Gene therapies (exon-skipping)- #2861

P11- 188- AAV genome maintenance in dystrophic muscles during AAV-U7snRNA-mediated exon skipping therapy

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Duchenne muscular dystrophy (DMD), the most common severe childhood muscular pathology, is due to the absence of the sub-sarcolemmal protein dystrophin. The dystrophin structure tolerates large internal deletions which led to the development of two main therapeutic strategies: gene therapy with transfer of micro-dystrophin cDNAs in muscles, and targeted exon skipping. Both approaches have shown encouraging results using adeno-associated viral (AAV) vectors, which allow efficient gene transfer into muscles. Exon skipping converts an out-of-frame mutation into an in-frame mutation leading to an internally deleted but partially functional quasi-dystrophin. Our team is working on the exon skipping strategy via AAVs expressing a U7 snRNA (AAV-U7). In preclinical models, a one-shot treatment of AAV-U7 was sufficient to attain substantial levels of restored quasi-dystrophin, which is associated with a significant improvement of the muscle force.

Despite the high efficiency of AAV-U7 strategy, we recently showed that quasi-dystrophin levels decreased significantly after one year in various skeletal muscles in the severely dystrophic dystrophin/utrophin knockout (dKO) mouse and GRMD dog. This decline in dystrophin was strongly correlated with viral genome loss, most likely due to alterations of the dystrophic myofiber membranes. In the context of an AAV-U7 clinical trial for DMD, AAV genome fate in dystrophic muscles is of major importance since the viral capsid immunogenicity currently limits repeated treatment. We recently investigated the viral genome fate in muscles of the moderately dystrophic mdx mouse and showed that non therapeutic viral genomes were lost quickly after the injection and that this loss was diminished when high doses of viral genomes restored the quasi-dystrophin at the sarcolemma. Our recent findings concerning AAV genome maintenance in dystrophic muscles will be presented.

Duchenne muscular dystrophy, AAV, exon skipping

P12- Metabolic and Mitochondrial Disorders- N° 189 to N° 207

Glycogenoses (except Pompe) and other metabolic myopathi- #2419

P12- 189- Grip test (non ischemic forearm exercise test) and cycle ergometer exercise comparison in McArdle disease (type V glycogenosis) and myoadenylate deaminase deficiency diagnosis.

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A retrospective study was designed to compare two exercise tests (grip test and a cycle ergometer exercise) and establish diagnostic thresholds for metabolic myopathies. 27 patients, experiencing exercise intolerance or myalgia, underwent a grip test and a cycle ergometer exercise. Simultaneously, blood lactate and ammonia were sampled. 4 patients had McArdle disease, 5 a complete MAD deficiency (MAD absent), 5 a partial MAD deficiency and were compared to 13 Controls experiencing exercise intolerance with normal muscle biopsy and acylcarnitine profile. McArdle patients showed decreased strength parameters (grip test Maximal Voluntary Contraction, p=0.038; Wmax, p=0.0025; mechanical energy, p=0.011 in grip test, p=0.006 for cycling). Work efficiency was impaired in Mc Ardle and MAD absent patients (15 and 13.1 mL O2.min-1.W-1, respectively). There was no difference between partial MAD deficiency patients and Controls. Most discriminating parameters for McArdle and MAD absent diagnosis were ammonia and lactate variations. Both tests perfectly discriminated (ROC curve AUC =1) McArdle patients, combining a low lactate variation (grip test: >1mmol/L, cycling: >0.45mmol/L) and a large ammonia variation (grip test: >100?mol/L, cycling: >20?mol/). Cycle ergometer exercise was superior to grip test to diagnose complete MAD deficiency, combining very low ammonia variation (grip test :> 10?mol/L, AUC=0.7955, p=0.066; cycling: > 8.7?mol/L, AUC=0.9045, p=0.0002) and mild lactate variation (AUC=0.7227, p=0.042 for cycling vs AUC=0.5273, p=0.79 for grip test). Grip test and cycle ergometer exercise are interesting diagnostic tools in metabolic myopathies, especially in McArdle disease, but cycle ergometer exercise is more efficient to diagnose complete MAD deficiency.

McArdle disease, type V glycogenosis, Grip test, cycle ergometer exercise, myoadenylate deaminase deficiency

Glycogenoses (except Pompe) and other metabolic myopathi- #2495

P12- 190- Characterization of a large sample of Brazilian patients with McArdle disease

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The objective was to analyze clinically, molecularly and epidemiologically 14 patients with McArdle disease (MD).Background MD is an autosomic recessive metabolical disorder characterized by onset of exercise intolerance, myalgia and painful cramping since childhood or adolescence triggered by physical activity. The incidence of MD is 1:100.000 newborn and can be attributed to mutations on PYGM gene leading to the absence of the enzyme miofosforilase b in the muscle.Methods We have studied 14 patients with MD from clinical, laboratorial and anatomical-pathological view. We have investigated the PYGM gene mutation by next generation sequencing.Results In our casuistic there are 8 men and 6 women. All of them refer symptoms beginning in

