alternative energy source to sustain muscle function. To this point, proteomic data indicate the presence of a metabolic rewiring that can lead to a shortage of glutamate-derived molecules with protective functions triggering lipogenesis, causing lipotoxicity over time. Moreover, results highlight the link between glucose depletion and ER stress. Since glucose is essential for protein glycosylation, if intracellular glucose levels decrease, the carbohydrate chain, that is used to glycosylate proteins, cannot be assembled and an improper protein glycosylation, protein misfolding, activation of the UPR, and increased ER stress is observed.

By the present study we identify specific metabolic nodes to be targeted with the possibility to ameliorate the course of the disease in DMD patients.

Dystrophinopathies (Duchenne, Becker, others)- #3271

**P08- 155- Loss of Dp140 dystrophin isoform and neuropsychological impairment in Tunisian boys with Duchenne muscular dystrophy**

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**Background**
Mutation of the dystrophin gene has long been recognized as a cause of intellectual impairment in Duchenne muscular dystrophy (DMD). However, underlying etiopathological mechanisms remain unclear.

**Objective**
To analyze neuropsychological profiles in a cohort of Tunisian boys with DMD and their correlation with causal mutations.

**Patients and methods**
Over 11 years (2004-2015), 20 boys were followed up in our department for a genetically confirmed diagnosis of DMD. Neuropsychological evaluation including full-scale intelligence quotient (IQ), memory assessment, verbal performances, attention processes, executive functions, mood and behavioral patterns. Correlation between dystrophin gene mutation and neuropsychological profiles were analyzed.

**Results**
Mean age at neuropsychological evaluation was 6.7 years. Causal mutations predicted to lead to a loss of the Dp140 isoform have been found in 15 (75%) patients. General intelligence assessments showed a mean IQ of 82 (range 50-110). Impairment in working memory was noted in 12 (60%) patients. Verbal performances and attention processes were altered in respectively 7 (35%) and 5 (25%) patients. Executive dysfunction was noted in 5 (25%) patients. Autism spectrum disorders were noted in 3 (15%) patients. We found that patients lacking Dp140 performed more poorly on all neuropsychological tests compared to those with preserved Dp140. There was no evidence of cognitive declining with the progression of muscular deterioration.

**Conclusion**
Our findings support emerging evidence of central nervous system involvement resulting in neuropsychological disorders in DMD. The loss of Dp 140 seems to be involved in the pathological mechanisms underlying neuropsychological disturbances and associated with a higher risk of cognitive impairment among patients with DMD. Neuropsychological deficits should be systematically detected in these patients in order to improve their quality of life.

**Cognitive impairment, Duchenne muscular dystrophy (DMD), Dp140 isoform**

Dystrophinopathies (Duchenne, Becker, others)- #4469

**P08- 156- Grip strength in Duchenne Muscular Dystrophy**
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Duchenne Muscular Dystrophy (DMD) is characterized by a progressive loss of muscle fibers, which are gradually replaced by fat and connective tissue. The disease typically develops from proximal to more distal muscles. Muscles of the hands are thus less affected throughout the disease course. Few studies have focused on the upper limb of DMD patients, despite the importance of maintaining independent function as long as possible for young men with DMD.

The results of grip strength of 157 DMD boys were gathered from several natural history protocols. 46 patients were ambulant and 111 were non-ambulant. They were aged from 5 to 30 years. Each patient was followed during at least one year. As a global picture, grip strength increases in ambulant boys while it decreases in non-ambulant patients when expressed in absolute value. However when expressed relatively to age or height using predictive models, grip strength decreases in both groups of patients.

These results confirm that growth and maturation partly compensate for disease progression in ambulant boys. Our study also suggests that stature, better than chronological age, is a major predictor of muscle strength. Height or other stature parameters (like hand circumference or ulna length for instance) must then be measured during clinical trials in order to express muscle strength in percentage of predicted values estimated for stature. Normalized variables should be used in future to provide markers of disease evolution, independently of growth and maturation.

**Duchenne Muscular Dystrophy, grip strength, outcome measure**

**P09- Facioscapulohumeral dystrophy (FSHD1, FSHD2)- N° 157 to N° 173**

Facioscapulohumeral dystrophy (FSHD1, FSHD2)- #2551
The purpose of this study was to investigate whether 8-week neuromuscular electrical stimulation (NMES) training of the tibialis anterior (TA) muscles in adults with facioscapulohumeral muscular dystrophy type 1 (FSHD1) would improve motor function, muscle strength and endurance. Eleven patients with FSHD1 and 10 age and gender matched healthy participants achieved a 8-week bilateral NMES training of the TA muscles 20 minutes per session, 3 sessions per week. Ankle dorsiflexion (DF) and plantar flexion (PF) maximal voluntary isometric contractions (MVC), a 2-minute sustained MVC ankle dorsiflexion with surface electromyography recordings (EMG) of the TA and the soleus (SOL) muscles were measured and functional tests were performed prior to and after the NMES training to disclose training effects. To assess the biological tolerance, plasma Creatine Kinase (CK) was measured before, at 4 weeks (W4), after the 8-week training (W8) and once randomly during the training. No training effect was found in any of the investigated variable for either group. Patients with FSHD showed lower MVC and lower maximal TA EMG amplitude during the DF MVCs. During the 2-minute sustained MVC, the percentage of force loss was lower for the FSHD patients. The percentage of TA EMG loss amplitude after the 2-minute MVC were found to be similar in both groups before and after the training but partly increased with training for the group of patients with FSHD1. Besides drastic differences between groups, none of the clinical motor function measures were improved with the training in patients with FSHD. CK did not change significantly during the NMES training period for both groups. Although the program was biologically tolerated, the NMES protocol was not strenuous enough and/or parameters of stimulation were not adequate to improve ankle strength, muscle endurance and motor function for the group of patients with FSHD1. Additionally, the absence of training effects may be explained by the NMES protocol which was designed for the patients with FSHD1, likely not appropriate to induce strength gains in healthy participants. Finally, the group of patients with FSHD1 showed lower force losses during the 2-minute sustained MVC, suggesting that they were experiencing a lower amount of muscle fatigue compared to the HP group.

