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PROCEEDINGS OF THE XIV MEETING OF THE ITALIAN ASSOCIATION OF MYOLOGY

Sirmione (BS), Italy

May 8-10, 2014

Associazione Italiana di Miologia

14° CONGRESSO NAZIONALE AIM

PalaCreberg Sirmione
8/10 maggio 2014

Azienda Ospedaliera "Spedali Civili" Brescia

Sin
SOCIETÀ ITALIANA DI NEUROLOGIA

Università degli Studi di Brescia

GIOVEDÌ, 8 MAGGIO

8.30 SALUTI E INTRODUZIONE
A. Padovani
Università degli Studi di Brescia
E. Agabiti Rosei
Università degli Studi di Brescia
M. Magoni
A.O. "Spedali Civili" Brescia

Apertura del Congresso
M. Moggio *(Milano)*, M. Filosto *(Brescia)*

8.45-9.45 **COMUNICAZIONI ORALI**

9.45-10.30 **Lettura magistrale**
Guidelines for diagnosis and treatment of inflammatory myopathies
M. De Visser *(Amsterdam)*

10.30-11.00 *Coffee break*

11.00-13.00 **Workshop congiunto AIM-SIR**
LE MIOPATIE INFIAMMATORIE: NEUROLOGI E REUMATOLOGI A CONFRONTO

11.00 Inquadramento clinico e diagnosi bioptica
M. Moggio *(Milano)*

11.30 Anticorpi miosite-specifici: significato clinico e patogenetico
A. Doria *(Padova)*

12.00 Approccio alla diagnosi ed alla terapia: l'esperienza del neurologo
R. Mantegazza *(Milano)*

12.30 Approccio alla diagnosi ed alla terapia: l'esperienza del reumatologo
R. Neri *(Pisa)*

13.00-14.00 *Pranzo*

14.00-15.30 **Visione e discussione poster**

15.30-16.30 **COMUNICAZIONI ORALI**

16.30 *Coffee break*

17.00-18.00 **Incontro con le Associazioni dei Pazienti**

18.00 **WORKSHOP**
NUOVI SVILUPPI NELLA TERAPIA DELLE MALATTIE NEUROMUSCOLARI

18.00 Distrofinopatie
G. Comi *(Milano)*

18.20 SMA
E. Mercuri *(Roma)*

18.40 Miastenia
A. Evoli *(Roma)*

VENERDÌ, 9 MAGGIO

8.30-9.30 **MUSCLE CLUB**

9.30-10.15 **Lettura magistrale**
Moderni aspetti nella diagnostica morfologica delle distrofie dei cingoli
R. Barresi *(Newcastle)*

10.15 *Coffee break*

10.45-12.45 **WORKSHOP**
IMPATTO DELLE METODICHE DI LABORATORIO NELL'ITER DIAGNOSTICO DELLE MALATTIE MUSCOLARI

10.45 Le indagini di laboratorio possono indirizzare verso una diagnosi piu' precoce?
A. Toscano *(Messina)*

11.15 L'imaging nella diagnostica differenziale delle miopatie.
G. Tasca *(Roma)*

11.45 Biomarkers nelle malattie neuromuscolari.
F. Gualandi *(Ferrara)*

12.15 Next Generation Sequencing e Whole Exome Sequencing: utilità e limiti.
V. Nigro *(Napoli)*

12.45-13.45 *Pranzo*

13.45-15.15 **VISIONE E DISCUSSIONE POSTER**

15.15-16.45 Aggiornamenti e proposte dei Gruppi di studio AIM

16.45-17.15 *Coffee break*

17.15-18.30 **COMUNICAZIONI ORALI**

18.30-20.00 Assemblea dei soci

21.00 Cena sociale

SABATO, 10 MAGGIO

8.30-10.30 **WORKSHOP**
NUOVI SVILUPPI NELLA TERAPIA DELLE MIOPATIE METABOLICHE

8.30 MNGIE ed altre malattie da deplezione del mtDNA.

9.00 La terapia genica nelle malattie mitocondriali.
M. Zeviani *(Cambridge)*

9.30 Glicogenosi V: dal modello animale allo sviluppo di nuove terapie.
A. Andreu *(Barcelona)*

10.00 Malattia di Pompe ad esordio tardivo: lo stato dell'arte.
M. Filosto *(Brescia)*

10.30-11.00 *Coffee break*

11.00-13.30 **COMUNICAZIONI ORALI**

13.30-14.00 Compilazione del questionario di valutazione ECM e verifica dell'apprendimento