childhood/adolescence and intolerance to physical exercise. CPK values were measured from 4 to 22 times the reference value (> 180). 11 patients performed muscle's biopsy that showed absent miofosforilase and presence of fosfofrutoquinase. At the present moment we have analyzed 8 biopsies which we have observed that 70% of muscle biopsies presented subsarcolemal vacuoles, 20% diffuse vacuoles and 10% internal vacuoles. Regarding the myophatic changes, we have found that 37,5% are mild, 37,5% are moderate and 25% are severe. 100% were PAS positive. Considering clinical symptoms, 71.4% of them refer cramps and dark urine, 78.6% myalgia, 100% fatigue. So far were carried out genetic test in 8 patients in the study. Five were homozygous for the common nonsense mutation in exon 1 Arg50Ter (R50X). One patient was a composed heterozygous for Arg50Ter and a synonymous p.Lys521 mutation in exon 15. One patient was heterozygous for the missense mutation Gln176Pro, in exon 4 and showed no other mutation and one patient showed two missense mutations in homozygosis: Pro659Thr and Asn708Ile, both in exon 17.Conclusions The importance of the study refers to the fact that MD is under diagnosis due to the genetic and clinical heterogeneity. A significant number of patients are only diagnosed in adult life, even though their symptoms/signals have been present since childhood/adolescence. This means the diagnosis passes by unnoticed in the majority, leaving the patient subject to fatal consequences. Although no study in literature could establish a correlation between genotype and phenotype, it is important to emphasizes that the patient with the most severe form of MD was the only one to present a specific mutation.

McArdle disease, PYGM, exercise intolerance

Glycogenoses (except Pompe) and other metabolic myopathi- #2531

P12- 191- Phosphoglucomutase type 1 deficiency: clinical and biochemical clues for the diagnosis

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Phosphoglucomutase 1 (PGM1) is a key enzyme between glycolysis and glycogenesis catalyzing the bidirectional transfer of phosphate from position 1 to 6 of glucose, leading to the production of Glucose 6-p. We describe 5 adult patients with PGM1 deficiency who underwent clinical, biochemical, imaging and histochemical studies. These patients suffered from exercise intolerance and rhabdomyolysis. Four presented mild proximal weakness and one had only muscle fatigability. Two had also hypoglycemic episodes. Four patients had also dysmorphic features such as uvula bifida, cleft palate or hexadactyly. One patient had hepatomegalia. No cardiorespiratory dysfunction was observed. CK was either mildly elevated or normal at rest. Mean CK level was 59 680 U/L during rhabdomyolysis. Electromyography was either normal or disclosed a myogenic pattern in proximal muscles. Muscle biopsy disclosed a slight increased of glycogen content on PAS reaction. Grip test showed high level of ammoniemia and slight increase of lactatemia during the exercise. In NMR spectroscopy, there was no suggestive abnormality of glycogen metabolism but mitochondrial dysfunction was suspected in one patient. Two-dimensional Western-blot of serum glycoproteins showed a combination of Congenital Disorder of Glycosylation (CDG) type I and CDG type II patterns. Dosage of the PGM1 enzyme activity was less than 1% in the muscle. Molecular analysis of the PGM1 gene showed homozygous mutations in 2 patients and 3 patients were compound heterozygous.

PGM1 deficiency encompass a wide range of symptoms extending fromexercise intolerance to more complex phenotype resembling to other inherited multisystemic metabolic disorders such as mitochondrial diseases. PGM1 deficiency may be evoked by a suggestive grip test and western blot of serum glycoproteins, which may be more suitable than muscle biopsy.

PGM1, glycogenolysis, Congenital Disorder of Glycosylation, rhabdomyolysis

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Glycogenoses (except Pompe) and other metabolic myopathi- #2533

P12- 192- STIM1 associated myopathies: a broad clinical spectrum ranging from tubular aggregate myopathy to Stormorken syndrome and beyond.

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STIM1 is the main Ca2+ sensor on the endoplasmic reticulum. Autosomal dominant mutations in the coiled coil 1 domain can cause Stormorken syndrome, a disease characterized by the association of hematological disorders (thrombocytopenia or thrombocytosis, asplenia), muscle fatigue, miosis, migraine, ichthyosis and mild hypocalcemia. Mutations in the highly conserved intraluminal EF domain have been identified in tubular aggregate myopathies (TAM), but never in Stormorken syndrome.

Here, we described 6 patients from 3 families with heterozygous STIM1 mutations. Two families (5 patients) with mutations in the intraluminal EF domain had an autosomal dominant (AD) TAM presenting with adult onset muscle weakness and pain predominantly in proximal lower limb, ophtalmoplegia with upward-gaze paresis and elevated CK levels (5xN).

A 38-years-old female, complaining of exercise intolerance, shared the clinical features presented above, but also had short stature, miosis, hypocalemia, anemia, thrombocytosis and asplenia consistent with Stormorken syndrome. Whole-body muscle Magnetic Resonance Imaging (MRI) showed diffuse fatty infiltration affecting proximal limb muscles, especially quadriceps, but also calf muscles and paravertebral muscles. Electroneuromyography showed mild myogenic changes in the trapezius muscle and some complex repetitive discharges. Antinuclear antibodies were elevated (>1/1280) with positive anti-Sp100 and anti-PML antibodies without clinical or biological symptoms of primary biliary cirrhosis. Muscle biopsy confirmed TAM. STIM1 gene analysis disclosed the novel c.252T>A, p.Asp84Glu missense mutation, which affects a highly conserved EF domain amino acid.

We described here the first case of Stormorken syndrome due to a heterozygous missense STIM1 mutation located in the EF domain, along with two other AD families presenting only with proximal lower limb predominant TAM and ophtalmoplegia with upward-gaze paresis. STIM1 gene mutations have also been previously described in asymptomatic patients with TAM on muscle biopsy. Recessive mutations of this gene are responsible for severe young onset immunodeficiency with muscle hypotonia, iris hypoplasia and auto-immune manifestations including auto-immune thrombocytopenia and hemolytic anemia. These reports demonstrate the broad clinical spectrum of STIM1 associated myopathies, ranging from TAM to Stormorken syndrome and beyond.

