Myasthenia gravis (MG) and chronic inflammatory demyelinating polyneuropathy (CIDP) are autoimmune neuromuscular disorders affecting both adult and children. There is currently very little data on the use of newer immunomodulatory agents, such as Rituximab (RTX), in these children.
Objective: To describe our single-center experience with RTX for the treatment of pediatric patients with MG or CIDp.
Methods: Retrospective chart review of all pediatric patients with MG or CIDP who received RTX at our institution since 2008. Clinical presentation, age at diagnosis, investigations, serological profile, medications, hospitalizations, time from diagnosis to RTX, pre- and post-treatment modified Rankin scale (mRS), pre-treatment Myasthenia Gravis Foundation of America (MGFA) class and postintervention status, adverse effects and follow-up length were recorded.
Results: We identified four pediatric patients with MG and two with CIDP who received RTX. Mean age at diagnosis was 8 years (range: 2-14). Average time from diagnosis to RTX initiation was 27 months (range: 8-43). Average length of follow-up after RTX was 37 months (range: 14-68). At the latest follow-up, four patients had improved compared to their pre-RTX baseline mRS status. Two patients, both of whom had MG, were clinically unchanged. No patient achieved complete remission and all six patients were still on at least one immunomodulatory treatment other than RTX. No adverse effect of RTX was noted.
Discussion: In four out of six patients, RTX therapy led to clinical improvement, but not to complete remission. These four patients achieved sustained clinical improvement after a single RTX course, whereas the other two had no significant change in clinical status after several RTX courses.
Conclusion: A single course of RTX can result in significant and sustained clinical improvement for some pediatric patients with MG or CIDP, but larger studies are needed to determine which patients would benefit most.

chronic inflammatory demyelinating polyneuropathy, rituximab, pediatric autoimmune neuromuscular disorders

P20 –Myotonic syndromes (dystrophic and non-dystrophic)- N° 299 to N° 322

Myotonias (except DM1 and DM2)- #2278

P20- 299- Electromyography in diagnosis of hereditary myotonic syndromes

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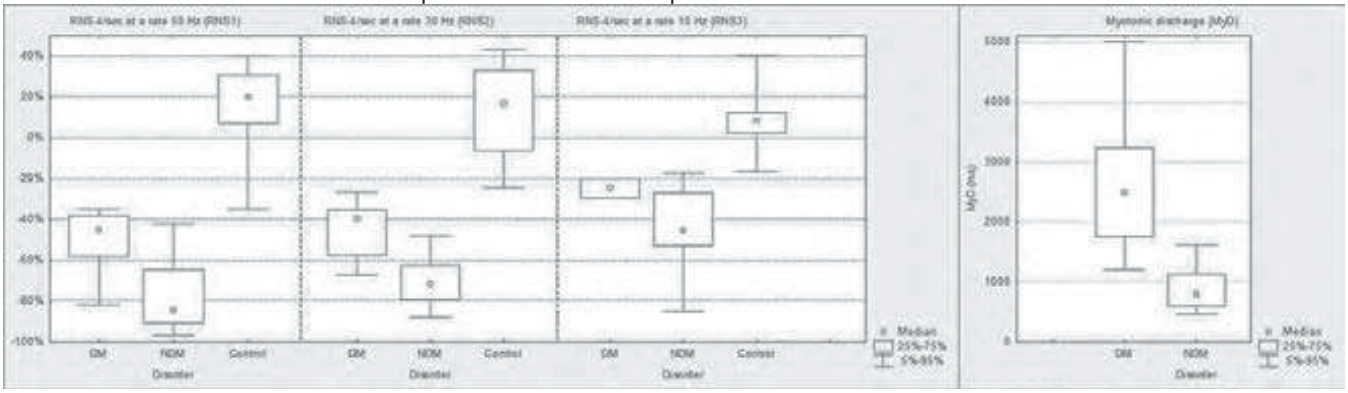
Hereditary myotonic syndromes (HMS)-a small genetically heterogeneous group of a muscle channelopathies with marked clinical polymorphism and often overlapping phenotypes. Myotonia is the leading symptom of HMS, is a result of the membrane excitability of muscle fibers, whose functional consequences can be studied by EMG. The correct EMG analysis can help in diagnosis of different types Myotonic dystrophy (DM), Nondystrophic myotonia (NDM) and to predict possible HMS causative mutations.
EMG was conducted in 88 DNA detected patients with HMS (Becker's disease (BD), Thomsen's disease (TD), DM type 1(DM1) and 2(DM2)). Repetitive nerves stimulation (RNS) 4/s at a rate of 50 Hz (RNS1) was done 64 patients, 57 patients of 30 Hz (RNS2), 58 patients of 10 Hz (RNS3). SET was held in 58 patients according to Fournier I, II, III patterns. 87 patients had needle EMG with an average duration of 3 typical myotonic discharge (MyD) (Table).
CF. Figure
We found no significant statistical differences in the values of the EMG in the patients with ?D and BD, similarly in the patients with DM1 and DM2. RNS2 and RNS3 were not enough in proving in diagnostic our patients' HMS.

The most significant changes were received in NDM (TD and BD) and DM (DM1 and DM2). The CMAP decrement was less than 60% in RNS1 in 18(78.3%) DM patients and only 5(16.1%) NDM patients. In NDM patients the decrement remained stable for over 5 years. One patient with homozygous CLCN1 gene mutations c.1936A>G did not have decrement in RNS1. MyD were longer than 1.5 seconds in 35(83.3%) DM patients, and only 4(9.5%) NDM patients, 2 DM1 patients did not have MyD.
Pattern II in SET was detected only 5(20.8%) NDM patients. Pattern III in SET was found in 42(80.8%) NDM and DM patients.
RNS1 and MyD allows to distinguish between NDM and DM patients (p>0,01) and the stable persistent CMAP decrement in the NDM will be possibly important in predicting of the individual mutations in the CLCN1 gene. The fact that Fournier's pattern II in SET was not informative in TD and BD patients needs to be explained in further researches.



Becker's disease, Thomsen's disease, EMG, repetitive nerves stimulation, myotonic discharge, short exercise test, channelopathies

Myotonias (except DM1 and DM2)- #2386

P20- 300- Pharmacological characterization of four hNav1.4 sodium channel mutations causing myotonia to address personalized therapy

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Paramyotonia congenita and sodium channel myotonia are two allelic human diseases characterized by a delay in muscle relaxation after contraction leading to skeletal muscle stiffness. The diseases are caused by gain-of-function mutations of the Nav1.4 voltage-gated sodium channel. Mexiletine was recently approved as orphan drug in non-dystrophic myotonias. By blocking muscle sodium channels, mexiletine reduces sarcolemma excitability and counteracts myotonia. Yet, lack of both efficacy and tolerability have been observed for a significant number of patients, who critically need alternative options. We previously showed that the G1306E hNav1.4 mutant causing myotonia permanens, a severe form of sodium channel myotonia, is less sensitive to mexiletine in vitro compared to wild-type channel, and that patients carrying G1306E can gain benefits by shifting treatment to flecainide, another sodium channel blocker, thereby opening the way toward a bench-to-patient pharmacogenetics approach (Desaphy et al., Neurology 2001; J. Physiol. 2004; Eur. J. Clin. Pharmacol. 2013). Here we studied the function and pharmacology of four other hNav1.4 mutations causing sodium channel myotonia or paramyotonia congenita, three of which are novel. As G1306E, the four mutations are located in channel structures involved in fast inactivation machinery.
The mutant channels were expressed in tsA201 cells and whole-cell sodium currents were recorded with patch-clamp technique. The four mutations induce functional defects in channel gating similar to G1306E, including a marked slowing of channel inactivation and a shift of fast inactivation voltage dependence toward positive voltages. Such effects likely account for the sarcolemma hyperexcitability and muscle stiffness in carriers. A reduced sensitivity of channel mutants to mexiletine was observed, while flecainide effects appeared to be preserved. These results indicate flecainide as a valuable antimyotonic drug, especially in patients carrying mutations inducing a positive shift of Nav1.4 fast inactivation voltage dependence. Accordingly, therapy was successfully shifted to flecainide in a patient carrying one of the new mutations. Supported by Association Française contre les Myopathies (grant #19027), Telethon-Italy (grant GGP14096), and Italian Department of Health (grant GR-2009-1580433).