Neuromuscular electrical stimulation training, facioscapulohumeral dystrophy type 1, surface electromyography

Facioscapulohumeral dystrophy (FSHD1, FSHD2): #2655

P09-158- A phase III clinical trial of autologous myoblast transplantation in facioscapulohumeral muscular dystrophy
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Facioscapulohumeral muscular dystrophy type 1 (FSHD1) is one of the most frequent adult myopathies. It is characterized by selective involvement of specific muscle groups resulting in a composite picture of affected and non-affected muscles. Clinically affected muscles are usually facial, scapular fixator, anterior foreleg, abdominal and humeral muscles while vastus lateralis (VL) muscle is usually spared until late stages of the disease. We previously observed that, under dedicated clinical-grade culture conditions, myoblasts harvested from clinically spared muscles behaved in vitro and in vivo similarly to myoblasts from control patients. This suggested the possibility to transfer cultured autologous cells from non-affected VL muscle into affected tibialis anterior (TA) muscle to improve locally the muscle regenerative capacities. Several preclinical and clinical trials developed in other indications underlined the critical need for a standardized methodology for administration of cells at multiple sites. Feasibility of cell preparation, safety of intramuscular administration, clinical and biological tolerance of the transplantations were investigated as the primary goal.

Three groups of patients included sequentially received 800 millions autologous cells, produced within 3 weeks from 1g VL muscle biopsy, and distributed into half volume of the TA muscle (approximately 40 cm3) under 10 ml according to 3 multilite models differing by the density of injections (1st group: 64 sites, 2nd group: 100 sites; 3rd group: 189 sites). The evolution with time of muscle force and composition were evaluated over two years period as secondary goals by mechanical testing of muscle strength and resistance to fatigue, surface electromyography, muscle MRI, FDG fixation by PET Scan. The biological and clinical follow-up of patients globally underlined the feasibility and tolerance of the procedures. Slight increases in twitch
response and slight decrease in fatigue were observed under the highest density of injection, suggesting that cell therapy may have positively affected the experimental leg, although little clinical benefit and no significant gain of function were noted over the follow-up. No significant changes were noted at muscle MRI and PET Scan data analysis. We suggest that the local FSHD1 degenerated muscle environment may be detrimental to the stability of the fibers or of the niches, and muscle regeneration may have been inefficient or too unstable.

Facioscapulohumeral muscular dystrophy, myoblast, cell transplantation, autologous

Facioscapulohumeral dystrophy (FSHD1, FSHD2): #2838

P09- 159- A phase III clinical trial of autologous myoblast transplantation in facioscapulohumeral muscular dystrophy
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Facioscapulohumeral muscular dystrophy type 1 (FSHD1) is one of the most frequent adult myopathies. Clinically is characterized by selective involvement of specific muscle groups resulting in a composite picture of clinically affected and non-affected muscles. Affected muscles are usually facial, scapular fixator muscles and anterior foreleg muscles, abdominal and humeral muscles while vastus lateralis muscle is usually spared until late stages of the disease. We previously observed that, under dedicated clinical-grade culture conditions, myoblasts harvested from clinically spared muscles behaved in vitro and in vivo similarly to myoblasts from control patients. This suggested the possibility to transfer cultured autologous cells from non-affected vastus lateralis (VL) muscle into affected tibialis anterior (TA) muscle to improve locally the muscle regenerative capacities. Several preclinical and clinical trials developed in other indications underlined the critical need for a standardized methodology for administration of cells at multiple sites. Feasibility of cell preparation, safety of intramuscular administration of cell suspensions, clinical and biological tolerance of the transplants were investigated as the primary goal.

Three groups of patients included sequentially received 800 millions autologous cells, produced within 3 weeks from 1g VL muscle biopsy, and distributed into half volume of the TA muscle (approximately 40 cm3) under 10 ml according to 3 multisite modalities differing by the density of injections (1st group: 64 sites; 2nd group: 100 sites; 3rd group: 189 sites). The evolution with time of muscle force and composition were evaluated over two years period as secondary goals by mechanical testing of muscle strength and resistance to fatigue, surface electromyography, muscle MRI, FDG fixation by PET Scan. The biological and clinical follow-up of patients globally underlined the feasibility and tolerance of the procedures. Slight increases in twitch response and slight decrease in fatigue were observed under the highest density of injection, suggesting that cell therapy may have positively affected the experimental leg, although little clinical benefit and no significant gain of function were noted over the follow-up. We conclude that the local FSHD1 degenerated muscle environment may be detrimental to the stability of the fibers or of the niches, and muscle regeneration may have been inefficient, too transitory, or too unstable.