STIM1, Stormorken syndrome, Tubular aggregate myopathies, TAM

Glycogenoses (except Pompe) and other metabolic myopathi- #2784

P12- 193- Long term muscle function follow up in patients with glycogen storage disease type III (GSDIII)

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Glycogen storage disease type III (GSDIII) is an autosomal recessive disorder caused by mutations in the AGL gene coding for the glycogen debranching enzyme. Current therapy is based on dietary modifications but new therapies are emerging. The identification of outcome measures which are sensitive to disease progression has become critical to assess the efficacy of new treatments in future clinical trials.
Here we present the longitudinal data of Motor Function Measure (MFM), Purdue pegboard test, timed tests and handgrip strength collected along 5 to 9 years of follow up in 12 patients between 13 and 56 years old with GSDIII. Eight of the 12 patients had 9 years of follow up.
In accordance with cross-sectional retrospective data, MFM D2 and D3 scores slightly and progressively worsened in patients aged between 13 and 56 years old. MFM D1 and total scores, as well as handgrip strength decreased with age in patients older than about 25, 30 and 40 years old, respectively. The Purdue pegboard score progressively reduced with age from 13 years of age but with large inter-visit variations. The time to stand from chair or to climb 4 stairs increased dramatically in some but not all patients older than 30 years old.
In conclusion, this preliminary longitudinal study suggests that MFM and handgrip strength are the most sensitive muscle function outcome measures in GSDIII patients from their third decade. Sensitive muscle outcomes remain to be found in younger GSDIII patients but is challenging as muscle symptoms typically develop at adulthood.

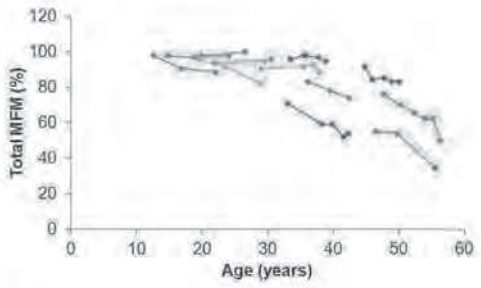

Fig. Long term follow up of 12 patients with GSDIII assessed by MFM

Glycogen storage disease type III; debranching enzyme deficiency; metabolic myopathy; outcome measures

Glycogenoses (except Pompe) and other metabolic myopathi- #2892

P12- 194- Diagnostic algorithm for metabolic myopathies based on exercise testing parameters: a prospective study

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Introduction:
The definitive diagnosis of metabolic myopathies requires an invasive muscle biopsy and subsequent highly specialised techniques for analysis. Our aim was to evaluate the accuracy of aerobic exercise testing as a non-invasive first line test to help the diagnostic approach.
Methods:
From December 2008 to September 2013, all the consecutive patients that both underwent a metabolic exercise testing and a muscle biopsy were prospectively enrolled, according to the Standards for Reporting of Diagnostic Accuracy (STARD recommendations). Subjects performed an incremental and maximal exercise testing on a cycle ergometer. Lactate, pyruvate, and ammonia concentrations were determined from venous blood samples drawn at rest, during exercise (50% predicted maximal power output, peak exercise), and recovery (2, 5, 10, and 15 min). Myoadenylate deaminase (MAD) activity was determined using p-nitro blue tetrazolium staining in muscle cryostat sections. Glycogen storage was assessed using PAS staining. The sensitivities and specificities of plasma ammonia, lactate, lactate/pyruvate and pyruvate/ammonia ratios to identify glycolysis defects, absent and decreased MAD activity were assessed using Receiver Operating Characteristic (ROC) curves analysis. Using a stepwise approach, a decision tree was generated.
Results:
70 patients were included. Omiting patients with glycolysis defects (n = 5), MAD staining was absent in 6, decreased in 9, and normal in 50 subjects. Lactate/rest at the 10th minute of recovery provided the greatest area under the ROC curves (AUC, 0.981 ± 0.034) to differentiate Absent from Present MAD activity. The pyruvate/ammonium ratio at the 5th minute of recovery from exercise displayed the best AUC (0.875 ± 0.076) to discriminate between Decreased and Normal MAD activity. The resulting decision tree achieved a diagnostic accuracy of 93.4%.
Conclusion:
The present algorithm provides a non-invasive test to accurately predict glycolysis defects, absent and decreased MAD activity, contributing to select patients for muscle biopsy and target appropriate histochemical analysis.

Metabolic Myopathies, Exercise Testing, ROC curves, Diagnostic algorithm

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Mitochondrial disorders- #2352

P12- 195- Cyclosporine A treatment increases muscle strength and prolongs life in mice with lethal mitochondrial myopathy

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Introduction: Mitochondrial myopathies are genetically heterogeneous metabolic disorders, originating from the dysfunction of one or more mitochondrial metabolic pathways1. Muscle weakness and exercise intolerance are hallmark symptoms in mitochondrial disorders. To date, no effective treatment exists. We previously reported that an excessive mitochondrial Ca2+ uptake in isolated muscle fibers, that could be inhibited by the cyclophilin D (CypD) inhibitor, cyclosporine A (CsA), may play a central role in the disease process2. In this study, we report the effects of a chronic administration of CsA in a mouse model of mitochondrial myopathy.

Methods: The muscle-specific Tfam knock-out (KO) mice were treated with CsA (120 µg/jour) for 4 weeks (from12 to 16 weeks of age). Maximal force production was assessed on whole EDL muscles and single FBD fibers. Cytosolic [Ca2+] was also measured with the fluorescent Ca2+ indicator indo-1. RT-PCR and western blot were used to assess the expression of genes and proteins related to mitochondrial and cytosolic Ca2+ handling. Muscles biopsies were obtained from control individuals and subjects presenting mitochondrial disease.