myotonia; sodium channel; pharmacogenetics

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2338

P20- 301- Retinal changes in myotonic dystrophy (DM1): a case report and a review of the literature

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Introduction
Ocular manifestations of myotonic dystrophy type 1 (DM1) include cataracts, ptosis, weakness of the ocular muscles, ocular hypotony and retinal changes. Ophthalmologists are familiar with cataract in DM1 patients, but retinal involvement is less well known, although the 1st publication dated in 1952.
Presentation of case
We report the case of a 55-year-old female, previously confirmed as DM1 who experienced visual acuity decrease on both eyes. Slit lamp examination revealed lens opacity. Optical coherence tomography (OCT), performed in 2008, 2013 and 2015, showed progression of epiretinal membranes (ERM).
Review of the literature
A searching in PubMed using key-words: ?retina? and ?myotonic dystrophy? has identified about 30 articles reporting cases or case-series of DM1 patients with retinal lesions.
Decrease of a- and b-waves amplitude was observed through electroretinography. Retinal pigmentary changes were identified by funduscopy and fluorescein angiography.
They were composed of butterfly-shaped pigmentary changes in the macula, reticular pigmentary retinal changes in the midperiphery, and peripheral atrophic polygonal-shaped changes. OCT, a non-invasive technique, is the most valuable advance in retinal diagnosis imaging. According to a study, 56.7% of DM1 patients had an ERM in at least one eye. The presence of ERM was significantly correlated with increasing age in the DM1 group.
Conclusions
In patients with DM1, the fundus should be examined carefully. In addition to clinical examination, OCT examination should be implemented as part of an ophthalmologic assessment for DM1 patients with reduced visual acuity.
It is of great relevance to analyze the presence of retinal changes that might limit the visual improvement following cataract extraction or ERM removal during vitrectomy. As therapeutic approach is available for ERM, the retinal screening is important for DM1 patients.
A better collaboration between ophthalmologists and neurologists from neuromuscular centers could optimize the eye care management of patients with DM1. This partnership could be facilitated through DM-scope, the longitudinal observational DM1 registry. To date, DM-scope collects information from more than 2,200 patients.

myotonic dystrophy, decreased visual acuity, retinal epiretinal membrane, optical coherence tomography

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Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2362

P20- 302- DM-Scope, a French nationwide registry to decipher pediatric myotonic dystrophies' clinical complexity

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Background: Myotonic Dystrophy type 1 (MD1) is known to exhibit a highly variable phenotype regarding its age of onset and the changing systemic involvement. However, most clinical data arise from observational studies that focus on MD1 adult forms. Pediatric descriptions are scarce, rely on restricted cohort of patients and thus remain incomplete.

Objective: We aimed to phenotypically characterize a wide MD1 pediatric population especially concerning the overall disease history, the severity of cognitive impairment and the extent of systemic manifestations.

Method: Since 2010, the French DM-Scope registry includes applications that aim to optimize the annual clinical evaluation of adult MD1 patients, and to promote clinical research in this field. Throughout 2014, we focused on the pediatric population and developed specific tools (i.e. standardized form, synopsis) in order to better characterize the disease in children and improve their standard of care. Currently, 24 neuropediatrics centers are involved in this observational study. Overall the French registry gather data from 2202 MD patients collected in 50 neuromuscular centers.

Results: DM-Scope has already enrolled 246 MD1 children (>18 years), 49% girls vs. 51% boys including congenital (38%), infantile (48%) and juvenile forms (14%). We will present this preliminary characterization of a large MD1 pediatric cohort and stress out some specific patterns observed in the 3 clinical forms.

Conclusion: DM-Scope, the French nationwide clinical network on myotonic dystrophies constitutes a strong task force. Up to date, the registry describes the largest adult and pediatric MD cohorts worldwide. DM-Scope therefore provides a powerful platform designed to optimize routine clinical management and clinical research in the field of myotonic dystrophies.

Myotonic dystrophy type 1, registry, phenotype, natural history

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Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2373

P20- 303- Upregulation of glutamate transport corrects cerebellum dysfunction in a mouse model of myotonic dystrophy

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Brain function is compromised in myotonic dystrophy, leading to debilitating symptoms in children and adult patients. DM type 1 (DM1) is mediated by the abnormal accumulation of toxic RNAs carrying expanded CUG repeats, which disrupt alternative splicing, transcription, polyadenylation and translation of downstream targets. In the central nervous system we do not know the extent of these molecular abnormalities, nor do we know the cell populations, neuronal circuits and pathways primarily affected. To address this question, we have been using the DMSXL transgenic mouse model of DM1 developed in our laboratory, which express a human DMPK transgene carrying more than 1000 CTG repeats. Mouse phenotyping revealed deficits in cerebellum-dependent motor coordination, while electrophysiological profiling in alert mice demonstrated abnormalities in Purkinje cell firing. These results are intriguing, since cerebellum is not typically associated with DM1 neuropathology. To investigate the mechanisms behind Purkinje dysfunction, we studied RNA foci distribution and found extensive foci accumulation in astroglial Bergmann cells surrounding the Purkinje layer, in both mouse and human cerebellum. Interestingly, abundant RNA foci were associated with more severe spliceopathy in laser microdissected Bergmann glia from DMSXL mice, relative to neighbouring Purkinje neurons. The pronounced RNA toxicity in astrocytes was associated with the downregulation of a glia-specific glutamate transporter in the brain of DM1 mice and patients. Pharmacological strategies to increase glial glutamate recapture in DMSXL mice corrected Purkinje cell electrophysiology, as well as motor discoordination, indicating that mouse cerebellum dysfunction is mediated by defective glutamate uptake by the supporting Bergmann glia. Our results demonstrate for the first time the critical role of astrocyte function and neuroglial communication in disease biology, and open new perspectives to the development of pharmacological approaches based on the manipulation of glutamate signalling in DM1 brains.