Chiusura del Congresso

ABSTRACTS

(in alphabetical order of the 1st Author)

Functional characterization of three new recessive ClC-1 mutations causing myotonia congenita in Southern Italy

C. Altamura [1], S. Portaro [2], N. Licata [2], C. Rodolico [2], O. Musumeci [2], M.M. Dinardo [1], P. Imbrici [1], A. Toscano [2], D. Conte Camerino [1], J.F. Desaphy [1]

[1] Department of Pharmacy & Drug Sciences, University of Bari - Aldo Moro, Bari; [2] Department of Neurosciences, Psychiatry and Anestesiology, University of Messina

Myotonia congenita is an inherited disease characterized by impaired muscle relaxation after contraction, resulting in muscle stiffness. It is caused by loss-of-function mutations of the muscle ClC-1 chloride channel. We report the functional analysis of three new missense mutations found in patients with recessive myotonia congenita. The T82A and R453W mutations were found in compound heterozygosis with the known G190S mutation in two unrelated families, and the G270V mutation was found in an homozygous patient. Recombinant hClC-1 channel mutants were expressed in a mammalian cell line for patch-clamp studies of chloride current properties. The G270V and G190S mutations were found to induce a dramatic shift of activation voltage-dependence toward more positive potentials, resulting in nearly zero chloride current within physiological voltage range. Thus the effect of G270V can explain the myotonia in the homozygous patient. Conversely, the T82A and R453W chloride currents showed current amplitude, kinetics, and voltage-dependence similar to WT currents. Studies of mutant cotransfection reproducing the patients heterozygosis are being performed in order to elucidate the pathomechanism of T82A and R453W mutations. Supported by Telethon-Italy (GGP10101).

Predictors for cardiac conduction abnormalities in DM1. A 33 Yrs. prospective study in 102 DM1 patients with normal ECG at baseline

G. Antonini [1], E. Bucci [1], E. Gabriele [2], A. Frattari [2], L. Licchelli [1], N. Vanacore [3], M. Testa [2]

[1] Dept. NESMOS and [2] Cardiologic Institute. Faculty of Medicine and Psychology. University of Rome; [3] National Institute of Health, Rome

Since 1980, 151 continuous DM1 patients have entered a prospective research project on genotype/phenotype correlations of the disease. Aimed to find genotypic and phenotypic predictors of cardiac conduction abnormalities (CCA), we have recorded yearly ECGs in 102 patients (M/F 57/45, aged 5-79 yrs. Median 37 yrs., CTG range 50-1700), who had normal ECG at baseline. Patients were followed from 1 to 33 yrs. (median 8 yrs.) During the F-U 43 patients developed CCA (AVB-I: 32%, AVB-II: 9%, BBB: 23%); 12 patients underwent PMK/ICD implantation; 13 patients died for cardiac cause; 59 patients showed an increase of MIRS.
Follow-up was similar in patients who developed CCA and in those who did not. CCA occurred more frequently in males ($p = 0.008$) and in patients who showed MIRS progression ($p = 0.015$). CTG expansion showed a significant inverse correlation with age at onset of CCA ($R2 = 0.16$; $p < 0.0001$). Logistic regression analysis showed that, after correction for CTG expansion and age, both male sex and MIRS progression were independent predictors for CCA development (OR = 3.15; 95%CI = 1.28-7.72; $p = 0.012$ and OR = 3.86; 95%CI = 1.47-10.15; $p = 0.006$, respectively).

