.
Aims: We aimed to generate mouse models of FSHD to reveal the DUX4 expression profile and dynamics, but avoiding its toxicity. These models will give insight into FSHD pathomechanisms and potential therapeutics.
Methods: To suppress the DUX4 ORF we constructed *DUX4p-nlacZ-pLAM* with the native human DUX4 promoter and pLAM region flanking a nuclear-localised *(n)lacZ* reporter gene. To additionally address the role of pLAM UTR region in the expression of native DUX4 locus, we also made a construct that lacks the pLAM region. Using pronuclear injection of these reporter constructs, we generated transgenic mice in which the expression dynamics of the pathogenic locus can be mapped.
Results: We first demonstrated the functionality of the *DUX4p-nlacZ-pLAM* construct in immortalised murine C2C12 and human myoblasts and myotubes. Comparatively, we discovered that the *DUX4p-nlacZ* construct without pLAM has a significantly higher level of expression *in vitro* in the immortalized cell lines. We then generated *DUX4p-nlacZ-pLAM* transgenic mice by pronuclear injection, and the analysis of the progeny reveals rare nuclei containing β-galactosidase in different groups of skeletal muscle of adult mice. We are now establishing multiple lines of *DUX4p-nlacZ* mice and will present their detailed analysis at the meeting.
Conclusions: Through generating transgenic reporter mouse lines that carries a native human configuration of DUX4 promoter with or without pLAM region, we aim to create an animal model that could be used for mapping the expression profile and dynamics of DUX4.

D29

Semi-quantitative muscle MRI in dysferlinopathy patients: pattern recognition and implications for clinical trials

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Background: The Jain Clinical Outcome Study (COS) is an international study of 203 adults with dysferlinopathy in 8 countries. Patients undergo six visits over three years, during which physiotherapy and medical assessments medical as well as muscle MRI are performed. Muscle MRI has been performed in 182 patients with a confirmed diagnosis of dysferlinopathy in 14 different centres, using 1.5T or 3T scanners from different manufacturers (Philips, General Electrics, Siemens).
Aims: To describe the pattern of pathology by muscle MRI in a large multinational cohort of patients with dysferlinopathy and correlate with physiotherapy assessments.
Methods: The semi-quantitative analysis was performed on axial T1 weighted sequences collected at baseline visits. This analysis was performed using the Mercuri scale modified by Fisher (0 to 4). 81–131 muscles were scored per patient.
The pattern of muscles involved was analysed using hierarchical analysis and presented it as heat maps. MRI results have also been correlated with appropriate functional tests for each region of the body analysed.
Results: The most frequently affected muscles were the gastrocnemius medialis and the soleus. A similar pattern of involvement was identified in most patients regardless of their clinical phenotype. Increasing muscle involvement on MRI correlated positively with disease duration and functional impairment, allowing us to define the most relevant regions of interest for quantitative MRI in longitudinal studies.
Conclusions: The study has expanded the characterization of the patterns that can be found in dysferlinopathy patients, regardless of their clinical phenotype. We have also shown a correlation of the muscle pathology as detected by T1w MRI with disease duration and the results of related functional tests, which will be of great help in the design of future clinical trials.

D30

A semi-automated image processing method for quantify dystrophin coverage at the sarcolemma membrane of each individual muscle fibre

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Background: In DMD clinical trials, one of the primary biological endpoints is the restoration of the dystrophin protein in muscles of treated patients. Dystrophin protein can be quantified by performing immunohistochemistry (IHC) assays and by Western Blot (WB). WB allows the quantification of the overall dystrophin expressed within the muscle but gives no indication if the protein is functionally localized at the sarcolemma. IHC detects dystrophin expressed at the sarcolemma therefore also provides information on its correct localization. Recent efforts have been focused on the feasibility of using techniques to quantify dystrophin mean intensity values at the sarcolemma as well percentage of dystrophin coverage for each individual fibre.