myotonic dystrophy, cerebellum, astrocyte, glutamate

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2411

P20- 304- Brain cell specificity of DM1 neuropathogenesis

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Myotonic dystrophy type 1 (DM1) is a complex, multisystemic disorder, in which the neurological manifestations severely affect the quality of life of patients and their families. The disease is caused by the abnormal expansion of a CTG repeat in the 3'UTR of the DMPK gene. Expanded DMPK transcripts accumulate in ribonuclear aggregates (or foci) in the nucleus of DM1 cells and disrupt cell and tissue functions.
Numerous clinical, neurophysiological and imaging studies have confirmed the involvement of the central nervous system (CNS) in DM1, but the cellular and molecular pathways affected in the brain are not yet fully understood.
We have generated the DMSXL transgenic mouse model, which expresses more than 1000 CTG repeats within the human DM1 locus and provides a useful tool to investigate the molecular mechanisms of the brain disease. These animals show foci accumulation and splicing defects in multiple tissues, notably in the CNS. As a result, DMSXL mice exhibit behavioral and cognitive phenotypes, electrophysiological defects, neurochemical changes and synaptic protein deregulation, indicative of neurological dysfunction.
RNA foci are more abundant in glial cells in vivo, in both DMSXL mice and DM1 brains, suggesting different cell type susceptibility to the accumulation of CUG repeats. We have used primary neuronal and glial cultures derived from DMSXL brains to investigate the cell type-specific disease mechanisms. Interestingly, DMSXL primary astrocytes show higher expression of expanded DMPK transcripts, more abundant RNA foci accumulation and more severe spliceopathy relative to primary neurons. The higher RNA toxicity in primary DMSXL astrocytes is associated with deregulated adhesion, growth dynamics and cell polarization during migration. All together these results indicate a marked deleterious effect of the expanded transcripts on astroglial biology. We are currently investigating the molecular and cellular phenotypes of DMSXL primary neurons through similar studies.
In order to dissect the molecular events and pathways specifically affected in individual brain cell types, we have performed global proteomics analysis of homogenous cultures of primary astrocytes and neurons. The molecular defects identified are currently being validated and functional tests will assess their implication in the disease biology.

Myotonic dystrophy type 1, central nervous system, transgenic mice, neuronal and astroglial biology

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Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2427

P20- 305- Emotional recognition impairment in the childhood-onset form of myotonic dystrophy type 1

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Brain involvement is now well-recognized as a common feature in a substantial proportion of patients with DM1. Depending on the phenotypic expression, the degree of cognitive impairment remains heterogeneous, ranging from mental retardation (in the congenital form) to selective executive and/or emotional deficits (in the adult-onset form). While somatic symptoms may be relatively discrete in the childhood form, deficits in visuo-constructive and visuo-spatial functions as well as verbal working memory weakness, poor attentional process and alexythimia have been reported. Scarce brain imaging studies suggest, in one hand, significant white matter abnormalities throughout the brain whereas other reported specific abnormalities in the bilateral temporal regions. In the adult phenotype, recent studies have shown significant correlation between lesions of the limbic system and dysfunction in the facial expression processing. The objective of the current study is to explore the nature and the extent of potential impairments on the recognition of emotional expressions in the childhood phenotype.
14 patients aged 6 to 20-year olds with the childhood DM1 (paternal or maternal transmission) and 14 typically developing children/adolescents matched to the DM1 group (according to age, gender and IQ) were included. All subjects took a computerized task were they had to select one label from a list choice that best described the emotion that was being expressed (visual, auditory or multimodal stimuli). Preliminary results highlight different patterns of performance according to (1) unimodal/multimodal emotional processing and (2) the valence of emotional expression (positive, negative and neutral). These data could provide a source of evidence of a continuum between neurocognitive deficits emerging during childhood and those occurring later in adults and emphasize the early vulnerability of social cognition in the childhood phenotypic expression of DM1.

DM1, Childhood phenotype, emotional processing , social cognition impairment

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Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2498

P20- 306- Atypical Myotonic Dystrophy type 1 families and CTG repeat contractions

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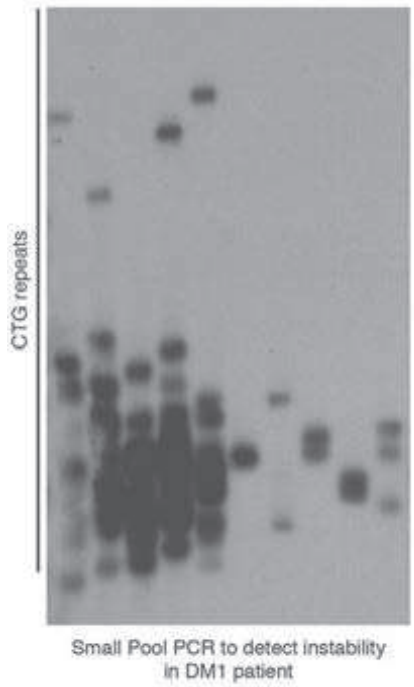
Myotonic dystrophy type 1 (DM1) is a dominant multisystemic disorder characterized by myotonia, cardiac and cognitive dysfunctions. DM1 is associated with a CTG repeat expansion in the 3' UTR of the DMPK gene. In the normal population, the CTG repeat is polymorphic and varies from 5 to 37 repeats, while in DM1 patients repeats ranged from 50 up to 4000 CTG. The

repeat usually increases from generation to generation, is generally correlated with clinical severity and age of onset, explaining anticipation observed in DM1 families. Somatic instability is particularly high and progresses with age towards larger repeats with different rates of expansion between tissues. DM1 family pedigrees have shown that >90% of transmissions result in expansions and 10% in contractions of the CTG repeat. To date, the mechanisms of contractions remain unknown. At the Necker hospital diagnosis center, we identified an unusual DM1 family (family A) showing contractions of the CTG repeat over three successive maternal transmissions and no anticipation suggesting genetic modifier(s) of the repeat instability. In order to determine if the somatic instability is also unusual in this family, we compared the CTG repeat somatic mosaic in blood from members of the family with the mosaic observed in blood from DM1 controls from DM1 families with intergenerational expansions. The CTG repeats appear more stable in patients from family A suggesting also a role of the genetic modifier(s) in somatic instability. By analyzing carefully the CTG sequences by TP-PCR developed in 5' of the repeat, we identified a CAG interruption. This finding suggests that this single CAG repeat in 5' could stabilize the CTG repeat across generations and in tissues. The perspective of this work is to demonstrate that this interruption stabilizes the repeat in model systems and to identify the mechanisms involved. Using the DM registry, DM-scope, developed by Dr Bassez, we identified new DM1 families showing CTG repeat stabilization across generations. We will also characterize the somatic instability in these families and look for possible genetics modifier(s). Understanding the mechanisms involved in the CTG repeat dynamic and particularly in the stabilization or contraction of the CTG repeat length will improve DM1 prognosis and can provide new clues for the development of therapeutically approaches.
E. Dandelot and S. Tomé contributed equally to this work.



Trinucleotide repeat, Instability, Contraction

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2509

P20- 307- Skeletal muscle and circulating microRNAs in Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is a multisystemic disorder that affects skeletal and smooth muscles as well as the eye, the heart, the endocrine, and the central nervous systems. DM1 is caused by abnormally expanded stretches of CTG DNA triplets in the DMPK gene. This mutation causes the accumulation of DMPK gene transcripts into nuclear foci and RNA toxicity due to dysfunction of mRNA splicing, protein translation and microRNAs (miRNAs). miRNA are short noncoding RNAs that, after maturation, are loaded onto the RISC effector complex that destabilizes target mRNAs and represses their translation. We and others established that the accumulation and the localization of specific miRNAs are altered in DM1 patients. However, the functional implications of these miRNA aberrations are still largely unknown. In order to discover the miRNAs that are functionally deregulated in DM1, we analyzed the RNAs associated to RISC immunoprecipitates of muscle biopsies derived from DM1 patients and matched controls. Using RNA-Sequencing, we identified a combination of miRNA/target mRNA couples that interact on the RISC complex and are enriched in DM1 patients. Specifically, we found miRNAs known to be involved in muscle damage and disease, as well as a number of deregulated mRNAs involved in energy metabolism and in muscle structure. Potential miRNA/mRNA pairs that could be involved in the dystrophic phenotype have been highlighted.