Results: CypD protein levels were increased in both mice and patients with mitochondrial myopathy (+60% and +200%, respectively). CsA treatment: extended lifespan of Tfam KO mice by 4 weeks; restored the free cytosolic Ca2+; prevented skeletal muscle weakness and the decrease in CASQ1 protein expression. COX1 protein expression was still lower in Tfam KO mice after treatment as compared to untreated mice.

Conclusion: CsA treatment improved Tfam KO skeletal muscle function by improving muscle fiber Ca2+ handling. The dominating problem in this model of mitochondrial myopathy may be the progressive muscle weakness rather than the energy deficiency. Overall, our results indicate that CsA treatment can be an effective therapeutic strategy to prevent muscle weakness in mitochondrial myopathies.

muscle weakness, mitochondria, therapy

Mitochondrial disorders- #2365

P12- 196- Growth and differentiation factor-15 is as sensitive and specific as FGF-21 as a biomarker for the diagnosis of mitochondrial diseases and is induced by mitochondrial dysfunction: an improved diagnostic method.

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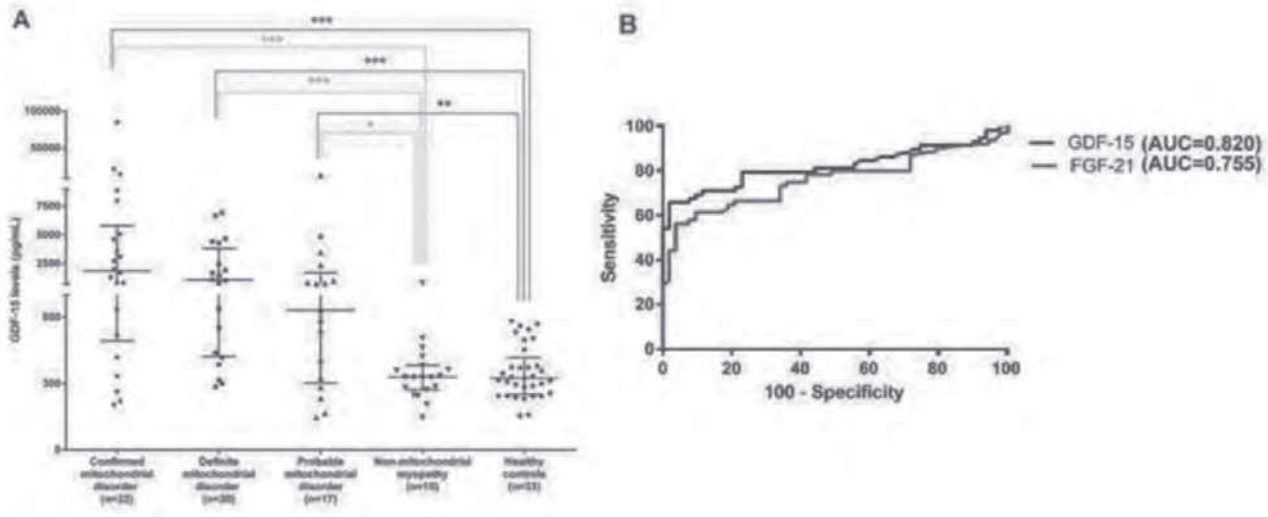
We previously described for the first time growth and differentiation factor 15 (GDF-15) as a potential novel biomarker for mitochondrial diseases. Here we evaluated the application of circulating levels of GDF-15 for the diagnosis of mitochondrial diseases affecting children and compared it to fibroblast-growth factor 21 (FGF-21). To investigate the mechanism of GDF-15 induction we studied its expression and secretion in response to mitochondrial dysfunction.
We analysed 59 samples from 48 children with a diagnosis of mitochondrial disease, 19 samples from children with non-mitochondrial neuromuscular disease and 33 samples from aged-matched healthy children. GDF-15 and FGF-21 circulating levels were determined by ELISA. GDF-15 expression and secretion were investigated in human and murine myotubes treated with respiratory chain inhibitors.
GDF-15 was significantly increased in patients (mean 4046pg/ml, 1492 SEM) relative to healthy (350, 21) and myopathic (350, 32) controls. There was a strong and significant positive correlation between GDF-15 and FGF-21 concentrations in patients (R= 0.78, p>0.0001). The area under the curve for the receiver-operating-characteristic analysis for GDF-15 was 0.82 (0.75 for FGF-21) indicating that it has a very good discriminatory power. The overall sensitivity and specificity for a cut-off value of 550pg/mL was 67.8%(95% CI54.4%-79.4%) and 92.3% (81.5%-97.9%) respectively. We found that elevated levels of GDF-15 and or FGF-21 correctly identified a larger proportion of patients than elevated levels of GDF-15 or FGF-21 alone. This was particularly helpful in those patients with less well defined diagnostic criteria.
GDF-15, as well as FGF-21, mRNA expression and protein secretion were significantly induced after treatment of myotubes with oligomycin and levels of expression of both factors significantly correlated.
To summarise, our data show that GDF-15 is a valuable serum quantitative biomarker for the diagnosis of mitochondrial diseases in children and that measurement of both GDF-15 and FGF-21 improves the disease detection ability of either factor separately. We propose to include both factors in the diagnostic workup of patients with mitochondrial disease to help guide further biochemical and molecular studies.
Finally, we demonstrate that GDF-15 is produced by skeletal muscle cells in response to mitochondrial dysfunction and that its levels correlate in vitro with FGF-21 levels.



GDF-15, FGF-21, biomarker, mitochondrial disease

Mitochondrial disorders- #2522

P12- 197- Immunohistochemical distribution of mHSP70 in muscle of children with various forms of myopathy.

