phenotype enables increased diagnostic rates for known genes in this cohort, while the use of WES provides scope both for new gene discovery and for additional research into disease modifiers and genotype-phenotype correlation with substantial cost effectiveness. The MYO-SEQ project is exploring 1000 exomes of patients that were aged 10 years and above and presented with unexplained limb girdle weakness and an elevated serum CK activity. Patients were recruited from more than 50 sites across Europe. Results from the project will be presented.

AAV-mediated transfer of FKRP shows therapeutic efficacy in a murine model of limb-girdle muscular dystrophy type 2i, but requires tight control of gene expression

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Limb Girdle Muscular Dystrophies (LGMD) type 2I, a recessive autosomal muscular dystrophy, is caused by mutations in the Fukutin Related Protein (FKRP) gene. It has been proposed that FKRP, whose function remains unclear, is a participant in α-dystroglycan (αDG) glycosylation, which is important to ensure the cell/matrix anchor of muscle fibers. A knock-in mouse model of LGMD2I was generated to express the most frequent mutation (L276I) encountered in patients. The introduction of the mutation did not alter the expression of FKRP, neither at transcriptional nor at translational levels, but did alter its function since abnormal glycosylation of αDG was observed. In this model, skeletal muscles were functionally impaired from 2 months of age and a moderate dystrophic pattern was evident by histology starting from 6 months of age. Gene transfer with a rAAV2/9 vector expressing *Fkrp* restored the biochemical defects, corrected the histological abnormalities and improved the resistance to eccentric stress in the mouse model was obtained. However, injection of high doses of the vector induced a decrease of αDG glycosylation and laminin binding. Finally, we showed that intravenous injection of the rAAV-*Fkrp* vector into a dystrophic mouse model suffering of dystroglycanopathy due to skeletal muscle-specific Fukutin (*Fktn*) knock-out caused toxicity. The dose-dependent worsening of the dystrophic phenotype suggests requirement for a precise control of its expression.

Symposium- Parallel Symposium Myotonic Dystrophies
• *Bernard JASMIN (CANADA)* • *Denis Furling (FRANCE)* • *Guillaume Bassez (FRANCE)*

Staufen1 Acts as a Disease Modifier in DM1.

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For several years, we have been interested in studying the molecular mechanisms that control expression of genes encoding synaptic proteins in both muscle and nerves. Although initially, the emphasis of this work was on elucidation of transcriptional events, we have become increasingly interested in studying post-transcriptional mechanisms. As part of our efforts to identify RNA-binding proteins that play a key role in skeletal muscle, we became interested in Staufen several years ago. Staufen is a RNA-binding protein that associates with RNA secondary structures, primarily through one or more double-stranded RNA-binding domains. The role of Staufen is perhaps best characterized in Drosophila, where it functions in the transport and localization of distinct mRNAs in oocytes and embryonic neuroblasts. Studies in mammals revealed that there are two genes, Staufen1 and Staufen2, and that Staufen1 also regulates mRNA stability in a mechanism referred to as Staufen1-mediated mRNA decay (SMD), as well as translation of a subpopulation of transcripts when bound to the 5'UTR.
Our studies focusing on the role of Staufen1 in skeletal muscle has shown that Staufen1 accumulates at the level of the post-synaptic membrane of the neuromuscular junction where its expression varies according to the state of differentiation and innervation of muscle cells, while being also influenced by the presence of agrin and heregulin (Bélanger et al., 2003). Our more recent work revealed that expression of Staufen1 is markedly increased in muscle samples from DM1 patients and DM1 mouse models, and that it can regulate alternative splicing of pre-mRNAs including the insulin receptor and chloride channel, while also promoting the nuclear-cytoplasmic transport of mutant CUG^{exp} DMPK mRNAs (Ravel-Chapuis et al., 2012). High-throughput RT-PCR assays with DM1 myoblasts further showed that Staufen1, in fact, has a broad impact on several alternative splicing events, with some events predicted to be beneficial for DM1 and others not (Bondy-Chorney et al., 2016). Moreover, we observed that Staufen1 levels decrease during embryonic muscle development. Sustained expression of Staufen1 negatively affects myogenic differentiation, independent of SMD, by controlling translation of c-myc (Ravel-Chapuis et al., 2014). Finally, we have also observed that Staufen1 is recruited into stress granules in normal myoblasts and myotubes. In contrast, DM1 myoblasts formed such granules much less efficiently in response to stress in a Staufen1-dependent and cell-specific manner (Ravel-Chapuis et al., 2016). Collectively, our findings show that Staufen1 is a novel splicing regulator that assumes multiple additional functions in normal and DM1 muscle cells thereby indicating its role as a disease modifier in DM1.
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Neutralize RNA toxicity induced by expanded-CUG repeats in Myotonic Dystrophy

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Myotonic Dystrophy type 1 (DM1), one of the most common form of inherited neuromuscular disorders in adults is characterized by myotonia, progressive muscle weakness and wasting, cardiac defects, endocrine troubles and cognitive impairments. This autosomal dominant disease is caused by an expanded tract of trinucleotide (CTG)n>50 repeats located in the 3' non-coding region of the *DMPK* gene. The size of the expansion is generally correlated with the clinical severity and the age of onset of the disease. Expression of pathogenic *DMPK* transcripts containing expanded CUG repeats (CUGexp-RNAs) results in a toxic RNA gain-of-function mechanism. CUGexp-RNAs are retained into the nucleus as riboprotein