Additionally, miRNAs are also present in bodily fluids, representing attractive potential biomarkers. Indeed, non-invasive biomarkers are a clear unmet need for DM1 diagnosis/staging evaluation. In recent works, we and others identified a subset of miRNA differently expressed in the plasma of a small group of DM1 patients. To validate these findings, an independent group of 100 DM1 and 100 controls were recruited identifying a DM1-miRNA signature of 8 miRNAs, 4 of which muscle specific, deregulated in DM1 patients. A "DM1 miRNA score" was elaborated, allowing to discriminate DM1 from controls (AUC: 0.84; p>0.0001). Of note, the DM1 miRNA score displayed an inverse correlation with muscle strength (r: -0.5; p>0.0001).
In conclusion, both skeletal muscle and plasma microRNAs are altered in DM1. Understanding miRNA regulation and role in DM1 is instrumental for unveiling DM1 molecular pathogenetic mechanisms and the development of new therapies.

DM1, microRNA, gene expression, biomarker

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2513

P20- 308- Post insulin receptor signalling abnormalities in myotonic dystrophy skeletal muscle cells.

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Myotonic dystrophies (DM) are autosomal dominant multisystemic disorders characterized by a variety of multisystemic features. Myotonic dystrophy type 1 (DM1) is caused by an expanded (CTG)n in the 3'untranslated region of DMPK gene, while the second form (DM2) is caused by an expanded (CCTG)n in the intron 1 of CNBP gene. The nuclear accumulation of CUG/CCUG- containing transcripts alters the function of specific alternative splicing regulators leading to aberrant alternative splicing of different genes that explain several DM features. Splicing alteration of insulin receptor (IR) gene is considered one of the causes of the metabolic dysfunctions, such as insulin resistance, hyperinsulinemia and a fourfold higher risk of developing Diabetes mellitus type 2, that characterize DM patients. However, at skeletal muscle level, there is still no mechanistic explanation for the muscle weakness and atrophy observable in DM patients or for the muscle histopathological features. The aim of this work is to investigate the mechanisms that contribute to the peripheral insulin resistance and to define if molecular defects in insulin signal might contribute to muscle atrophy and weakness observable in DM patients. Human skeletal muscle cultures provide a powerful tool for the investigation of the biochemical and genetic basis of peripheral insulin resistance, since glucose uptake and glycogen synthesis remain responsive to insulin in cultured muscle cells. Our study was performed on myotubes at 5 days (T5) of differentiation derived from myoblasts isolated from muscle biopsies of 3 DM1, 3 DM2 and 3 healthy subjects. IR splicing alteration was analysed by RT-PCR. Myotubes were stimulated with 10 nM insulin for 0, 5, 15 and 30 minutes. The activation of several proteins involved in PI3K or RAS pathway, such as IRS-1, AKT, p70, MAPK and GSK3?, was analysed by western blot. Our results show that at T5 both control and DM myotubes express more foetal than adult IR isoform. However, the activation of the insulin pathway appears to be lower in DM myotubes and this alteration seems to be more evident in DM2 muscle cells. These data indicate that post receptor signalling abnormalities might contribute to DM insulin resistance regardless the alteration of IR splicing. Further investigations will be necessary to identify novel biomarkers that could be target for therapeutic intervention to improve the quality of life of DM patients.

myotonic dystrophies, insulin resistance, insulin receptor, skeletal muscle cells

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2518

P20- 309- Involvement of microtubule network in myotonic dystrophy insulin resistant skeletal muscle cells

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Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are multisystemic disorders linked to two different genetic loci. DM1 is caused by an expanded (CTG)n in the 3' UTR of the DMPK gene, while DM2 is caused by the expansion of a (CCTG)n repeat in the intron 1 of the CNBP gene. In both forms, expanded repeats cause the nuclear accumulation of mutant transcripts thus deregulating the activity of some RNA-binding proteins and providing an explanation for the multisystemic phenotype. DMs are characterized by metabolic dysfunctions such as insulin resistance and a fourfold higher risk of developing Diabetes mellitus type 2. It has been suggested that the splicing alteration of insulin receptor (IR) may play a role in peripheral insulin resistance. However it cannot be excluded that post receptor signalling abnormalities could also contribute to this feature of DM. The binding of insulin to its receptor activates a complex pathway culminating in the translocation of the glucose transporter GLUT4 into the plasma membrane. Since the cytoskeleton provides a platform for intracellular transport, the aim of this work is to investigate if actin and microtubule organization is impaired in DM skeletal muscle cells. Myotubes at 5 days of differentiation (T5) from 3 healthy subjects, 3 DM1 and 3 DM2 patients have been stimulated with 10 nM insulin from 0 to 60 minutes. Actin and microtubule organizations and GLUT4 translocation have been analyzed by immunofluorescence. Moreover, the expression of two kinases (ERK1/2 and GSK3?) involved in the stabilization of microtubules has been analyzed by western blot. Fluorescent analysis of actin remodelling in response to insulin stimulation did not show any difference between control and DM myotubes. However, microtubule nucleation analyzed by ?-tubulin staining has shown that DM muscle cells exhibit a defective microtubule reorganization after insulin stimulation. This alteration is associated with an increased activation of ERK1/2 and of GSK3?. These data indicate that microtubule abnormalities might contribute to a lower glucose uptake observed in DM myotubes. Further investigations will be necessary to identify if microtubule instability might be related to other clinical features of this disease.

microtubule, cytoskeleton, myotubes, actin, insulin resistance

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2552

P20- 310- Histopathological alterations in myotonic dystrophy: molecular evidences for the premature aging of skeletal muscle

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Myotonic Dystrophies (DM) are autosomal dominant neuromuscular disorders characterized by a variety of multisystemic features. To date two types of Myotonic Dystrophies have been described: Myotonic Dystrophy type 1 (DM1) and type 2 (DM2). DM1 is caused by an expanded (CTG)n in the 3' untranslated region of the DMPK gene, while DM2 is caused by the expansion of a tetranucleotidic repeat (CCTG)n in the intron 1 of the CNBP gene. In both forms, the nuclear accumulation of mutant transcripts alters the function of alternative splicing regulators, leading to an aberrant alternative splicing (spliceopathy) of several genes that causes the multisystemic phenotype of the disease. However, spliceopathy does not completely explain skeletal muscle histopathological alterations commonly observed in DM patients. There are evidences that many symptoms of DM adult form, such as muscle weakness and wasting, cataracts and cardiac arrhythmias are reminiscent of normal aging. Sarcopenia is the age-related condition characterized by the progressive loss of mass, strength and function of skeletal muscles. Interestingly, skeletal muscle of DM patients shares apparent similarities with the aging muscle. Indeed, in both conditions, fiber size variability, central or clumped nuclei and decreased muscle regeneration capability are observed.
In this background, the aim of this work was to analyze the molecular similarities between aging-related skeletal muscle atrophy and DM histopathological alteration. In particular, the expression of atrogin 1/MURF1, Lamin A/C and activated ERK1/2 was investigated both in proliferating and senescent myoblasts and in muscle samples obtained from biceps brachii skeletal muscle.
In DM skeletal muscle atrogin1 and MURF1 protein levels appeared to be similar to those observed in age-matched healthy controls. These data are in line with those reported in aged skeletal muscles that do not show an increase in protein degradation mediated by atrogin1/MURF1 upregulation. Moreover, the expression of Lamin A/C, proteins involved in premature aging syndrome progeria, decreased during in vitro DM myoblast aging. Furthermore, an increase in the activation of the ERK1/2 kinases in both proliferating and senescent myoblast was observed in DM muscle cells.
In conclusion, these data suggest that the histopathological alterations observable in DM skeletal muscle can be considered the result of a premature muscle aging.

sarcopenia, aging, skeletal muscle, atrogenes

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2630

P20- 311- Study design of an assessment protocol for central and peripheral fatigue in myotonic dystrophy type 1 DM1).