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Mitochondrial heat shock protein 70 (mHSP70) is a component of the mitochondria, which controls the import of the proteins into mitochondria and plays important role in the biogenesis of mitochondrial complex IV. Taking into consideration the significance of mitochondrial morphological and functional changes in various forms of myopathy in children, it seems relevant to study mHSP70 distribution in muscle fibers in cases of various myopathies.

Materials and methods: Paraffin embedded muscle tissue sections obtained from 30 patients with mitochondrial myopathies and 30 patients with congenital myopathies (centronuclear, nemaline, central core myopathy, etc.) were stained. mHSP70, HIF1-alpha, mitochondrial complex IV subunit I (Cox1), mitochondrial complex V d-subunit and marker of mitochondrial outer membrane were visualized with help of fluorescence immunohistochemistry, then measured by morphometric analysis and compared to clinical and laboratory findings.
Results: In mitochondrial myopathy mHSP70 average intensity of fluorescence (AIF) positively correlated with patients' muscle strength and negatively correlated with "fatigue/strength" ratio, which is typically high in patients with mitochondrial myopathy. AIF of mHSP70 correlated with several lab parameters, among which were negative correlation with plasma lactate/pyruvate ratio before glucose load, and positive correlation with this parameter after the load; AIF of mHSP70 negatively correlated with area of mitochondrial pool. In congenital myopathies AIF of mHSP70 didn't correlate with patients' muscle strength and positively correlated with "fatigue/strength" ratio. It positively correlated with plasma lactate/pyruvate ratio before glucose load, and negatively correlated with this parameter after the load; AIF of mHSP70 positively correlated with area of mitochondrial pool. Peculiar changes were found in central core myopathy: immunohistochemistry revealed the absence of Cox1 in the core area and the area of Cox1 negatively correlated with AIF of mHSP70.

Conclusion: Obtained results indicate that mHSP70 activation plays compensatory role in various forms of mitochondrial lesions with the exception of mitochondrial myopathy, in which it may be decompensated probably according to sighificant number of defective organelles. mHSP70 compensatory role can provide a new insight into the pathogenesis of neuromuscular diseases. Investigation of mHSP70 as a diagnostic marker is of the special interest.

Mitochondria, heat shock protein 70, complex IV, complex V, HIF1-alpha, immunohistochemistry, mitochondrial myopathy, congenital myopathy, central core myopathy, diagnostic marker

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Mitochondrial disorders- #2596

P12- 198- Clinical presentation and response to treatment of patients with primary mitochondrial encephalomyopaties.

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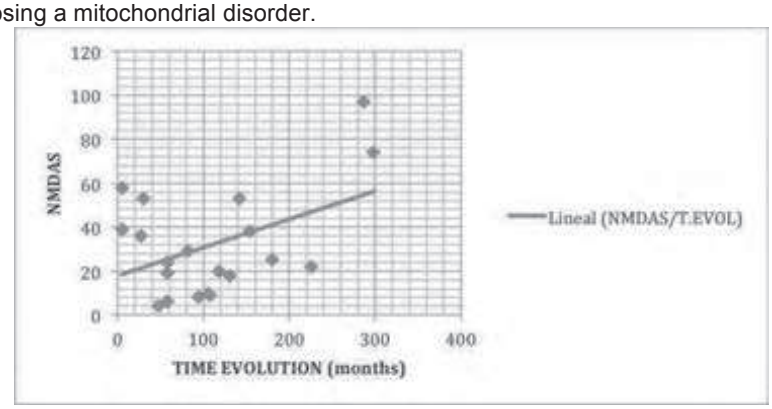
OBJECTIVE
To present our experience in the clinical presentation, diagnosis, treatment and outcome of a cohort of primary mitochondrial encephalomyopathies, diagnosed and followed-up in the Hospital Clinic of Barcelona.
PATIENTS AND METHODS
We realized an observational analysis of a retrospective cohort of 30 patients who received the diagnosis of mitochondrial encephalomyopathy. The diagnosis was made on the basis of clinical features together with muscle biopsy, genetic studies and analysis of the mitochondrial respiratory chain (MRC). The time of follow-up was 5- 310 months; the progression and treatment response were measured by the Newcastle Mitochondrial Disease Adult Scale (NMDAS).
RESULTS
The male/female ratio was 43%/57%. Mean age was 46.2 years (range: 2-77). The most common symptoms at diagnosis were weakness (20%) and palpebral ptosis (20%) but other symptoms such as stroke-like episodes, seizures, cardiac block and deafness among others also occurred. The most frequent diseases were MELAS and KEARNS-SAYRE syndromes. Sixteen DNA genetic studies were performed, with mutations in 68,75%. Muscle biopsies were performed in 28 cases. Pathological changes compatible with mitochondrial myopathy (mainly ragged-red fibers) were observed in 26 cases. In 26 (86,66% of the patients) polarographic analysis of the MRC were performed, with abnormal results in 47% of them. Twelve (40%) patients were on specific treatment. Sixteen (53%) patients worsened over time, ten (33%) remained stable, three (10%) died and only one (3%) improved. In the NMDA scale the mean value was 33,4 points (range 4-97). Twelve (40%) patients were on specific treatment with a mean value of 41,9 points in the NMDA scale, and the patients with symptomatic treatment had a mean value of 25, 7 points in the NMDA scale (see figure).
CONCLUSIONS
In our experience the clinical expression of mitochondrial encephalomyopathies is extremely variable, involving multiple organs and showing heterogeneous and unpredictable progression. The muscle biopsy, a rapid and cost-effective procedure, remains an important tool for diagnosing a mitochondrial disorder.



mitochondrial, encephalomyopathies, NMDAS

Mitochondrial disorders- #2635

P12- 199- DRP-1, AIF and mTOR: a link between mitochondria morphology and muscle degeneration in C. elegans ?

