diagnosis of FTD is difficult due to lack of uniformity in clinical presentation. Diagnosis is made after excluding other causes of myopathy and on the basis of histological evidence of type 1 muscle fiber hypotrophy. Genetic analysis shows also a great heterogeneity. Our data shows that presentation at birth accounts only for half of the cases. An integrated multidisciplinary approach of neuromuscular experts, geneticists, neuropathologists, will improve and optimize the diagnosis in this group of congenital myopathies.

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P.111

Modelling autosomal dominant centronuclear myopathy in zebrafish

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Mutations in DNM2, encoding dynamin 2, an ubiquitously expressed GTPase, cause the autosomal dominant centronuclear myopathy (AD-CNM). Most of the AD-CNM mutations in DNM2 (such as S619L) are gain-of-function mutations leading to an increased GTPase activity. So far, DNM2 AD-CNM mutations have only been modelled transiently in zebrafish through the injection of mutated mRNA. mRNA injected larvae disclosed a severe phenotype with structural and functional abnormalities of the triad (Gibbs et al 2013). Precise genome editing can be achieved with CRISPR-Cas9 and a single-stranded oligonucleotide (ssODN) stimulating the double strand break repair through the homology-directed repair mechanism. We have efficiently generated a knockin of the S619L mutation in dnm2a. No additional indel was detected after Sanger sequencing. Unexpectedly, heterozygous *dnm2a*$^{S619L/WT}$ larvae have a preserved locomotor function (Zebrabox at 5 and 7 days post-fertilization (dpf)). They are able to survive until adulthood and to breed. Homozygous *dnm2a*$^{S619L/S619L}$ larvae also have a preserved locomotor function and are able to survive until adulthood. Whole embryo immunofluorescence (WIF) for RyR is not different between heterozygous, homozygous and WT larvae at 5 dpf, suggesting that triad structure is not impaired. Calcium imaging was performed after injection of the mylz2: GCaMP3 plasmid. The peak amplitude of calcium response is not different between heterozygous, homozygous and WT larvae, suggesting that triad function is not impaired. WIF for Islet1 and Zn8 at 5 dpf does not show any structural abnormality in primary and secondary motoneurons. At the muscle cDNA level, the full-length dnm2a ^{S619L} allele is expressed at a similar level as the WT allele in heterozygous larvae. In conclusion, we have efficiently generated a knockin of the S619L mutation in dnm2a. In contrast to the previously reported severe phenotype of the S619L mRNA injected larvae, S619L knockin larvae have normal locomotor function, normal triad structure and normal triad function despite the fact that the S619L allele is expressed in muscle. A higher level of expression of the S619L allele may be required to induce a phenotype in zebrafish. We are thus generating a transgenic line overexpressing the mutated *dnm2a* under the control of a muscle-specific promoter: unc503.

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Two murine models for tubular aggregate myopathy with mutations in *Stim1* and *Orai1*

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Calcium (Ca^{2+}) is a key mediator in a wide range of cellular functions. Ca^{2+} released from the sarcoplasmic reticulum (SR) induces sarcomeric contraction of myofibrils, which is a primary mechanism mediating Ca^{2+} homeostasis in skeletal muscle. At the depletion of Ca^{2+} in the SR, store-operated Ca^{2+} entry (SOCE) is induced to obtain Ca^{2+} from the extracellular area. SR protein, STIM1 and Ca^{2+} channel protein on the plasma membrane, ORAI1 work on SOCE in skeletal myofibers. Tubular aggregate myopathy (TAM) has been known to be caused by constitutively activated SOCE owing to the dominant mutations in *ORAI1* or *STIM1* consequently. For further investigation of the mechanism in TAM, we generated knock-in mice with Orai1$^{G100S/+}$ or Stim$^{H109Q/+}$ mutations, respectively, both of which corresponding mutations in human have been identified as causative of TAM. Orai1$^{G100S/+}$ mice represented a significant reduction in contractile forces in TA muscles. Muscle pathology shows the presence of tubular aggregates (TAs) but the absence of internally nucleated or necrotic fibers. On the other hand, Stim1$^{H109Q/+}$ mice also showed muscle weakness and muscle atrophy. Muscle pathology showed variation in fiber size, the presence of internally nucleated fibers with mild endomysial fibrosis, but no TAs. In blood, the level of creatine kinase is mildly elevated in Stim1$^{H109Q/+}$, while serum calcium level is significantly decreased in Orai1$^{G100S/+}$. From the above findings, both mice clinically mimic TAM patients, but each mouse partly recapitulated pathological phenotypes. These mice may contribute to elucidate the mechanism of appearance of the specific pathological features and to establish a therapeutic strategy to TAM.

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P.113

Phenotype, genetics and natural history in 131 *SEPN1*-related myopathy patients: towards clinical trial readiness

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Autosomal-recessive mutations of the *SEPN1* (SELENON) gene cause SEPN1-related myopathy (SEPN1-RM). Patients present with early-onset severe axial weakness, scoliosis, spinal rigidity and life-threatening respiratory insufficiency, contrasting with fairly preserved limb strength and ambulation. SEPN1-RM natural history is poorly documented, hindering the implementation of clinical trials. We analyzed retrospectively the largest series of SEPN1-RM patients so far. We included patients aged 2-59 years, thus revealing disease evolution in late adulthood. First symptoms appeared within the first two years in 84.69% of cases (mean 18.2±29.8months) and were mostly neonatal hypotonia, poor head control and delayed motor milestones. Scoliosis was present in 86.1% patients from 8.9±4years; 36.8% required arthrodesis. All patients developed restrictive respiratory failure from the age of 10.1±6.2years and 81.7% required assisted ventilation from 14.7±8.9years while fully ambulant. Polysomnography detected nocturnal hypoventilation and frequent apneas in 92.9% of patients from early ages. Loss of ambulation occurred in 8 patients (8-54 years). Strikingly, we found a significant correlation between body weight and disease severity (p 0.02). While most patients were underweight (<4th percentile) and mean BMI in adults was 16.9±4, the most severe patients with early loss of ambulation and rapidly progressive respiratory failure were overweight with trunk fat accumulation. From the molecular point of view, we found 64 different SELENON mutations and we identified Exon 1 as a hot spot. We also describe the first genotype-phenotype correlations in SEPN1-RM. Homozygous mutations leading to protein absence were significantly associated with more severe forms (p 0.003). Our results improve understanding of the SEPN1-RM phenotype and natural history,