Novel *SEPN1* Mutation in 3 Patients: diagnostic clues of neck weakness and MRI pattern

A. Ardissone [1], C. Bragato [2], F. Blasevich [2], M. Mora [2], I. Moroni [1]

[1] Child Neurology Unit, [2] Neuromuscular Diseases and Neuroimmunology Unit, Foundation IRCCS C. Besta Neurological Institute, Milan, Italy

Mutations in *SEPN1* cause Selenoprotein N-related myopathy (*SEPN*-RM), characterized by early onset axial and neck weakness, spinal rigidity, respiratory failure and four distinct histopathological entities. A typical MRI pattern has been described in lower limbs, represented by a selective involvement of the sartorius muscle. We report on the clinical, histopatological and genetic findings in 3 patients from two families, presenting with an heterogenous phenotype and carrying novel *SEPN1* mutations. Two siblings arrived to the observation at the age of 10 and 14 years respectively, presenting with mild myopathic signs, neck weakness and spinal rigidity; the female showed also a severe diffuse muscle wasting. After two years follow-up the myopatic signs were stable, while a marked respiratory involvement was detected. The third patient, a three years old boy, presented with severe axial weakness, leading to a "dropped head" appearance, and lower limb girdle muscle weakness. The typical MRI pattern was present in both cases. *SEPN1* gene analysis disclosed the presence of the c.1176delA mutation in the siblings and of the c.726_727InsTCC mutation in the other patient. We underline the clinical diagnostic clues of early neck and axial weakness and of muscle MRI in addressing the diagnosis of *SEPN*-RM, and confirm the importance of investigating the progressive respiratory impairment in spite of mild myopathic course.

Beneficial effects of salbutamol in congenital myasthenic syndrome associated with new mutations in *CHRND*

G. Astrea [1], L. Maggi [2], R. Trovato [1], D. Kapetis [2], D. Cassandrini [1], S. Frosini [1], R. Brugnoni [2], P. Bernasconi [2], R. Mantegazza [2], R. Battini [1], F.M. Santorelli [1]

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Congenital myasthenic syndromes (CMS) are disabling, but potentially treatable disorder characterized by a neuromuscular transmission defect. Cholinesterase inhibitors are effective in most cases, but harmful in specific CMS. Salbutamol, a beta-adrenergic agonist, has demonstrated a partial improvement of clinical symptoms in some CMS.
A 15-year-old girl received a clinical diagnosis of CMS in her first year of life. The patient was put on acetylcholinesterase inhibitors since age 2.5 years and on 3,4 DAP since age 9 with limited improvement. At the age of 14 years, salbutamol was gradually added on (4 mg /day and, after six months, 6mg/day) because of lost of independent ambulation. Response to therapy was evaluated by using the MFM scale. We identified a novel missense mutation (c.215 A>C/p.Thr72Asn) heteroallelic to the c.521_524dupATAC/p.Ala176tyrfs* in CHRND. Molecular modeling demonstrated that replacement of Asn72 modifies local H-bond interaction environment between β1, β6 sheet domains and β1/ β2 loop.
Comparison of the pre- and post-treatment examinations showed a beneficial response to salbutamol with no side effects. Our findings highlight the importance of a molecular diagnosis in CMS and proposes salbutamol's use when conventional therapies fail to achieved a stable response.

Next Generation Sequencing in facioscapulohumeral muscular dystrophy patients supports the idea that FSHD is a complex genetic disease

E. Attico [1], M. Savarese [2 3], G. Ricci [1 4], A. Nikolic [1], J. Daolio [1], F. Mele [1], M. Govi, G. Vattemi [5], L. Vercelli [6], L. Villa [7], L. Ruggiero [8], M. Sciacco [7], C. Rodolico [9], L. Morandi [10], G. Siciliano [4], C. Angelini [11], Italian Network for FSHD, A. Di Muzio [12], A. Berardinelli [13], G. D'Angelo [14], M.A. Maioli [15], L. Santoro [8], M. Moggio [7], T. Mongini [6], G. Tomelleri [5], V. Nigro [2 3], R. Tupler