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Muscle degeneration is a common feature of muscle aging and muscular dystrophies such as Duchenne Muscular Dystrophy (DMD). Our lab has established a Caenorhabditis elegans mutant with progressive dystrophin-dependent muscle degeneration called the C. elegans DMD model. This model exhibits dramatic changes in mitochondrial morphology compared to wild-type worms suggesting a deregulation in the fusion/fission mitochondrial balance. My goal is to shed light on the role of mitochondria dynamics in the molecular mechanisms leading to muscle degeneration.

To start, we first focused on DRP-1, a protein required for mitochondrial fission. We introduced in the C. elegans DMD model a null mutation drp-1(tm1108). The DMD;drp-1 mutants presented reduced mitochondrial fragmentation compared to DMD mutants. Furthermore, DMD;drp-1 mutants exhibited reduced muscle degeneration and increased mobility. DRP-1 is well known to be implicated in cell death processes. In C. elegans, the pro-apoptotic function of DRP-1 is dependent on its cleavage by the CED-3 caspase. We generated DMD;drp-1 transgenic lines expressing DRP-1 with mutated CED-3 cleavage site. Interestingly, our data suggest that DRP-1 cleavage by CED-3 is required for DRP-1 to decrease muscle degeneration. All together, the pro-fission and pro-apoptotic protein DRP-1 seems to be implicated in pathological muscle degeneration.

After these first observations, we aimed at identifying other genetic suppressors of dystrophin dependent muscle degeneration playing a role in mitochondria dynamics. Large screen on the C. elegans DMD model allowed for the identification of 60 genes, which decrease muscle degeneration after RNAi knock-down. Among them, we identified 28 candidate genes that participate in mitochondria biology. We tested the 28 RNAi candidates for an effect on mitochondria morphology of DMD worms. We found that knock-down by RNAi of wah-1 (AIF homolog) and let-363 (mTOR homolog) can reduce mitochondrial fragmentation of DMD mutants. Our findings provide the first evidence that WAH-1 affects mitochondria morphology in muscle cells.

Moreover, reducing WAH-1 or LET-363 levels in absence of DRP-1 can further decrease muscle degeneration of the C. elegans DMD model than wah-1 RNAi or let-363 RNAi or drp-1(tm1108) mutation alone suggesting at least in part distinct molecular pathways.

As mitochondria processes are highly conserved among species, our study is likely to be relevant to Human muscle degeneration.

mitochondria dynamics, muscle degeneration, DRP-1, AIF, mTOR

Mitochondrial disorders- #3048

P12- 200- Phenotypes of mitochondrial diseases in the adult Slovenian patients

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We analysed 33 patients referred to muscle biopsy with the clinical diagnosis of possible mitochondrial disorder during the period, January 2004-October 2015. Clinical diagnosis was based on the phenotype considered to be typical for mitochondrial disease (as PEO or PEO+, MNGIE, MELAS, MERRF) or combination of peripheral and central nervous system involvement combined with abnormalities of other systems (as endocrine, musculoskeletal, visual , hearing system, kidney, liver and heart abnormalities). Increased levels of CK, pyruvate and lactate were in favour of mitochondrial disease, but normal levels of these parameters did not exclude referral of patients for muscle biopsy. EMG was seldom myopathic; sensory-motor neuropathy either axonal or demyelinating was much more frequent; occasionally EMG was normal. Unspecific EEG abnormalities were observed often with suspected »encephalopathy« in cognitive or personality abnormalities. In such cases MRI of the head revealed unspecific cortical atrophy and in one case increased lactate peak at MRI spectroscopy. Cerebellar atrophy was observed occasionally with ataxia. The most useful morphological investigation was the histochemical demonstration of fibres not possessing cytochrome-oxidase activity. The abnormality could be combined with ragged red or ragged blue fibres and was always associated with ultrastructural mitochondrial abnormalities. It was considered specific for mitochondrial disorder for persons less than 50 years of age. All cases, except one, of PEO or PEO+ syndromes, bilateral ptosis and phenotypes which encompass lipomas irrespective of the associative signs, had typical morphological abnormalities of cytochrome-oxidase negative fibres. In one case with MELAS and MNGIE phenotype ultrastructural abnormalities of mitochondria were detected only. In one case of MELAS and MERRF cytochrome c-negative fibres were detected as expected for mitochondrial disorder. In the minority of patients determination of RC complexes was possible. Enzyme abnormalities could be associated with normal muscle morphology, but were always associated with abnormalities in mitochondrial /nuclear DNA.

phenotype, mitochondrial disease, muscle biopsy

Pompe disease- #2379

P12- 201- Novel mutations of GAA gene in Iranian late-onset Pompe patients

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Background: Pompe disease (glycogen storage disease type II or acid maltase deficiency) is a rare autosomal recessive disorder due to absence or deficiency of acid ?-glucosidase. Different clinical presentations occur based on residual enzyme activity. Hundreds of mutations in GAA gene are known to be responsible and novel mutations are increasingly reported.
Methods: We collected clinical and paraclinical data of 15 patients with diagnosis of late-onset Pompe disease. They all had reduced enzyme activity on DBS analysis. Genetic investigation was performed to find out the underlying mutations.
Results: The age of symptom onset in our patients varied between less than 2 to 38 years. The clinical presentations were heterogeneous and distinctively respiratory involvement was less frequent. The most common mutation was c.-32-13T>G and we found four novel mutations including c.(2040+2dup), c.(1650delG), c.(1837T>G) and c.(2596delG).

Conclusion: This is the first comprehensive report of late-onset Pompe disease in Iranian patients. Distinct phenotypic and genotypic features of Pompe disease in this population are highlighted.