Facioscapulohumeral muscular dystrophy, myoblast, cell transplantation, autologous

Facioscapulohumeral dystrophy (FSHD1, FSHD2): #2847

P09- 160- Sensitivity and specificity of DR1 bisulfite sequencing in detecting SMCHD1 mutation in a cohort of FSHD1 and FSHD-like patients

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Facioscapulohumeral muscular dystrophy (FSHD) is characterized by typical, progressive and asymmetric weakness of selective muscle groups. The variability of clinical expression and the existence of myopathies mimicking FSHD clinical phenotype may complicate diagnosis. We can distinguish autosomal dominant FSHD1, associated with a contracted and permissive D4Z4 repeat array on chromosome 4 (71, 11 repeated units). FSHD2 patients display hypomethylation of the D4Z4 repeats both on chromosomes 4 and 10, while in FSHD1 patients, only the pathogenic contracted allele is hypomethylated. We recently described patients with severe clinical phenotype carrying both FSHD1 and FSHD2 mutations. This multicentric study assessed the sensitivity and specificity of DR1 methylation analysis by bisulfite sequencing (MABS) of blood DNA in detecting SMCHD1 pathogenic mutations. We analyzed 68 FSHD1 patients and 42 patients with typical FSHD clinical features and no contraction of the D4Z4 repeats on chromosome 4. All patients underwent clinical examination, haplotyping of 4q allele, targeted sequencing of the SMCHD1 gene by NGS and DR1 MABS (threshold level for DR1 hypomethylation > 30%). The pathogenicity of SMCHD1 mutations was computationally predicted and additional functional analyses were performed for new mutations. Among FSHD1 patients, we found 25 patients with DR1 methylation levels below the threshold, 6 of them carrying pathogenic mutations in SMCHD1 and a permissive 4Q allele, while 5 of them carry rare polymorphisms. The sensitivity of DR1 MABS in detecting patients with pathogenic SMCHD1 mutation was 100%, while specificity was 69%. Among 42 FSHD-like patients, we found 32 patients with DR1 hypomethylation, 29 of them carrying SMCHD1 pathogenic mutations and a permissive 4Q allele, 1 carries a rare polymorphism. For 6 other patients, an alternative diagnosis was confirmed and none shows DR1 hypomethylation. In this cohort, the sensitivity of DR1 MABS in predicting SMCHD1 pathogenic mutations was 96% and specificity was 75%. In conclusion, DR1 MABS is highly sensitive to identify patients with SMCHD1 mutations in FSHD1 and FSHD-like cohorts, but has moderate specificity. This may be improved by accurate sizing of chromosome 4 and 10 D4Z4 loci.

Facioscapulohumeral muscular dystrophy, SMCHD1, hypomethylation

Facioscapulohumeral dystrophy (FSHD1, FSHD2): #2878
P09- 161- Segregation between a frameshift SMCHD1 mutation, D4Z4 hypomethylation and Facio-Scapulo-Humeral Dystrophy.
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Facio-Scapulo-Humeral muscular Dystrophy (FSHD) is linked to a copy number reduction (n>10) of the 4q D4Z4 subtelomeric macrosatellite, in association with a DUX4-permissive haplotype. This main form of the disease is indicated as FSHD1. FSHD-like phenotypes may also appear, in 5% of cases, in the absence of D4Z4 copy number reduction (FSHD2). In 70-80% of these FSHD2 patients, variants of the SMCHD1 gene have been reported to segregate with DUX4-compatible haplotypes and associate with D4Z4 hypomethylation.

Here, we describe a family presenting neuromuscular symptoms reminiscent of FSHD but without D4Z4 copy number reduction. Bisulfite sequencing showed significant hypomethylation in a proximal region within D4Z4 in symptomatic cases of the family.

Exome sequencing revealed a heterozygous insertion of 7 bp in exon 37 of the SMCHD1 gene (c.4614_4615 insTATAATA) segregating with clinical signs and producing a frameshift in the protein with a premature stop codon 4 amino acids after the insertion (p.A1539Yfs*4), potentially leading to a truncated protein with a putative dominant negative effect. At the transcript level, this insertion (p.A1539Yfs*4) was detected, excluding haploinsufficiency as pathological mechanism. Ongoing analysis of nonsense mediated degradation of the mutated transcript and quantification of relative allelic expression will allow elucidating the cause of truncated protein absence. By this work, we aim to shed light on SMCHD1 mutated protein complex contribution to FSHD pathogenesis.