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Facioscapulohumeral muscular dystrophy has been associated with reduction of the number of D4Z4 repetitive elements at 4q35. FSHD is characterized by great clinical variability within families in which D4Z4 reduced allele (DRA) segregates. An increasing number of cases are sporadic with no other affected relatives and several findings suggest that additional factors (genetic modifiers) might modulate FSHD expression. Thus molecular diagnosis, prognosis and genetic counseling have become more challenging.
To gain additional information on the complexity of FSHD, we tested 40 FSHD patients belonging to families with reduced penetrance by testing, a broad core panel of 93 genes involved in myopathies (Motorplex). This Next Generation Sequencing-based workflow permit the analysis of 2,544 exons.
We studied 40 samples from FSHD patients belonging to families in which other DRA carries are healthy. In all subjects we found putative pathogenic variations in genes causing different myopathies. In addition to DRA, all patients carried at least one damaging variations in other disease genes. These variants, if they had been detected alone in the context of a single gene testing, would have been considered as causative. The high number of damaging mutations identified in each sample support the hypothesis of "multiple factors" leading to the FSHD phenotype.
In conclusion, the use of a reliable, sensitive and specific method has been able to identify putative pathogenic mutations that can explain the variable penetrance of DRA. Importantly these large set of mutations observed in FSHD patients highlight the genetic complexity that might contribute to the disease expression.

Lack of association between CNS and muscle involvement in Steinert's Disease (DM1). What about a link between CNS and behaviour?

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Introduction. Myotonic dystrophy is a genetic, multisystemic disorder due to polynucleotide-expansions being only partially reliable to predict phenotypic expression. Beyond muscular involvement DM1 can be characterized by functional/morphological brain abnormalities to different extents. From a neuropsychological point of view executive, visuo-spatial dysfunctions, mood and personality impairments are reported.
Methods. Forty subjects with established clinical-genetic diagnosis, underwent complete neurological assessment, including psychological interview and neuropsychological evaluation. Main caregiver underwent patient's Quality-of-life interview. A subgroup of 15patients underwent brain-MRI investigation.
Results. We found reduced scores in neuropsychological tests for frontal (61%) and visuo-spatial abilities (66%); interestingly verbal abilities were rather preserved (80%). Behaviour was characterized by mixed-mood conditions (anxiety, depression, apathy) and by variable sets of pathological personality traits, though without fulfilling diagnostic criteria for major psychiatric disorder according to DSM-IV. Patient's and main caregiver's reports showed internal discrepancies (63%), with patients

tending to denial some aspects of their condition. Brain imaging revealed white-matter involvement in frontal (53%), parietal (27%) and temporal (73%) lobes. Statistical analysis showed significant relationships between reduced spatial memory performances and temporal lobe white-matter changes (Fisher-Exact-Test, $p < 0.05$).

Conclusions. Muscle and brain appear independently involved in DM1; white-matter lesions are common in DM1 patients independently from muscle involvement (MIRS). In our study CNS involvement in DM1 is characterized by cognitive/psychopathological dysfunctions, heterogeneously distributed; this could be a prominent feature in DM1, leading to an increased burden in management in health-institutions and at home. Cognitive/behavioural disorders could have significant relationships with white-matter lesions and should be investigated since the early phases of illness, in order to plan proper management.

Early limb-girdle and congenital muscular dystrophy co-occurrence within the same family carrying a new homozygous *ISPD* mutation

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Dystroglycanopathies represent an important subgroup of recessively inherited disorders within the group of muscular dystrophies. They are associated with a reduction in the functional glycosylation of dystroglycan, and their severity may vary from the mild forms of adult-onset limb-girdle muscular dystrophy (LGMD), to the severe congenital muscular dystrophies with cerebral and ocular involvement. Although mutations in at least 17 genes have been identified so far, about 50% of the cases with dystroglycanopathy still remain unsolved. Mutations in the isoprenoid synthase domain containing (ISPD) gene has been associated with loss of dystroglycan glycosylation. Similarly to other genes associated with dystroglycanopathies, ISPD gene mutations have been at first reported as a frequent cause of Walker–Warburg syndrome. More recently, ISPD mutations have been reported in seven families, with phenotypes ranging from congenital muscular dystrophy to LGMD. We report clinical, histopathological, immunochemical, genetic and muscular MRI findings in 2 consanguineous children of Pakistani origin, carrying a new homozygous missense mutation (Gly123Arg) in the ISPD gene. Case 1 is a 8 year-old female with an early limb-girdle phenotype, who lost ambulation at the age of 7.5 years. Case 2 is a 2.5 year-old male and second degree cousin of case 1, showing a congenital muscular dystrophy phenotype. Cognitive development, brain MRI, eye examination, electrocardiogram and echocardiogram were normal in both the patients. To our knowledge, this is the first report on the co-occurrence of both early limb-girdle and congenital muscular dystrophy within the same family carrying a new homozygous *ISPD* mutation.