Pompe disease, glycogen storage disease type II, acid ?-glucosidase, GAA gene mutation

Pompe disease- #2537

P12- 202- Development of VAL-1221, a bifunctional antibody-enzyme replacement candidate for treating glycogen storage disorders

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Valerion has developed a humanized monoclonal antibody, VAL-1205, capable of serving as a cell-penetrating delivery vehicle. VAL-1205 is a modified form of a naturally occurring murine anti-DNA binding, 3E10MAb, derived from an inbred lupus mouse model. The cell-penetrating ability of 3E10MAb was found to be dependent on expression and function of the equilibrative nucleoside transporter (ENT-2). ENT-2 is found in all tissues but expression is highly elevated in human skeletal muscle. Functional fragments of VAL-1205 recombinantly-fused to different deficient proteins and enzymes have demonstrated efficacy in mouse models of Myotonic Dystrophy, Myotublar Myopathy, Pompe disease and Anderson's disease. Specifically, a recombinant fusion, VAL-1221, consisting of VAL-1205 FAb fused to the alpha-glucosidase (GAA) enzyme was demonstrated to penetrate cells via both antibody driven and mannose-6-phosphate receptor (M6PR) mediated uptake pathways. VAL-1221 had demonstrated glycogen clearance in both GSDII and GSDIV mouse models as well as patient derived fibroblasts. In mouse biodistribution studies, Zr89 labeled VAL-1221 demonstrated improved muscle targeting compared to GAA-alone. In addition to this improved muscle targeting, VAL-1221 has unique functional capabilities based on the ability to reach intracellular locations outside the lysomes (ex. low pH autophagic vacuoles) affording both lysosomal and extralysosomal glycogen clearance. Based on these novel properties, Valerion is currently preparing a Master Cell Bank to produce and release material under full cGMP compliance for clinical study. To date, both single- and multi-dose tox studies in primates have been completed and no adverse events reported. As additional safety and efficacy pre-clinical studies are nearing completion, Valerion is preparing a clinical strategy designed to utilize the VAL-1205 platform to treat multiple orphan genetic muscle diseases.

Pompe Disease, Glycogen Storage, muscle delivery

Pompe disease- #2631

P12- 203- Infrared Micro spectroscopy with the synchrotron light, an innovative approach to investigate tissue chemical changes in mouse model of Pompe of disease (glycogenosis type II) and to assess efficiency in gene therapy.

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Pompe disease is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA). The disease is characterized by lysosomal glycogen storage in heart and muscles, and manifests as a fatal cardiomyopathy in infantile form. Cardiac correction by enzyme replacement therapy (ERT) has recently prolonged the lifespan of these patients, revealing a new natural history. The emergent neurologic phenotype and the poor correction of skeletal muscles in survivors are currently partly attributed to central nervous system (CNS) glycogen storage, uncorrected by ERT. A gene therapy strategy using AAV vectors delivered to cerebrospinal fluid has been set up to restore GAA activity into the CNS. We demonstrate the use of Infrared Micro spectroscopy with synchrotron light as an innovative tool to map glycogen at the subcellular level in motor neurons and cardiac fibers. Principal Component Analysis (PCA) of infrared spectral data from motor neurons and cardiac fibers show that both treated and wild-type animals are merged in the same cluster whereas infrared spectra obtained from untreated Pompe mice are characterized by increase of the bands assigned to the carbohydrates of glycogen.

This new analytical approach that allows an highly sensitive and resolutive direct probing of tissue glycogen is required to explore early biochemical change at a subcellular level and therefore to assess therapeutic efficiency for Pompe disease.

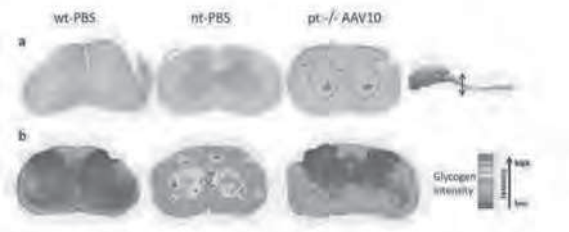

Figure 1 *:a- Representative sections of cross-sectioned cervical spinal cord, paraffin embedding, PAS-luxol fast blue stain. The glycogen storage appears purple on a blue background, motor neuron of spinal cord are located in ventral horn (vh). b- Chemical imaging performed after the infrared chemical mapping showing the relative concentration of glycogen infrared spectra absorption acquired from cross-section cervical spinal cord of the dewaxed formalin fixed paraffin.*

Pompe disease, glycogen, gene therapy, Infrared Micro spectroscopy

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Pompe disease- #2814

P12- 204- Simplified desensitization protocol for enzymotherapy in adult Pompe Disease.

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5. Service d'Allergologie, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, Lyon, France

Pompe disease (PD) is an inherited lysosomal disease in which there is a decrease or absence of acid alpha glucosidase (GAA) activity. This enzyme defect induce a glycogen storage in different tissus, specially muscle and heart, resulting in muscle weakness, respiratory failure and cardiopathy. There are two different phenotypes that we can name early and late onset. The first, extremely severe, lead to death in the childhood, the second one, milder, is responsible for a progressive disability, and a lifespan reduce. Substitutive Enzymotherapy (Enzyme Replacement Therapy (ERT)) dispensed every two weeks is the only treatment that has shown benefits. However, this treatment induces hypersensitivity for half of the treated patients. Those reactions can be mild to severe, some times requiring ERT suspension and anti anaphylaxis drug administration. Easily understanding, high amount of GAA infusion seems to be identify by the immune system as a DAMP, and induce a immune reaction, involving sometime, but not always, Immunoglobulin E (IgE) production, and activating Masts Cells and Basophile Polynuclear. The precise process of these reactions remains still unclear.