FSHD, Facio-Scapulo-Humeral Dystrophy, SMCHD1

Facioscapulohumeral dystrophy (FSHD1, FSHD2): #2885
P09- 162- Nuclear location of DUX4 is required for its corepressor activity on the progesterone nuclear receptor
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Our laboratory first presented evidence indicating that DUX4 is a negative co-regulator of the human progesterone receptor (PR). We also showed that progesterone protects cultured cells from DUX4-mediated cytotoxicity. DUX4 is normally expressed in gonadal tissues of healthy individuals, coincidental with a potential role for DUX4 in the endocrine pathway. Because DUX4 is a toxic protein, we hypothesize that cells from germinal tissues have specific regulatory and protective mechanism allowing normal expression of the DUX4 gene and bypassing the toxic effect of DUX4, respectively. The hypothetical protective mechanism(s) would not be present in tissues where ectopically/improperly expressed DUX4 leads to cell death. We have previously shown that DUX4-mediated toxicity is dependent on the subcellular location of DUX4. In this work we explored alternative subcellular locations of DUX4 regulates/modifies its coregulatory activity on the PR. Mutations at the more relevant NLSs from DUX4 (i.e. NLS1 and NLS2) partially delocalized DUX4 from the cell nucleus and were analyzed in these studies. It was observed that DUX4 with altered transit to the nuclei lose its corepressor activity on the PR. Thus, re-located DUX4 (i.e. mostly cytoplasmic) does not have any effect on the activity of the PR. These results indicate that the corepressor effect of DUX4 on the activity of the PR is exerted at the cell nucleus. DUX4-NLS mutants carrying the NLS from the virus SV40 would...
allow to re-direct DUX4 to the nuclei to analyze if the DUX4 NLS sequences per se participate in the DUX4 regulatory activity on the PR. To explore if alternative macromolecular structures of DUX4 disturb its activity on the PR, DUX4 fusions to GFP were analyzed. All the studies were performed on breast cancer cells endogenously expressing the PR. In this work we also analyzed the protective effect of progesterone on the toxicity of DUX4 NLS mutants. The toxic effect of DUX4 mutants was analyzed using a modified assay described in our laboratory and quantified using FACS. Results from these experiments indicate that progesterone synergize the low-toxicity of DUX4 NLS mutants. Taken together these studies strongly support our previous contention about the negative co-regulatory activity of DUX4 on the PR as well as the protective effect of progesterone on DUX4 toxicity. These results are relevant to both the normal and pathological function of DUX4 as well as the future rational therapies in FSHD.

FSHD, DUX4, toxicity, hormone

Facioscapulohemeral dystrophy (FSHD1, FSHD2)- #2951
P09- 163- The FSHD-modifier gene FAT1 patterns neuromuscular development through a cross-talk between mesenchyme, muscles, and spinal motor neurons
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FSHD is a human myopathy affecting selective groups of muscles in the face and shoulder, caused by 4q35 abnormalities leading to excess amounts of the transcription factor DUX4. However, patients can remain asymptomatic in spite of enhanced DUX4 expression, implying the contribution of disease modifiers to trigger symptoms. We recently identified the FAT1 cadherin as a key player in myogenesis and as a novel modifier gene in FSHD (Caruso, 2013 [1]): 1) Fat1 ablation in mice causes FSHD-like muscle phenotypes [1]. 2) Reduced FAT1 RNA levels were found in FSHD muscles at fetal [1] and adult (Mariot, 2015) stages. 3) We identified alterations of the FAT1 gene in FSHD-like patients, including deletions of a FAT1 regulatory element, also segregating with classical FSHD [1], or pathogenic variants altering protein or RNA structure (Puppo, 2015). Thus, alterations of FAT1 functions can cause FSHD-like symptoms, and may contribute to their appearance in FSHD. We have explored the roles of Fat1 at the root of muscle phenotypes, by genetically dissecting its functions during neuromuscular development. Fat1 patterns muscle shapes by coordinating myoblast migration polarity and by positioning muscle attachment sites [1]. The muscle shape defects of Fat1 knockouts are associated with motor axon guidance and specification phenotypes in motor neurons (MNs) innervating affected muscles. Interestingly, Fat1 is also expressed in muscle-associated mesenchymal cells, and in the MNs corresponding to the muscles vulnerable to Fat1 deletion. Deleting Fat1 activity in migrating myogenic cells [1] only partially recapitulates the knockout muscle phenotype. Instead, Fat1 ablation in the limb mesenchyme causes severe alterations of muscle shapes and attachment sites, further disrupting axonal arborization of affected muscles and acquisition of MN pool markers. This identifies mesenchyme as a source of Fat1-dependent muscle- and MN-patterning cues. Adult mice with Fat1 ablation in mesenchymal cells display significant loss of forelimb grip strength and altered NMJ integrity. Finally, Fat1 expression in MNs also contributed to refining motor pool identity and NMJ integrity in Fat1-vulnerable target muscle, but was dispensable for myogenesis and motor axon guidance. These results identify a Fat1-driven cross-talk between peripheral tissue and motor neurons that contributes to neuromuscular circuit assembly, alteration of which appear at the origin of FSHD-like symptoms.