Pilot study of flavocoxid in ambulant DMD patients

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Muscle degeneration in Duchenne muscular dystrophy (DMD) is exacerbated by the endogenous inflammatory response and increased oxidative stress. A key role is played by nuclear factor(NF)- κB. We previously showed that flavocoxid, a flavonoid with antioxidant and anti-inflammatory properties, ameliorates muscle pathology and function in *mdx* mice. This effect seemed to be mediated by the inhibition of NF-κB, tumor necrosis factor-α, cyclooxygenase-2/5-lipoxygenase and MAPKs expression in muscle. Moreover, flavocoxid has been shown to decrement serum levels of IL-1β and TNF-α in *in vivo* studies.

Primary end-point of this pilot study was to evaluate safety and tolerability of flavocoxid administered daily *per os* for one year in ambulant DMD patients. We also evaluated function, muscle strength and quality of life. The effects of flavocoxid on selected biomarkers was also assessed. We enrolled 20 patients. We did not report any treatment-related adverse event and clinically meaningful change in laboratory findings. Serum expression analysis of inflammatory cytokines showed a significant reduction of TNF-α and IL-1β and oxidative stress markers ($p < 0.05$). The results of the multidimensional clinical evaluation showed an overall stabilization of clinical course, even in patients older than 7 years and which showed deterioration in the year before baseline. Moreover, a significant worsening of North Star Ambulatory Assessment was shown at 6 months after end of treatment compared to all trial time points ($p < 0.05$). We demonstrated that flavocoxid at this dosage is safe, also in pediatric age and in association with corticosteroids, and able to exert its biological effects on inflammatory pathways relevant to DMD pathogenesis.

Mutations in *GMPPB* cause α-DG: report of an additional highlighting

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Congenital muscular dystrophies with hypoglycosylation of alpha-dystroglycan (α-DG) are a heterogeneous group of disorders in which the spectrum of severity observed ranges from classical congenital muscle dystrophy (CMD) presentation to children showing mild limb-girdle weakness variably associated with mild intellectual disability and/or structural brain anomalies. Recently, mutations in the guanosine diphosphate mannose (GDP-mannose) pyrophosphorylase B (*GMPPB*) gene have been associated with muscular dystrophy with hypoglycosylated α-DG.

Through an international collaborative effort, we identified recessive *GMPPB* mutations in an additional patient. LS is a 5-year-old boy who presented axial muscle weakness in the first months of life followed by ataxia and nystagmus, hyperCKmia, brain MRI with enlargement posterior fossa, moderate intellectual disability and autistic-like behaviour.
In light of no evidence of perturbed transferrin glycoforms and a reduced muscular immunostaining for glycosylated α-DG, we performed exome sequencing and detected a reported p. R287Q (Carss et al., 2013) and a novel p.I219T variant in *GMPPB*. The latter mutation is not present in Exome Variant Server or dbSNP but a variant in the same place is seen 3 of 9464 cases in the UK10K project Considering that there were no other suggestive variants in the exome of LS, most probably the 2 mutations in *GMPPB* are causative of CMD with low α-DG and autistic-like features.