Considering the lack of therapeutics alternatives, and the proved benefit of ERT, desensitization finds here its place.
We report here the case of a patient for who a simplified desensitization protocol has been successfully achieved, allowing ERT to be follow/pursuit, resulting at least in clinical improvement.

Pompe Disease, ERT, desensitization

Pompe disease- #3044

P12- 205- Long-term neurologic and cardiac correction by intrathecal gene therapy in Pompe disease

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Pompe disease is a lysosomal storage disorder caused by acid-a-glucosidase (GAA) deficiency, leading to glycogen storage. The disease manifests as a fatal cardiomyopathy in infantile form. Enzyme replacement therapy (ERT) has recently prolonged the lifespan of these patients, revealing a new natural history. The neurologic phenotype and the persistence of selective muscular weakness in some patients could be attributed to the central nervous system (CNS) storage uncorrected by ERT. GAA-KO 6neo/6neo mice were treated with a single intrathecal administration of adeno-associated recombinant vector (AAV) mediated gene transfer of human GAA at one month and their neurologic, neuromuscular, and cardiac function was assessed for one year. We demonstrate a significant functional neurologic correction in treated animals from 4 months onward, a neuromuscular improvement from 9 months onward, and a correction of the hypertrophic cardiomyopathy at 12 months. The regions most affected by the disease i.e the brainstem, spinal cord, and the left cardiac ventricular wall all show enzymatic, biochemical and histological correction. Muscle glycogen storage is not affected by the treatment, thus suggesting that the restoration of muscle functionality is directly related to the CNS correction. This unprecedented global and long-term CNS and cardiac cure offer new perspectives for the management of patients.

Pompe disease, gene therapy, Intrathecal

Pompe disease- #3284

P12- 206- Lack of robust satellite cell activation and muscle regeneration during the progression of Pompe disease

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Pompe disease (PD), a recessively inherited metabolic myopathy caused by inactivating mutations in the acid alpha glucosidase (GAA) gene. GAA deficiency causes glycogen accumulation in several tissues that is particularly damaging in skeletal muscle. Classic infantile Pompe patients, which also develop a cardiac hypertrophy, are characterized by a rapidly progressing disease course and death within 18 months. Patients with a later onset of disease develop muscle weakness more slowly and may become wheelchair- and ventilator-dependent eventually. Skeletal muscle has a remarkable capacity to repair damage that is dependent on a rare population of muscle-resident stem cells, called satellite cells. It remains enigmatic why satellite cells fail to compensate the progressive muscle damage characterizing neuromuscular disorders. To address this, we have analyzed muscle fiber pathology, the satellite cell response and muscle regeneration activity in muscle biopsies from Pompe patients across all ages and stages of disease. Pathology included muscle fiber vacuolization, loss of cross striation, and immune cell infiltration. The total number of Pax7-positive satellite cells in muscle biopsies from infantile, childhood- and adult-onset patients was indistinguishable from age-matched controls, indicating that the satellite cell pool is not exhausted in Pompe disease. However, immunohistochemical analysis of PAX7/Ki67 and MyoD/Myogenin expression suggested that the levels of satellite cell activation and differentiation, respectively, were low. In line with this, the expression of embryonic Myosin Heavy Chain was weak in samples from the rapidly progressing classic infantile patients and undetectable in those from the childhood- and adult-onset Pompe disease patients. We conclude that satellite cells are not properly activated during Pompe disease progression and that this contributes to an impaired muscle regenerative response. The preservation of the satellite cell pool may offer a venue for the development of novel treatment strategies directed towards the activation of endogenous satellite cells.

Pompe disease, muscle regeneration, satellite cells, muscle stem cells

Pompe disease- #4498

P12- 207- Specific and redundant roles of the TEAD family of transcription factors in C2C12 cell and primary myoblast differentiation.

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The TEAD family of transcription factors recognise the MCAT element found in the promoters of muscle-specific genes. We previously used shRNA-mediated silencing to show that Tead4 plays an essential role in C2C12 cell differentiation with Tead4 silenced cells giving rise to shortened myotubes. Here, we have used siRNA-mediated silencing to address the role of the Tead factors in primary myoblast differentiation. In contrast to C2C12 cells where Tead4 plays a critical role, its silencing in primary myoblasts had little effect on their differentiation. Silencing of individual Tead factors had no significant effect on primary myoblast differentiation, whereas combinatorial silencing led to inhibition of their differentiation indicating redundancy amongst these factors. In C2C12 cells also, combinatorial Tead silencing had much more potent effects than silencing of Tead4 alone indicating a contribution of other Teads in this process. By integrating Tead1 and Tead4 ChIP-seq data with RNA-seq data following combinatorial Tead1/4 silencing, we identify distinct but overlapping sets of Tead regulated genes in both C2C12 cells and primary myoblasts. We also integrated the Tead1/4 ChIP-seq data with public data sets on Myog and Myod1 ChIP-seq and chromatin modifications to identify a series of active regulatory elements bound by Tead factors alone or together with Myog and Myod1. These data dissect the specific and combinatorial functions of these transcription factors in muscle differentiation regulatory networks.

P13- Hereditary neuropathies /- N° 208 to N° 211

Hereditary neuropathies- #2388

P13- 208- Motoneuron electrical activity, Na+/K+ pump and neuromuscular junction defects in Andermann syndrome

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Andermann syndrome is an autosomal recessive disease characterized by peripheral neuropathy with variable agenesis of the corpus callosum with most affected individuals being hypotonic and amyotrophic. This neurodevelopmental and