contribute to improve diagnosis, management and follow-up and pave the way towards clinical trial readiness.

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Clinical, histological, and genetic characterization of *PYROXD1*-related myopathy

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Congenital myopathies are a heterogeneous group of hereditary disorders characterized by early-onset muscle weakness and distinctive morphological abnormalities in skeletal muscle fibers. Recently, recessive PYROXD1 mutations were reported in families with slowly progressive muscle weakness, and here we describe three novel *PYROXD1* families at the clinical, histological, and genetic level. Histological analyses on muscle biopsies from all families revealed fiber size variability, endomysial fibrosis, and muscle fibers with multiple internal nuclei and cores, potentially representing a histopathological hallmark of the disorder. Further characterization of the structural muscle defects uncovered aggregations of myofibrillar proteins, and provided evidence for enhanced autophagy and fiber degeneration and regeneration. Sequencing identified homozygous or compound heterozygous *PYROXD1* mutations including the first deep intronic mutation. To assess the impact of the intronic mutation, muscle RNA was extracted and sequenced. The c.415-976A>G mutation reinforces a cryptic donor splice site and results in the exonization of 110 coding nucleotides between exons 4 and 5 containing an in-frame stop codon. Overall, this work expands the *PYROXD1* mutation spectrum and sheds light on the muscle histology of the disorder. Western blot revealed that HSP70 and glutathione reductase proteins were both significantly more abundant in our patients. This suggests that the *PYROXD1* mutations result in oxidative stress, which presumably contributes to the skeletal muscle pathology of the patients. Comparison of all new and published cases uncovered a genotype/phenotype correlation with a more severe phenotypic presentation of patients harboring splice mutations and leading to a reduction of the overall PYROXD1 protein level.

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McARDLE DISEASE

P.115
Natural history of McArdle disease in a cohort of 220 patients

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McArdle disease is caused by recessive mutations in the gene encoding muscle glycogen phosphorylase (*MGP*) which results in enzyme deficiency. The condition is considered to cause a 'pure' muscle phenotype with symptoms including: exercise intolerance, inability to perform isometric activities. Known associated complications include: hyperuricaemia and gout, acute rhabdomyolysis with myoglobinuria leading to compartment syndrome and acute renal failure. We retrospectively assessed case records of 220 patients with genetically confirmed McArdle disease from 2011-2019 and will report data relating to genotype, phenotype (including frequency of known associated complications) and functional capacity based upon a 12 minute walking test. We will also report results of prospective screening for other 'unexpected' co-morbidities in our cohort including the frequency of cardiovascular disease, thyroid disease and pattern retinal dystrophy. We also assessed the frequency of other systemic disorders. Our data suggest that MGP deficiency is not such a benign condition and may be associated with systemic issues beyond the skeletal muscles. The role of MGP in both skeletal and non-skeletal muscle tissues may give a clue as to the underlying pathogenesis of these co-morbidities. Prospective regular monitoring of these patients may be worthwhile.

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P.116
The existence of the 'Third Wind' phenomenon in McArdle disease

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The objective of this work was to confirm or refute the contention suggested by patients of the existence of a 'Third Wind' that is; around 2 hours of exercise they experience a further lessening of symptoms which usually restricts physical activity in those with McArdle disease. A Second Wind has been well-known in McArdle disease for many decades where after c8 minutes of exercise, due, it is thought, to increased blood flow and more stable ATP production via β-oxidation of lipids, muscle pain is reduced and patients feel more comfortable exercising. Fourteen participants walked at a self-selected pace on a motorised treadmill for 151 min (2 hr 31 min). Six were genetically confirmed GSDV (McArdle group) and eight were pathology free individuals (Control group). Expired gas samples were collected at selected timepoints throughout and calculations of fat and carbohydrate use were made using RER. Preliminary findings suggest that those in the McArdle group utilise energy differently in prolonged exercise compared with the control group. In addition, between minute 121-151 the McArdle group demonstrated a non-linear rise in fat use. In prolonged exercise up to 121 minutes those with McArdle disease appear to use different metabolism to those without pathology. Between minute 121 and 151 the non-linear rise in fat use in the McArdle group does suggest that there is a change at c2hr that could substantiate the suggestion of a 'Third Wind'.

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Rhabdomyolysis due to unaccustomed exercise: experiences from a multidisciplinary clinic

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The highly specialised service for McArdle disease and related disorders in the United Kingdom receives many referrals to investigate the cause of rhabdomyolysis. An increasing number of these referrals are following unaccustomed exercise. Since January 2017 fifteen such patients have been assessed in clinic. A typical history reveals either a period of inactivity followed by an intense training session at the gym, often with a personal trainer, prior to an episode of rhabdomyolysis, or very physically fit and active people who have done several sessions of unaccustomed exercise on consecutive days resulting in raised CK and hospital admission. We present