Acute ataxia and psychomotor regression due to SDHAF1 mutation responsive to riboflavine

A. Berardinelli [1], C. Ravelli [1], C. Baldassari [1], M. Rossi [1], U. Balottin [1*], A. Pichiecchio [2], C. Uggetti [3], I. Colombo [4], D. Ronchi [5], V. Melzi [5], F. Fortunato [5], M. Moggio [4], G.P. Comi [5]

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Deficiency of complex II (succinate dehydrogenase, SDH) represents a rare cause of mitochondrial disease and is associated with a wide range of clinical symptoms. Recently, mutations of SDHAF1, the gene encoding for the SDH assembly factor 1, were reported in SDH-defective infantile leukoencephalopathy with typical brain MRI and spectroscopy features. No satisfactory treatment is currently available, and affected patients undergo a relentlessly progressive motor and mental deterioration. Riboflavin has been reported as possibly effective in reducing the progression of disease or even preventing from developing signs of neurological involvement when administered before the onset of the disease, but so far only few cases have been described.
We describe here the case of child with SDHAF1 mutation and typical features in brain MRI and spectroscopy with onset at 2ys 4mths, treated with riboflavin and CoQ and with a positive clinical and neuroradiological outcome after 3 years

Cognitive impairment in mitochondrial encephalomyopathies

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Objective. Mitochondrial encephalomyopathies are a genetically heterogeneous group of diseases characterized by multisystem involvement. We studied the neuropsycological profile, magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT) to look for common or specific cognitive defects and a possible correlation with neuroimaging data.
Patients and methods. 53 patients (thirthy three females, twenty males) aged 17-80 years: fourty-three PEO, five MELAS, four MERRF and one MNGIE. All the patients underwent neuropsychological evaluations and MRI neuroimaging; eighteen patients had also SPECT. Four main cognitive areas were explored: visuospatial perception, memory, executive, speech and praxic functions.
Results. Mental control, short term memory and visual selective attention were selectively impaired in 75% of patients., but we did not found any correlation between neuropsychological profile, and age, clinical phenotypes and genetics. SPECT was abnormal in nine patient with parietal and temporal hypoperfusion in six of them. A 60–year old woman with PEO showed selective hypoperfusion in right temporal lobe with temporomesial atrophy and developed an Alzheimer's like syndrome. MRI neuroimaging revealed signs of subcortical white matter impairment in thirty-four patients. Finally we observed four patients with short term memory or mental control impairment without SPECT abnormalities.
Conclusions. Cognitive impairment is frequent in mitochondrial diseases revealing a consistent pattern independently from phenotype; moreover no correlation were observed with MRI and SPECT imaging.

Body composition and energy expenditure in Duchenne muscolar distrophy: longitudinal study

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Background. Duchenne muscolar distrophy (DMD) is characterised by decreased fat-free mass (FFM) and increased fat mass (FM). Skeletal muscle metabolism is the major determinant of the resting energy expenditure (REE). A reduction of REE, according to the severe muscle loss, is hypothetical in DMD subjects but in the literature there are few and conflicting data regarding this relationship.
Objective. To provide longitudinal data about the natural evolution of body composition and REE in DMD and to investigate their relationship.
Methods. At baseline we studied 11 subjects with DMD median age 11 years (IQR: 9-13). They were divided in normal-weight and obese according to Italian BMI growth norms table. Only five patients were assessed at follow-up after 12 months. Body composition (FFM, FM, FFMI) was measured using DEXA; REE was assessed by indirect calorimetry; dietary energy intake was also investigated.
Results. At baseline in obese subjects mean FM% was significantly greater than in normal-weight (51.2; IQR:50.2-58.1vs39.1; IQR:29.7-46.4, $p = 0.014$). Also the FFM was greater in obeses. The REE values were smaller in normal weight

subjects (1325.5;IQR:1006-1467.5vs1633;IQR:1402-1683 kcal/day) but similar when adjusted for kg/FFM (50.5; IQR:48.1-57vs51.1; IQR:48.8-53.2). The primary longitudinal outcomes show a mean weight gain of 3 kg and a mean FM% increase; even the mean FFM significantly increase (26.2; IQR:20.4-31.6, kg p = 0.043). REE and REE/FFM mean values decreased. The caloric intake was stable respect to basal observation.