FSHD, FAT1, Neuromuscular development, Mesenchyme

Facioscapulohemeral dystrophy (FSHD1, FSHD2)- #2958
P09- 164- The translocation of DUX4 and DUX4c during myoblast differentiation allows their association with nucleo-cytoplasmic proteins associated with mRNP granules.
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Hundreds of double homeobox (DUX) genes map within 3.3-kb repeated elements dispersed in the human genome and encode DNA-binding proteins. Among these, we identified DUX4, a potent transcription factor that causes facioscapulohemeral muscular dystrophy (FSHD). In the present study, we performed yeast two-hybrid screens and protein co-purifications to identify protein partners of DUX4, DUX4c (identical to DUX4 except for the end of the carboxyl terminal domain) and DUX1 (limited to the double homeodomain). Unexpectedly, we identified and validated (by co-immunoprecipitation, GST pull-down, co-immunofluorescence and in situ Proximal Ligation Assay) the interaction of DUX4, DUX4c and DUX1 with type III intermediate filament protein desmin in the cytoplasm and at the nuclear periphery. Desmin filaments link adjacent sarcromeres at the Z-discs, interact with mitochondria and contribute to positioning of the nuclei. All these functions are altered in FSHD muscles. Another Z-disc protein, LMCDC1 was also validated as a DUX4 partner. The functionality of DUX4 or DUX4c interactions with cytoplasmic proteins is underscored by the observation of DUX4 and DUX4c nucleo-cytoplasmic translocation upon myoblast fusion. In addition, we also validated (by co-immunoprecipitation, co-immunofluorescence or in situ PLA) several RNA-binding proteins involved in mRNA splicing and translation. Among these, the nuclear FUS and SF3B1 are reported to translocate to the cytoplasm of neuronal cells where they associated with ribonucleoparticles (RNP). These complexes contain untranslabeled mRNAs, bring mRNAs to subcellular areas where their translation is required at specific times. Several other validated or identified DUX4/4c partners are also contained in mRNP-granules, and the co-localization with cytoplasmic DAPI-positive spots is in keeping with such an association. Large muscle RNPs were recently shown to exit the nuclei via a novel mechanism of...
nuclear envelope budding. Following DUX4 or DUX4c overexpression in muscle cell cultures, we observed their association with similar nuclear buds. In conclusion, in addition to their transcripational activities, DUX4 and DUX4c induction in FSHD muscle cells might disturb cytoskeletal dynamics and mRNA splicing, location and translation by their association with proteins regulating these processes. Further investigations are on-going to confirm such function that might be common to proteins encoded by hundreds of DUX genes.

Facioscapulohumeral dystrophy (FSHD1, FSHD2): #2969

**P09-165- Topological organization of the 4q35 locus upon differentiation reveals common pathways between FSHD1 and FSHD2**

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Over the recent years, nuclear topology and sequence distribution within the nuclear space has emerged as a key element in the determination of cell fate and the regulation of gene expression, with repressed loci mainly located at the nuclear periphery. Dynamic interactions between DNA sequences or subnuclear domains have also been identified. However, only a little number of sequences with topological activity has been characterized so far. Among them, we described the D4Z4 macrosatellite element linked to Facio-Scapulo-Humeral Dystrophy (FSHD) as the first genomic element able to tether a telomere at the nuclear periphery and to modulate the topology and replication timing of its abutting telomere. We have also recently shown that telomere length influences the organization of the 4q35 locus and participate in the regulation of 4q35 genes by a mechanism named Telomeric Position Effect Over Long Distance (TPE-OLD). Nevertheless, the partitioning of the 4q35 region by domains of attachment at the nuclear periphery raises the question of how long distance interactions regulate this subtelomeric region, on the role of the D4Z4 element in the regulation of this locus and on the trans-acting factors involved in this regulation in the disease context.

To address this question, we used induced pluripotent stem cells derived for FSHD patients with different number of D4Z4 elements and FSHD2 patients carrying SMCHD1 mutations. We investigated the topology of the 4q35 region in the different genomic context and upon muscle commitment. We show that the topology of the 4q35 region is similar in FSHD1 and FSHD2 patients and is dynamically modulated upon skeletal muscle differentiation. The trans-acting factors and consequences on the regulation of the locus will be discussed.

**Facio-Scapulo-Humeral Dystrophy, induced pluripotent stem cells, muscle differentiation, telomere, DNA methylation, D4Z4, SMCHD1**

Facioscapulohumeral dystrophy (FSHD1, FSHD2): #2973

**P09-166- DNA Methylation analysis in pluripotent stem cells reveals a dynamic epigenetic regulation of the D4Z4 repeat in patients affected with Facio Scapulo Humeral Dystrophy upon muscle differentiation**

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FSHD is an autosomal dominant myopathy characterized by a progressive and asymmetric weakening of facial, shoulder, and upper body muscles with a progression to the lower body. At the molecular level, for 95% of patients, the pathology is linked to the deletion of a number of repetitive macrosatellite elements at the 4q35 locus (D4Z4). D4Z4 is extremely GC rich (70%) and highly methylated in normal individuals but hypomethylated in patients with FSHD (FSHD1) and in patients with phenotypic FSHD but without the deletion on both chromosomes 4 (FSHD2) (Gaillard et al, 2014). We studied the distribution of DNA methylation along the D4Z4 macrosatellite repeat in ES and induced pluripotent stem cells from FSHD1 and 2 patients and control cells.

To increase the number of D4Z4 allele analyzed, we developed a new methodology of high throughput targeted bisulfite sequencing using fusion of bisulfite sequencing primers (BSP) and the Ion Torrent PGM technology. We applied this technology to pluripotent stem cells such as human Embryonic Stem cells (hESC) and human induced Pluripotent Stem Cells (hiPSC) from FSHD patients and population of non-carriers but also cells differentiated toward the skeletal muscle lineage. Using this high throughput methylation methodology, we show that hypomethylation in FSHD1 patients is observed in both allele of D4Z4 and not restricted to the disease-carrying allele. We also demonstrate that the methylation level is not uniform along D4Z4. In the hiPSC from both control and FSHD patients, we demonstrate an increase of D4Z4 methylation. We confirmed in the hESC that no difference of methylation might be observed in the two situations control or disease. This mechanism is restricted to D4Z4 since other subtelomeric regions were not modulated by the disease. These data shed light on a dynamic regulation of D4Z4 methylation and on the absence of correlation between the reduction of the D4Z4 array and the hypomethylation. Upon differentiation toward the skeletal muscle lineage, D4Z4 methylation is reduced in patient's cells suggesting a key role for epigenetics in the regulation of this complex repetitive subtelomeric array. Interestingly, human stem cells from FSHD patients do not carry the hypomethylation feature the FSHD somatic cells and might be an interesting model for studying the physiopathological mechanisms of this still enigmatic disease.