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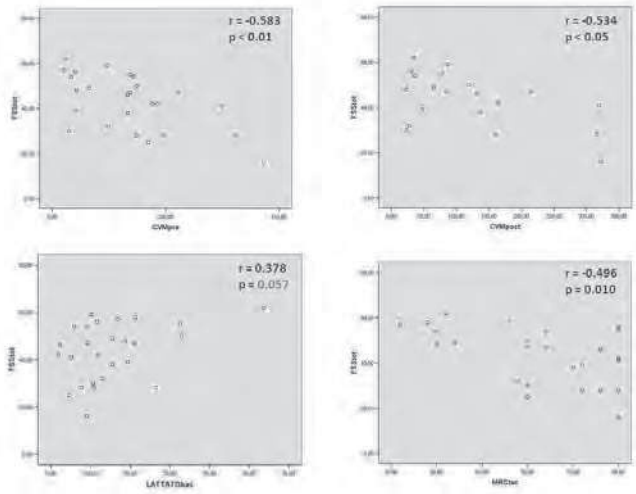
DM1 is an autosomal-dominant disorder characterized by muscle weakness, myotonia, and multisystemic involvement. According to current literature fatigue and daytime sleepiness are among the main symptoms of DM1 (Kalkman et al. 2005). Oxidative stress has been proposed to be one of the pathogenic factors of fatigue consequent to DM1 (Angelini and Tasca, 2012). Bray et al. (2012) suggested that the decrease in maximal force production observed during repeated muscular effort can be caused by a central mechanism, specifically the expenditure of CNS resources.
To get an overview of different types of fatigue, we studied the dimensions of experienced fatigue and of physiological fatigue in a sample of 26 DM1 patients (17 males, 9 females, mean age 41.6 years, SD ± 12.7) meeting both genetic and clinical criteria for DM1; experienced fatigue was studied through Fatigue Severity Scale (FSS), and physiological fatigue was measured through an intermittent incremental effort of the forearm muscles using a myometer; oxidative stress balance blood markers trend during aerobic exercise test were collected. Our exercise protocol proved to be easily deployable and well-tolerated thus we consider it a suitable tool for the intended purposes; however statistical analysis revealed no significant differences between oxidative stress balance markers before and after the effort. The occurrence of central fatigue in the sample means that central activation worsens during the motor contraction; interestingly FSS score was significantly correlated to MVC (before and after the effort, $r_{before} = -0.583$, $p > 0.01$, $r_{post} = -0.534$, $p > 0.05$), and to motor disability measured by MRC ($r = -0.496$, $p > 0.05$); moreover we found a strong tendency towards significance in the association to lactate baseline ($r = 0.378$, $p = 0.057$). The presence of daytime sleepiness was also assessed (ESS) but was not correlated to any of the other variables. Multiple regression analysis have then been performed to see whether and how experienced and physiological fatigue measures relate to the other variables.
Results are discussed to define whether or not, based on clinical and laboratory grounds, such exercise training protocol may be suitable for proper management of DM1 patients. Proper assessment of fatigue should therefore be included in algorithms for data collection in DM1 patient registries.



fatigue, dm1, central, peripheral

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2643

P20- 312- Deciphering myotonic dystrophy type 1 phenotypic spectrum: a comprehensive registry-based multicentre observational study

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Background: Myotonic Dystrophy type 1 (DM1) is one of the most variable neuromuscular disorder. Variation of age at onset, multisystemic involvement and severity limit prognosis, medical care, and clinical trials design. Yet, comprehensive large scale studies in the DM1 population are lacking.
Objective: To shed light on the frequency of clinical manifestations in a large cohort of DM1 adult patients. We focused, particularly, on symptoms prevalence and their respective relationship.
Methods: In a multicenter observational study, univariate and multivariate analyses of symptoms standardized data were performed in an unprecedented large cohort of DM1 adult patients (France, n=1910; Quebec, n=1103) gathered in the DM-Scope international registry.
Results: We show that the majority of patients have juvenile or adult clinical forms. Frequencies of DM1 clinical manifestations were as follow: myotonia (90%), obvious limb muscle weakness (MIRS 3 to 5) (85%), cataracts (64%), dysphagia (60%), digestive tract symptoms (46%), cardiac defects (44%), respiratory dysfunction (35%), hypersomnia (29%), and severe school or job limitations. Endocrine disturbances included thyroid dysfunction (17%), diabetes (8%) and 26% had hypofertility. The frequency of some symptoms differed, to some extents, between the French and Quebec populations. Furthermore, we observed that some clinical features were positively or inversely correlated, and discriminated specific phenotypic manifestations correlating with the CTG repeat expansion mutation.
Conclusion: Assessing a large cohort of patients will allow an accurate description of the complex temporal and clinical DM1 phenotypic spectrum. Furthermore, the identification of distinct clinical patterns will facilitate both design and patients recruitment in upcoming clinical trials.

myotonic dystrophy, epidemiology, registry, phenotype

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2644

P20- 313- Refining myotonic dystrophy type 1 clinical classification

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Background: Myotonic dystrophy (DM) is considered as one of the most variable genetic diseases. Since innovative therapeutic strategies are approaching for DM it is becoming of crucial importance to reach harmonization for disease classification. Despite several experts groups are working on classification, no consensus is yet available. Databases provide powerful tools to remove the uncertainty.
Objective: We assessed the robustness of a DM1 classification model divided into five clinical forms based on age of onset.
Methods: The study has been performed on a large collection of standardized data obtained in 1962 French DM1 patients (>18yrs) from the nationwide DM-Scope registry. The five clinical forms were first compared for distribution of CTG expansion

size, frequency and age of onset of the main symptoms. Then, using a particular two-dimensional method combining time and frequency as parameters, we determined the clinical profile of DM1 manifestations for each clinical form.
Results: Analyses validate the DM1 classification model divided into five clinical forms with regard to the triplet repeats expansion size, frequency, age of onset and clinical profiles of the main symptoms. Patients were classified as follow: congenital (3.7%), infantile (17.8%), juvenile (25.9%), adult (39.4%) and late onset forms (13.1%). We show that the continuum assumption from congenital form to late onset form is a reality. Furthermore, our data allow us to highlight clinical manifestations specific for some clinical forms.
Conclusion: This study provides strong evidence supporting a five-grade DM1 clinical classification model. In addition we observed specificities in clinical profiles related to particular DM1 forms. Together these results may refine the DM1 classification and improve clinical trial design.
myotonic dystrophy, registry, disease classification