aggregates or foci that sequester MBNL splicing factors leading to functional loss of MBNL and alternative splicing misregulations of a subset of pre-mRNAs. Thus, mis-splicing of *CLCN1*, *INSR* and *BIN1* pre-mRNAs were associated respectively to myotonia, insulin resistance and muscle weakness in DM1. Recently, we determine that abnormal splicing switch of *DMD* exon 78 in DM1 skeletal muscles compromises muscle fiber maintenance leading to ultrastructural abnormalities such as ringed fibers and sarcoplasmic masses. Finally, new abnormal spliced events have been identified in heart tissues of DM1 patients, and we showed that splicing alteration of *SCN5a* contributes to cardiac conduction abnormalities and arrhythmia, both symptoms of DM1.
Currently there is no cure for DM1 however several strategies are under development to reverse toxic CUGexp-RNAs dominant effects. Here we propose to neutralize RNA toxicity in DM1 cells by interfering with the abnormal CUGexp-RNA:MBNL interaction in order to release sequestered endogenous MBNL factor and restore its function. For this purpose, we have engineered a modified MBNLΔ polypeptide that keeps its RNA binding property but lacks its splicing activity. To evaluate its ability to inhibit CUGexp-RNA toxicity, we first expressed a GFP-MBNLΔ construct in DM1 muscle cells by using lentiviral vectors. We found that GFP-MBNLΔ colocalized with nuclear CUGexp-RNA foci and splicing misregulations as well as differentiation defects were corrected in DM1-treated muscle cells. To further assess this strategy *in vivo*, intramuscular injections of AAV-GFP-MBNLΔ vectors were performed in DM1 mice (HSA-LR) expressing 220CTG in skeletal muscles. As observed *in vitro*, colocalization of GFP-MBNLΔ with nuclear CUGexp-RNA foci in myofibers indicates that MBNLΔ is able to compete and release endogenous MBNL from these aggregates. More, splicing alterations of several transcripts were normalized or nearly corrected in injected HSA-LR mice and the myotonia was also abolished. In conclusion, we propose that a MBNLΔ-decoy gene therapy approach could represent an alternate or complementary therapeutic approach for Myotonic Dystrophy.

Structuring translational research in myotonic dystrophy: current approaches towards novel therapies
Guillaume Bassez, Neuromuscular Reference Center, Henri Mondor University Hospital, Créteil, France

Myotonic dystrophy type 1 (DM1) is the most prevalence autosomal dominant disease affecting the muscular function for which new treatments are being developed. Recent progress in understanding DM1 pathophysiology led to several molecular and pharmacological therapeutic approaches. Consequently, drug development and upcoming clinical trials further stress the need for dedicated translational research studies. This overview will present successful synergistic initiatives in France to foster collaborative research toward clinical trial readiness. This collaborative approach encompass various tools, infrastructure and network, including (1) the characterization of a DM mouse model, (2) the study of CUG expanded nuclear foci as therapeutic biomarker in mouse and human muscle, (3) a natural history of DM1 patients over 3 years, to prospectively better characterize neuromuscular outcome measures (4) a clinical network of reference neuromuscular centers, (4) the national DM-scope registry, (5) collaborative epidemiological and observational studies aiming at effective selection and enrolment of DM patients in clinical trials.

Plenary Session- Pharmacotherapy
• *Olivier DORCHIES (SWITZERLAND)* • *Zohar Argov (ISRAEL)* • *Ichizo Nishino (JAPAN)* • *Ana Ferreiro (FRANCE)*

Pharmacotherapy of Duchenne muscular dystrophy: an overview on nutraceuticals and repurposed drugs
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Duchenne muscular dystrophy (DMD) is a severe X-linked disorder caused by the lack of dystrophin, a large protein that confers mechanical stability to muscle fibres and ensures proper signalling across the sarcolemma. Boys affected by DMD develop progressive muscle wasting, cardiac and respiratory failure, and early death. Currently, corticosteroids are among the only drugs prescribed to boys with DMD. These drugs have limited efficacy and many adverse effects. Recently, there have been tremendous efforts towards gene and cell therapy to repair or replace the defective dystrophin gene. As these approaches are facing many hurdles, pharmacological strategies using small molecular weight compounds offer numerous advantages. This presentation will discuss briefly the pros and cons of gene therapy, cell therapy, and pharmacotherapy. It will then focus on dietary supplements (nutraceuticals) and repurposed drugs for time-effective translation of data obtained on animal models into clinical trials, hopefully, for the benefit of patients.
Of note, although DMD is a monogenic disease, many signalling pathways and cellular processes are altered downstream of the missing dystrophin. These include impaired calcium homeostasis, mitochondrial function, energy production, protein synthesis, kinase activity, regeneration from stem cells, and excessive production of reactive oxygen/nitrogen species, exposure to cytokines, inflammation, fibrosis, etc. Fortunately, most, if not all, of these features are not specific for DMD but are shared by other disorders such as inflammatory diseases, diabetes, age and cancer-related loss of muscle mass, heart failure, and fibrotic disorders. This situation offers the opportunity to mitigate DMD symptoms via a variety of pharmacological targets and using diverse classes of drugs and nutraceuticals that are already approved for human use and are readily available and affordable. The preclinical evaluation of nutraceuticals and repurposed drugs in DMD animal models and their facilitated translation into clinical trials will be illustrated using examples of our research as well as from other groups. These compounds are creatine, green tea polyphenols and other antioxidants, melatonin, pentoxifylline, rimeporide, losartan, halofuginone, Viagra and related compounds.
Finally, we will show that estrogenic signalling is a previously unrecognized pathway that critically controls dystrophic disease in model mice. Based on published and unpublished work from our group, we will show that tamoxifen, a drug used for more than 30 years to treat breast cancer, efficaciously ameliorates muscle function and structure in dystrophic mice and may become a symptomatic treatment for DMD boys in the next years.