**DNA methylation; Embryonic Stem Cell; induce pluripotent stem cell; Facio Scapulo Humeral Dystrophy**

Facioscapulohumeral dystrophy (FSHD1, FSHD2): #2974

**P09-167- Induced pluripotent stem cells (hiPSC) and skeletal muscle differentiation as a tool to investigate the epigenetic regulation of the 4q35 subtelomeric locus in Facio-Scapulo-Humeral Dystrophy**
Induced pluripotent stem cells (hiPSC) and skeletal muscle differentiation as a tool to investigate the epigenetic regulation of the 4q35 subtelomeric locus in Facio-Scapulo-Humeral Dystrophy.

Facioscapulo-humeral dystrophy (FSHD) is a common muscular dystrophy characterized by weakness in the face, shoulder and upper muscles with a progression to almost the lower body. In 95% of cases (FSHD1), this pathology is linked to shortening of an array of macrosatellite elements, D4Z4, at the subtelomeric 4q35 locus. In the 2-3% of the remaining patients, the pathology is associated to mutations in the SMCHD1 gene (FSHD2). For the rest of patients, the cause of the disease is still undetermined. At the molecular level, the pathology is associated with hypomethylation of D4Z4, an activation of the DUX4 retrogene and a modulation in cis of a number of 4q35 genes. We investigated the link between the mutations in the SMCHD1 gene, DNA methylation and the FSHD physiopathology.

Using fibroblasts of 4 FSHD1 patients, 3 FSHD2 patients and 2 controls, we generated human induced pluripotent stem cells (hiPSCs). Without the use of chemical pathways inhibitors, we developed a new and efficient protocol to generate muscular progenitors from these hiPSCs. The progenitors can be amplified, frozen and thawed and further differentiated into contractile multinucleated skeletal muscle fibers. This rapid and efficient method allows us to produce differentiated cells within 40 to 50 days after induction. These skeletal muscle cells express myogenic markers such as MyoD, Myosin Heavy Chains, Myogenin and Desmin.

We performed DNA methylation analysis using bisulfite sequencing on hiPSCs and muscle progenitors from healthy and FSHD patients. We demonstrate that D4Z4 methylation is dynamically regulated after reprogramming and differentiation. We also observed differences between FSHD1 and FSHD2 cells suggesting a role at the epigenetic level for SMCHD1 during the differentiation process. Furthermore, skeletal muscle differentiation is associated with a tri-dimensional reorganization of the 4q35 locus within the cell nucleus.

Altogether, our data emphasize induced pluripotent stem cells as an interesting model to investigate the early events regulated by SMCHD1 and contraction of the D4Z4 array in the patho-mechanisms leading to FSHD.

**FSHD, epigenetic, methylation, muscle differentiation**

**Faciocapulohumeral dystrophy (FSHD1, FSHD2)- #2980**

**P09- 168- The Role of D4Z4-Encoded Proteins in the Osteogenic Differentiation of Mesenchymal Stromal Cells Isolated from Bone Marrow.**

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The D4Z4 repeat array in 4q35 is hypomethylated in Faciocapulohumeral muscular dystrophy (FSHD). We have previously identified the Double Homeobox 4 (DUX4) gene within the D4Z4 unit that inappropriately expresses a toxic 52-kDa protein in FSHD muscle cells. DUX4 is also a retrogene that is normally expressed in germline cells and is submitted to repeat-induced silencing in adult tissues. DUX4 mRNAs have been detected in human embryonic and induced pluripotent stem (hES and iPS) cells. We have now investigated whether DUX4 could be expressed in human mesenchymal stromal cells (hMSCs) isolated from bone marrow. These cells can differentiate along several lineages (osteoblasts, chondroblasts, adipocytes,?).

We have unexpectedly found that DUX4 expression was induced upon hMSC differentiation to osteoblasts. This process involved 52-kDa DUX4 known in FSHD muscles and a new longer protein form (58 kDa). The 52-kDa DUX4 protein was expressed at very low levels in the undifferentiated and differentiated cells while the 58-kDa DUX4 protein was detected at increasing levels from day 8 to 21 following the induction of osteogenic differentiation. During osteogenic differentiation of human embryonic stem cells (hESC) that carry the FSHD1 genetic defect, a DUX4 mRNA with a more distant 5' start site was characterized: it presented a 60-codon reading frame extension and encoded the 58-kDa protein (DUX4M60). Transfections of hMSCs with an antisense oligonucleotide targeting DUX4 mRNAs decreased both the 52- and 58-kDa protein levels and confirmed their identity. Gain and loss of function experiments in hMSCs suggested these DUX4 proteins had roles in osteogenic differentiation as evidenced by the alkaline phosphatase activity and calcium deposition. The differentiation was increased by 52-kDa DUX4 but was delayed by 58-kDa DUX4 expression, showing opposite roles in osteoblastic differentiation. Neither of these proteins appeared cytotoxic in hMSCs during their differentiation in the osteogenic lineage.