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2680

P20- 314- Implication of BIN1 in myotonic dystrophy type 1 disease

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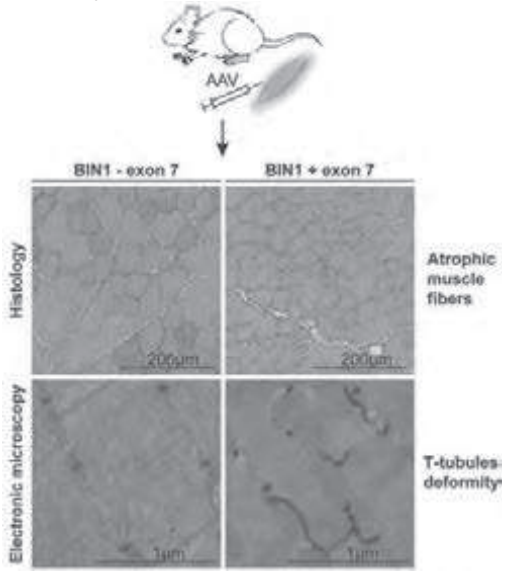
Myotonic dystrophy of type 1 (DM1), the most common dystrophy in adults, is an autosomal disorder characterized by muscular myotonia, muscle weakness, heart conduction defects and some others common features like ocular cataract and insulin resistance. DM1 is due to a large expansion of CTG repeats located in the 3'-UTR of the DMPK gene. Expanded CTG repeats are transcribed in pathogenic RNA, which sequester RNA-binding proteins such as the splicing regulator MBNL1, resulting in alternative splicing defects in DM1 patients.
We identified that the alternative splicing of the Bridging Integrator-1 (BIN1) mRNA is altered in DM1. BIN1 has important functions in skeletal muscle, notably in the biogenesis of muscle T-tubules, which are key actors of the excitation-contraction coupling machinery. Moreover, mutations in BIN1 or Dynamin 2 (DNM2) genes can leads to Centronuclear Myopathy, which shares some histopathological features with DM1.
We analyzed the splicing regulation of BIN1 exon 7, and found that its inclusion is repressed by MBNL1, which binds to CUG motifs located upstream of BIN1 exon 7. Consequently, exon 7 is aberrantly expressed in DM1 skeletal muscle. Also, we confirmed previous in vitro findings demonstrating that the presence of exon 7 enhances the interaction of BIN1 protein with DNM2 (Ellis et al., 2012).
Next, we addressed the in vivo impact of exon 7 inclusion using AAV-mediated expression of BIN1 isoforms (-/+ ex.7) in skeletal muscle of wild type mice. We found that muscle overexpressing BIN1 +exon 7 displays t-tubules deformity, higher number of small atrophic fibers and increased muscle fatigue compared to control mice.
These results suggest that aberrant inclusion of BIN1 exon 7 in DM1 skeletal muscle leads to forced interaction of BIN1 with DNM2 and would contribute to the muscle atrophy and the muscle weakness observed in DM1 patients.



BIN1, DNM2, myotonic dystrophy type 1, muscle weakness, alternative splicing, AAV2/9

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2782

P20- 315- A decoy-based gene therapy to neutralize CUG-expanded RNA toxicity in DM1

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Myotonic Dystrophy type 1 (DM1) is characterized by myotonia, progressive muscle weakness and wasting as well as cardiac and cognitive defects. This autosomal dominant disease is caused by an expanded CTG repeats located in the 3' UTR of the

DMPK gene. At the molecular level, expression of pathogenic DMPK transcripts containing expanded CUG repeats (CUGexp-RNA) results in a toxic RNA gain-of-function mechanism. CUGexp-RNAs are retained into the nucleus as riboprotein aggregates that sequester MBNL splicing factors leading functional loss of MBNL and specific alternative splicing misregulations. Thus, mis-splicing of CLCN1 and BIN1 pre-mRNAs contributes respectively to myotonia and muscle weakness. Currently several strategies are under development to reverse toxic CUGexp-RNAs dominant effects.
The aim of this study is to restore functional level of MBNL using a novel decoy-based gene therapy approach to release endogenous sequestered MBNL factors from CUGexp-RNA aggregates. For this purpose, we engineered a modified MBNL?-decoy tool displaying high binding property for expanded CUG repeats and lacking splicing activity. Thus, a GFP-MBNL? construct was expressed in DM1 muscle cells using lentiviral vectors to evaluate its ability to inhibit CUGexp-RNA toxicity. GFP-MBNL? is able to bind to CUG-expanded transcripts and splicing misregulations as well as differentiation defects were corrected in MBNL?-treated DM1 muscle cells. Next, intramuscular injections of AAV-GFP-MBNL? vectors were performed in DM1 mice expressing 220CTG in skeletal muscles. As observed in DM1 cells, GFP-MBNL? tools colocalize with nuclear CUGexp-RNA aggregates in myofibers and splicing alterations of several transcripts were normalized in injected DM1 mouse muscles. In addition, myotonia was also abolished in MBNL?-treated DM1 mouse muscles indicating that MBNL? can compete and release functional endogenous MBNL from CUGexp-RNA aggregates. In conclusion, we propose that a MBNL? decoy-based gene therapy approach could represent an alternate or complementary therapeutic approach for DM1.

Myotonic Dystrophy, gene therapy, expanded CTG, MBNL

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2790

P20- 316- Abnormal splicing switch of DMD's penultimate exon compromises muscle fiber maintenance in Myotonic Dystrophy

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Myotonic Dystrophy type 1 (DM1) is characterized at the skeletal muscle level by progressive weakness, wasting and myotonia. DM1 is an autosomal dominant disorder caused by an expanded CTG repeat in the 3'UTR of the DMPK gene, for which expression of pathogenic RNA leads to muscular dysfunction. CUG-expanded RNAs are retained in nuclear aggregates that sequester MBNL RNA binding factors involved in the regulation of alternative splicing during development. Functional loss of MBNL1 results in abnormal splicing of a subset of pre-mRNAs in DM1. Among them, missplicing of CLCN1, and BIN1 pre-mRNAs have been associated with myotonia and muscle weakness, respectively. Additional missplicing events have been described in skeletal muscles of DM1 patients, however their consequences on muscle function remain largely unknown. In this study, we were interested to the aberrant splicing of DMD exon 78 that strongly correlates with muscle disease severity in DM1 patients.

DMD gene is composed of 79 exons encoding a 427-kDa subsarcolemmal dystrophin protein in skeletal muscle. Alternative splicing of DMD exon 78 is regulated by MBNL1 during skeletal muscle development and modifies the dystrophin C-terminus structure leading to a ?-sheet C-terminus in the adult isoform in place of an amphipathic ?-helix C-terminus in the embryonic isoform. This developmental transition is required for muscle function since forced exclusion of dmd exon 78 using an exon-skipping approach in zebrafish severely impairs mobility and muscle architecture. Moreover, expression of micro-dystrophin constructs in dystrophin deficient mice demonstrates that the presence of the amphipathic ?-helix C-terminus is not able to improve muscle function in contrast to the ?-sheet C-terminus. Finally, we show that forced Dmd exon 78 skipping and subsequent embryonic dystrophin re-expression in wild-type mice leads to muscle fiber remodelling and ultrastructural abnormalities. Similar changes have been described in affected muscles of DM1 patients suggesting that abnormal splicing of DMD exon 78 due to pathogenic CUGexp-RNA mediating MBNL1 functional loss could contribute to the progressive dystrophic process in this disease.