Several therapeutic approaches for FSHD are being developed that aim to interfere with DUX4 expression. Our present study indicates essential functions in MSC differentiation that should be maintained and demonstrates the need for specific muscle targeting of DUX4-suppressing agents.

**DUX4 variants, FSHD, MSC, osteogenesis, differentiation**

Facioscapulohumeral dystrophy (FSHD1, FSHD2)- #3003
The French National Registry for Facio-Scapulo-Humeral muscular Dystrophy (FSHD) has been launched in June 2013. The principal aims of this project are to collect epidemiological data, to promote clinical research, and to develop standards of care for FSHD patients.

A dedicated database and website (www.fshd.fr) have been developed to enable online data input. Molecular and clinical curators validate genetic and clinical data.

Patients included are FSHD1 patients genetically confirmed (inclusion possible through clinical evaluation form and/or the self-reported form), and patients presenting with FSHD phenotype without the typical D4Z4 contraction (FSHD2 and/or FSHD-like patients, only through clinical evaluation form). Data collected are related to genetic diagnosis, muscular and extra-muscular involvement, pain and patient care.

So far, nearly 500 patients have been included. Not only this value represents about 30% of expected French FSHD patients, but also it’s one of the largest cohort of FSHD patients data collected so far worldwide. The aim of this study is to present preliminary statistical data analysis on this cohort focusing on FSHD1 patients whose clinical evaluation form will be available at the end of November 2015. We will present demographic and general characteristics of this population, genotype/phenotype correlation based on number of D4Z4 repeated units, age of onset and severity of clinical involvement (age related CSS, MMT score on 36 muscles corrected by disease duration). We also performed statistical analysis concerning the correlation between the distribution of respiratory and extra-muscular involvement (ocular, hearing loss, cardiac, gastrointestinal) with the age of onset, with the severity of clinical involvement (see above) and with the number of repeated units. Moreover, correlation between BMI and clinical severity; metabolic and endocrine involvement, BMI and clinical severity; pain, BMI and clinical severity will also be analysed. These preliminary analyses will allow us to develop future directions of clinical research and will contribute in drawing national evidence based patients guidelines.

Facioscapulohumeral muscular Dystrophy, data base

Facioscapulohumeral dystrophy (FSHD1, FSHD2)- #3011

P09- 170- New outcome measures for home monitoring in fshd

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Current clinical studies in FSHD lack reliable and sensitive outcome measures. In neuromuscular field, the effectiveness of treatment is mainly evaluated by testing standard strength and function measures, usually done in a hospital environment that could lead to a bias in the evaluation of patients. To overcome this, ActiMyo©- a home-based activity recording device with two sensors, each containing a tri-axial accelerometer, gyroscope, magnetometer and barometer- was designed to assess ambulant and non-ambulant patients with neuromuscular diseases at home. This device is provided to patients involved in clinical studies and allows recording of different type of movements of the upper and/or lower limb, during their daily life activity, in their natural environment.

The Institute of Myology, in collaboration with Sysnav, a French company specialized in navigation systems and motion estimation, developed a new algorithm to characterize ambulant patients’ activity with facioscapulohumeral muscular dystrophy (FSHD), allowing their monitoring at home.
Preliminary tests were performed on a cohort of 9 volunteers with FSHD, aged from 13 to 54, 3 males and 6 females, who wore ActiMyo at the most affected wrist and the ipsilateral ankle during different tests. Using ActiMyo during the 6 minute walking distance (6MWD) test, the ankle trajectory of each patient was calculated with every step. The signal processing allowed to precisely define the length of each step of the different patients and thus, to accurately determine the total distance traveled during a 6MWD. In all patients tested, the difference between the distance measured by the clinician and the distance calculated by the ActiMyo was less than 3%.

Recordings were also carried out with sensors attached to the upper limbs (above the elbow) and chest (on the sternum) during sessions mimicking movements of daily life and extracted from conventionally accepted tests, among FSHD and control patients. Preliminary analyses of these recordings enabled to differentiate positive controls from healthy subjects using features such as trajectories of the upper limbs, proportion of jerks during movements or strong trunk compensation, which emerge as potential monitoring criteria.

Results from recordings by ActiMyo are particularly important to address the lack of outcome measures, necessary to respond to treatment efficacy issues in current or future clinical studies involving FSHD patients.
NGS technologies make possible to focus the sequencing to the exome or to a more specific DNA target bringing the genetic testing to a higher level of complexity and this is especially true for NMDs since they present diagnostic challenges due to the phenotypic variability sometimes discordant with the segregation of Mendelian traits and leading to the hypothesis that additional factors might modulate phenotypes of each intra- and inter-familial condition. Considering the wide FSHD inter- and intra-familial clinical variability and severity observed, we applied the NGS technology to 89 FSHD patients recruited from families in which the disease has a reduced penetrance to the hypothesis that the presence of subsets of genetic variants could contribute to the disease onset, signs and manifestations once inherited in defined genetic backgrounds. We used the Motorplex core panel covering a genomic landscape of 160 genes involved in neuromuscular diseases. The NGS analysis highlighted the presence of 508 variants belonging to 99 genes of the panel. Interestingly, we identified 372 variants characterized by frequency >1%; out of 372, 246 variants have been predicted to be detrimental. To define the significance of these variants we conducted segregation studies in selected families. The final output of this wide NGS study supports the hypothesis that family genetic background contributes to disease expression with consequences for clinical practice. It will be therefore necessary to perform in depth genotype-phenotype characterization of FSHD patients and their families. This approach will lay the basis for proper genetic counseling.