DM1, Dystrophin, alternative splicing,MBNL1, muscle fiber maintenance, dystrophy

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Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2852

P20- 317- Enhanced systemic delivery of antisense oligonucleotides using cell-penetrating peptide to reverse RNA toxicity in Myotonic Dystrophy

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4. Laboratory of Molecular Biology, Medical Research Council, Cambridge, Royaume Uni
5. Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, Royaume Uni
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7. Généthon, Evry, France
8. Inserm UMR 1163, Paris Descartes-Sorbonne Cité University, Imagine Institute, Paris, France

Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease, characterized by progressive muscle atrophy and weakness, myotonia, cardiac defects and other multisystemic symptoms. DM1 mutation consists in an abnormal expansion of a CTG repeat in the 3'UTR of the DMPK gene. As a consequence, mutant transcripts containing expanded CUG repeats (CUGexp-RNA) are retained in nuclear aggregates (or foci) that sequestered MBNL splicing factors. Functional loss of MBNL1 leads to alternative splicing changes in several transcripts including CLCN1, BIN1 and DMD, which have been related to patients' symptoms. Recently, antisense oligonucleotides (AON) strategies have shown very promising results in DM1 models. AON were delivered to target mutant DMPK transcripts containing expanded-CUG repeats in order to either degrade these pathogenic RNAs or interfere with abnormal binding/sequestration of RNA-binding proteins like MBNL1. Thus, injection of steric blocking PMO-CAG in DM1 mice reverses molecular changes induced by CUGexp-RNA however AON systemic delivery is still an ongoing challenge for this multisystemic disease.
Here we described the use of a PMO-CAG AON conjugated to an arginine-rich cell-penetrating peptide designated as Pip6a, to enhance its systemic delivery. As preliminary experiments, we confirmed in vitro that treatment of human DM1 myoblasts with Pip6a-PMO-CAG induces MBNL1 release from foci and relocalization into the nucleus that correlates with the normalization of DM1 splicing defects (including PDLIM3, DMD, SORBS1 and SMTN genes). Next we assess Pip6a-PMO-CAG in vivo using a DM1 transgenic model (220CTG in the 3'UTR of the Human Skeletal Actin gene) expressing CUGexp-RNA in skeletal muscles. Intravenous (IV) injection of a single dose of Pip6a-PMO-CAG (12,5 mg/kg) in HSA-LR mice results in a significant correction of alternative splicing defects as well as myotonia. Subsquent experiments revealed that a complete correction of DM1 splicing defects in skeletal muscles of HSA-LR mice is already reached after two IV injections. The beneficial effect is maintained for several weeks after the last injection without adverse effects but additional toxicology analyses in kidney, liver and blood serum are under investigation. This ongoing study suggests that systemic delivery of Pip6a-PMO-CAG could represent a promising therapeutic approach for DM1.

Therapy, antisense, oligonucleotide, Cell-penetrating peptide, mice model, myotonic dystrophy

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Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2895

P20- 318- Identification of a novel myogenesis pathway involving MyoD, Twist-1 and miR-206 implicated in Myotonic Dystrophy.

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2. Paris, France

DM1 is the most common form of muscular dystrophy in adults, and the second most common type of muscular dystrophy after Duchene Muscular Dystrophy. Statistics show that 1 in 8000 individuals globally are affected by DM1. It is an inherited autosomal dominant, neuromuscular disorder. The diseases affect firstly the skeletal muscles through a progressive skeletal muscle weakness, wasting and myotonia. Some patients with DM1 observe congenital, juvenile or adult-onset form of the disease and this in dependable on the age of symptom onset. Congenital form of Myotonic Dystrophy type 1 (CDM1) demonstrates the most severe phenotype of the disorder with 25% neonatal mortality proportion.
Twist-1 is mostly expressed during development and has been previously shown to control myogenesis. Since its regulation in muscle has not been fully exploited, the aim of the project was to identify miRNAs in muscle which regulate Twist-1. miR-206, one of the most important myomiRs, was identified as a possible candidate for Twist-1 mRNA. Luciferase assays and transfections in human foetal myoblasts showed that miR-206 is a direct target for Twist-1 and through this pathway muscle cell differentiation is promoted. We next investigated whether MyoD, a major myogenic transcription factor regulates Twist-1, since it is known that MyoD induces miR-206 gene expression. We found that forced MyoD expression induces miR-206 up-regulation and Twist-1 down-regulation through miR-206 promoter binding, followed by increase in muscle cell differentiation. As a next step, we wanted to investigate whether this novel pathway is implicated in a disease with defective muscle cell differentiation and most specifically in DM1. Experiments were performed in muscle cells from patients with CDM1 which fail to differentiate to myotubes. Our results showed that CDM1 myoblast cells which have a defective differentiation program have low levels of MyoD and miR-206 but high Twist-1 levels. This seems rational based on the properties which characterise these three molecules during muscle cell differentiation. MyoD overexpression inhibited Twist-1 through miR-206 induction, followed by an increase in muscle cell differentiation. These results reveal a novel mechanism of myogenesis which might also play an important role in Myotonic Dystrophy.

DM1, Twist-1, MiR-206, MyoD, Myogenesis

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2899

P20- 319- Human muscle cell expressing conditional CTG expansion as a cellular model for Myotonic Dystrophy

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Myotonic Dystrophy type 1 (DM1) is an autosomal inherited disease characterized by myotonia, progressive muscle weakness and atrophy, cardiac conduction defects, as well as other multisystemic defects. This disorder is caused by expanded (CTG)n repeats of 50 to several thousand triplets in the 3'UTR of the DMPK gene. Pathogenic CTG expansions are transcribed but mutant RNAs containing expanded CUG repeats (CUGexp-RNA) are retained within the nucleus as discrete aggregates or foci. These RNA foci sequester MBNL binding proteins leading to their functional loss. In affected skeletal muscles of DM1 patients, altered activities of MBNL1 results in alternative splicing misregulation of a specific group of transcripts. To date more than forty mis-spliced events have been described in affected muscles of DM1 patients but only few of them were correlated with symptoms. Thus, altered splicing of ClCN1 and BIN1 pre-mRNAs have been associated with myotonia and weakness respectively. However the mechanism responsible for muscle atrophy is still unclear yet.

To identify new candidates or pathways that are altered by the expression of CUGexp-RNA, a global approach using massive parallel sequencing will be performed on a DM1 muscle cell model. To avoid the effect of CUGexp-RNA on myogenic differentiation, we developed a cellular model of human muscle cells expressing conditional CUGexp-RNA. As a first step, myoblasts containing a large CTG expansion under the control of an inducible promoter (Tet-on) were differentiated under non-permissive conditions. Then, doxycylin was added to these differentiated muscle cell cultures to induce the expression of CUGexp-RNA. Our preliminary results show the presence of nuclear RNA-foci in myotubes and specific DM1 splicing alterations following the expression of expanded CTG repeats. This model is currently under characterization and an RNAseq approach will be performed to analyze the modification of the human muscle cell transcriptome by the abnormal expression of pathogenic CUGexp-RNA.

cellular model, CTG conditional expression , myotonic dystrophy

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Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #2994

P20- 320- HnRNP L augmentation as a therapeutic approach for myotonic dystrophy

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Myotonic dystrophy is an autosomal dominant multi-system disorder that affects the skeletal muscles, central nervous system, heart, pancreas, and eyes. It is a severe, life-threatening disease for which no definitive therapy has been discovered. Myotonic dystrophy type 1 (DM1) is caused by a trinucleotide repeat expansion in the 3' untranslated region of one allele of the DMPK gene. The expanded RNA transcript binds and sequesters the MBNL1 protein, impairing its function. MBNL1 deficiency leads to aberrant splicing of multiple downstream RNA targets, thus leading to the multi-organ system complications. We recently demonstrated that the conserved splicing factor heteronuclear ribonucleoprotein L (hnRNP L) is a binding partner of MBNL1. In silico analysis suggests that hnRNP L and MBNL1 share a subset of targets that are aberrantly processed in DM1. We show that hnRNP L plays an important role in muscle development and homeostasis. Downregulation of the hnRNP L ortholog in Drosophila, or in Danio rerio, leads to marked muscle abnormalities and decreased survival of organisms. Similarly, silencing of hnRNP L in normal human myoblasts results in the death of corresponding myotubes early after differentiation. By conducting a targeted drug screen, we have identified a small molecule which elevates the levels of both hnRNP L and MBNL1 in normal, as well as DM1-diseased, myoblast cell lines. This compound is well tolerated both in vitro and in vivo (in Drosophila and mice). We have initiated experiments aimed at assessing the effect of our candidate drug and structural analogs in an established mouse model of DM1. Our small molecule therapeutic lead may open new possibilities to impact DM1 disease progression, thus complementing other experimental approaches focused on neutralizing the toxic RNA repeats.

myotonic dystrophy, MBNL1, hnRNP L, therapeutic, small molecule

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Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #3002

P20- 321- A protocol to exacerbate the DMSXL mouse model skeletal muscle phenotype

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The DMSXL mouse model has been created with a large genomic fragment containing the human DMPK gene carrying >1000CTG. This model shows molecular, physiological and histological defects that recapitulate most of the DM1 phenotype multisystemic manifestations such as brain, heart and skeletal muscle features. Nevertheless, reminiscent to DM1 patients background, the mouse phenotype is variable and sometimes moderate. In particular, reduced myopathic alterations in young

animals can limit the assessment of therapeutic interventions. Here, we aimed at worsening the muscular phenotype in 2 month-old DMSXL mice using a forced eccentric excercise protocol to optimize the evaluation of biotherapies. Our results suggest that eccentric exercise car worsen the muscular weakness observed in DMSXL vs. WT, with a significant decrease of their specific maximal force (sPO) in gastrocnemius muscle. This acts independently to body weight gain, muscle weight changes, DMPK mRNA nuclear foci or HE staining histological abnormalities suggesting molecular deregulation pathways. Preliminary isoform quantification for candidate genes in WT gastrocnemius revealed that the splicing profile depend on state of development and can be affected for LDB3 and MBNL2 mRNA in non exercised DMSXL vs. WT opening to further molecular investigations in exercised DMSXL. We suggest that an eccentric exercise protocol could optimize biotherapy preclinical evaluation in the DMSXL model.

Myotonic dystrophy, biotherapy, mouse model

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #3027

P20- 322- Lower-limb Muscle Weakness, Postural Instability, and Gait Abnormalities in Patients with Myotonic Dystrophy Type 1

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Mechanisms underlying gait and balance impairments in patients with myotonic dystrophy type 1 (DM1) remain incompletely understood. This study aimed to: evaluate gait using lower-trunk accelerometry in twenty two patients with DM1 and twenty healthy controls; investigate potential relationships between muscle strength, postural stability, and gait parameters. Analysis of acceleration patterns of the lower-trunk was performed during a 25-m walking trial at self-selected pace. Participants also underwent a standard 6-min walking test, lower-limb muscle strength assessment, and postural stability assessment. Percentage predicted 6-min walking distance in DM1 correlated with percentage predicted strength of ankle dorsiflexors (? = 0.72, P > 0.05), plantar flexors (? = 0.44, P > 0.05), and knee extensors (? = 0.45, P > 0.05) expressed as percentage of predicted values. Patients had reduced postural stability that was correlated to interstep and interstride regularity in the vertical direction (? = -0.62 and ? = -0.58; both P > 0.05). At self-selected pace, patients displayed reduced walking speed, stride frequency, step length, gait regularity, and gait symmetry. Patients also exhibited higher lower-trunk acceleration power in the mediolateral direction and greater entropy (i.e. index of signal organization) in all directions. In patients, ankle plantar and dorsiflexors strength correlated interstride regularity in the vertical direction (? = 0.57 and ? = 0.59, respectively; both P > 0.05). No significant correlation was found between gait parameters and percentage predicted strength of hip flexors. These findings highlight the important contribution of distal muscle weakness to gait alterations in patients with DM1. In addition, impaired postural regulation may also contribute to gait impairments. Systematic gait analysis might provide a sensitive marker and offer an additional endpoint. Longitudinal follow-up of patients with DM1 is ongoing to investigate the progression of gait abnormalities in conjunction with changes in functional capacities, muscle strength, and postural control. To conclude, this study provides novel insights regarding balance and gait impairments in patients with DM1 that could be valuable for characterization of patients and optimization of therapeutic strategies.

myotonic dystrophy; gait; balance; muscle; weakness; strength; accelerometer.

P21 – Nuclear envelopathies (lamin A/C, emerin, others)- N° 323 to N° 330

Nuclear envelopathies (lamin A/C, emerin, others)- #2320

P21- 323- Muscular dystrophy-associated mutations in sun1 and sun2 impair myonuclear positioning through defective nuclear-microtubule connection

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Emery-Dreifuss muscular dystrophy (EDMD) is a genetically heterogeneous disorder involving progressive muscle wasting and weakness, tendon contractures and cardiac conduction defects. EDMD has been linked to mutations in several genes encoding proteins of the nuclear envelope (NE), most commonly lamin A/C and emerin.
We recently identified SUN1 and SUN2 as novel EDMD-associated genes. SUN1 and SUN2, together with nesprins, form the LINC complex, which spans the nuclear envelope (NE) and connects the nucleus to the cytoskeleton. The SUN proteins, which reside at the inner nuclear membrane, connect the complex to the nuclear lamina and chromatin. In turn, the nesprins reside in the outer nuclear membrane and form direct connections with cytoskeletal filaments. A major role of this connection is to facilitate nuclear positioning within the cell. This is particularly important in muscle, where the multiple myonuclei are regularly positioned along the length of the myocyte, just below the sarcolemma. Myonuclear positioning is controlled by the microtubule (MT) network and MT nucleating proteins relocate from the centrosome to the NE early in myogenesis. However, the precise molecular connections involved are poorly understood.
In cultured myotubes from a patient with compound heterozygous SUN1 mutations, we observed grossly abnormal myonuclear clustering, suggesting a defect in LINC complex connection with the MT network. In support of this, we observed a defect in recruitment of the centrosomal protein, pericentrin, to the NE and a failure of MT nucleation from the NE. We have used various approaches to investigate the nature of these connections and found that SUN1 and SUN2 act redundantly to recruit pericentrin and MT motor proteins to the NE and that this recruitment is specifically mediated by nesprin-1.