**FSHD, NGS, segregation**

Facioscapulohumeral dystrophy (FSHD1, FSHD2)- #3036

P09- 173- Epigenetic investigations of FSHD families from the Italian National Registry of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is considered as an autosomal dominant disorder, causally related to reduced number (78) of 3.3 kb tandemly arrayed D4Z4 repeats on 4q35. The current model explaining FSHD pathogenesis favours the possibility that D4Z4 repeat array governs 4q35 chromatin conformation leading to aberrant overexpression of nearby genes. Consistent with this model it was reported that patients carrying 4q allele with a D4Z4 array less than 11 repeats (FSHD1) and contraction-independent patients (FSHD2), who carry D4Z4 alleles of normal size on both chromosomes 4q, display D4Z4 decreased level of methylation (?25%).

D4Z4 hypomethylation might be a marker of a more complex mechanism as suggested by recent studies showing that SMCHD1 deficiency affects several genomic loci beside D4Z4. The possibility that a complex genetic and epigenetic network is altered in FSHD is highlighted by genotype-phenotype studies of FSHD families suggesting that some FSHD cases develop because of complex genetic conditions.

In order to test this possibility we designed a research strategy for the identification of these elements. We performed a systematic study on a cohort of 82 FSHD families (85 FSHD1 patients, 24 non-manifesting relatives and 35 FSHD2 patients), 49 healthy controls and 10 subjects affected with other muscular diseases. We focused our attention on the FSHD1 families with the reduced penetrance to test the possibility that differential clinical expression might correlate with the different degree of D4Z4 methylation between FSHD patients and their healthy relatives carrying the same DRA (1-10 D4Z4 units) and FSHD2
patients which are sporadic and with milder phenotype. To this purpose we analyzed the level of the D4Z4 methylation on both 4q35 and tested DNA for the presence of SMCHD1 mutation. Our study revealed that the methylation status of the D4Z4 region does not strictly correlate with the presence and severity of a FSHD clinical phenotype as we detected both D4Z4 methylation profiles, hypomethylation (725%) and normal level of methylation (735%), in all analyzed subgroups. Interestingly, we observed FSHD patients carrying hypomethylated D4Z4 alleles of normal size with no SMCHD1 mutation suggesting the presence of additional epigenetic factors determining the D4Z4 methylation status. Studies on well clinically characterized families will foster the dissection of epigenetic mechanisms involved in developing FSHD.

FSHD, D4Z4 hypomethylation, SMCHD1, complex genetic disease

P10- Gene therapies (except exon-skipping) /- N° 174 to N° 182

Gene therapies (except exon-skipping)- #2337

P10-174- NF kappa B sites on plasmid DNA enhance transfection of skeletal muscles following hydrodynamic limb vein injection in healthy mice

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Hydrodynamic injection via the limb saphenous vein (HLV) is an in vivo loco-regional administration procedure useful to deliver plasmid DNA (pDNA) into skeletal muscles. This method has been successfully used in mice, dogs, and non-human primates and it was recently shown to be safe and feasible in humans. Despite it allows to deliver pDNA at the same time in all the muscles of a limb, only few pDNA copies actually reach the nucleus of each muscle cell. Besides, it has been shown that a sequence comprising 3 NF motifs (3NF) can greatly enhance the nuclear import of a pDNA under inflammatory condition. In the present study, we examined the effect of 3NF contained into a luciferase-encoding pDNA (p3NF-CMVLuc-3NF) on the transfection efficiency of skeletal muscles following HLV injection in normal healthy mice. As compared with a pDNA lacking 3NF, injection of p3NF-CMVLuc-3NF resulted in 20-30 times more intense in vivo bioluminescence in the whole injected leg. This advantage was observed throughout our experiments which lasted at least one month. When considering each muscle separately, we found that p3NF-CMVLuc-3NF actually mediated up to 200 times higher luciferase activity, especially in muscles of the lower limb, which are more difficult to transfect via this administration procedure. After repeated HLV injections of p3NF-CMVLuc-3NF, the luciferase activity was increased for several days, a ~10-fold higher bioluminescence being measured after injection of a 10-fold higher dose. All these results suggest that 3NF would be very beneficial to enhance the transfection efficiency of therapeutic genes following HLV injection, especially in the context of Duchenne Muscular Dystrophy in which the chronic inflammation could play as an in situ catalyzer.

non viral transfection, skeletal muscle, Hydrodynamic limb vein injection, plasmid DNA, nuclear import, NFkB; skeletal muscle, nuclear import

Gene therapies (except exon-skipping)- #2572

P10-175- Neutral amphiphilic triblock copolymers promote minidystrophin expression in skeletal muscles of mdx mice following hydrodynamic limb vein injection.

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