Discussion. In the obese patients FM was greater but also FFM values. This higher value of FFM in obese may be due to the difference in mean age between groups beside possible genetic determinants of body size. The REE was significantly lower than the value obtained from the literature in healthy children of the same age and it was significantly lower in the normal-weight children than in the obese subjects. The REE/FFM, nevertheless, was similar between the two groups, due to the higher values of FFM in the obese subjects. At the follow-up the significant increase of FFM is probably due to the influence of growth and of sexual hormones. Moreover we can suppose that the absence of significant changes in REE was secondary to the too short follow-up.

Conclusion. DMD patients suffer from progressive weight gain and increase fat mass but in young boys the hormonal pattern probably influence FFM and its decrease may be detected later.

MLASA syndrome: a new pathogenic mutation in the *PUS1* gene

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Myopathy-Lactic-Acidosis-Sideroblastic-Anemia (MLASA) syndrome is a rare autosomal recessive disease that involves skeletal muscle and erythroid cells. Mutations on *PUS1* and *YARS2* genes have been reported. Both genes are involved in post-transcriptional modifications of mitochondrial and cytoplasmic tRNAs. We studied a 33 years old female presenting since childhood a mild cognitive retardation and a severe sideroblastic anemia that required several blood transfusions. She developed hepatopathy, cardiomyopathy and insulin dependent diabetes. Slowly progressive and generalized muscle weakness appeared in adolescence and, at the age of 33, patient was unable to walk and showed a diffuse muscle hypotrophy with severe weakness that involves both proximal and distal muscles. Patient had normal CK, but elevated lactic acid. Brain MR showed cerebral atrophy with hyperintensity of the cortical-spinal tract and muscle MR a severe muscle atrophy with fibro-fatty infiltration. Muscle biopsy showed myopathic changes with ragged red fibers and a decrease of COX activity. *YARS2* gene study was normal, but two different mutations in the *PUS1* gene were identified: c.487delA and a novel c.884 G > A resulting in p.R295Q. This patient showed all MLASA stigmata but a classic phenotype is hard to define. Severity of cognitive, muscular and systemic involvement differ in all reported cases. Clinical and molecular findings of this patient widen genotype-phenotype spectrum in MLASA syndrome.

Toll-like receptors and innate immunity: new key players in the pathophysiology of oculopharyngeal muscular dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a late-onset muscle disease caused by short (GCN)11–17/polyalanine expansions within the polyadenylate-binding protein nuclear 1 gene (*PABPN1*). *PABPN1* plays a key role in the regulation of RNA metabolism, by modulating post-transcriptional processes including transcript stability, nuclear export and translation. Here we hypothesized that accumulation of the expanded mutant PABPN1 protein and the consequent impairment of protein homeostasis might represent an endogenous danger signal able to activate Toll-like receptors (TLRs) and innate immunity, promoting degenerative downstream processes in skeletal muscle. The analysis of mRNA transcript levels of TLR3, TLR4, TLR7 and TLR9, molecules involved in the recognition of endogenous and exogenous nucleic acids, and of the TLR-inducible cytokine interferon beta, showed their up-regulation in OPMD muscle samples compared to controls. By immunofluorescence we observed a highly positive staining for all the TLRs investigated, particularly for TLR4. TLRs were expressed on the sarcolemma or diffusely in the cytoplasm of some muscle fibers.

Overall, our findings suggest that TLRs might play a pathogenetic role in OPMD. These results might have important implications for new therapeutic approaches.

Cardiac imaging in emerinopathies: a cohort study

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The X-linked Emery Dreifuss muscular dystrophy is a clinical entity characterized by cardiac compromise, muscle weakness on a humero-peroneal distribution and contractures. We performed cardiac magnetic imaging studies on a small cohort of subjects carrying the same STA gene alteration. The cohort includes three affected males, whose cardiac compromise varies from mild to severe, and three females carriers. The pattern of heart alterations detected by heart magnetic imaging techniques is peculiar and sheds light on the likely pathophysiology mechanisms responsible for heart manifestations.

Progression of muscle histopathology but not of spliceopathy in myotonic dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is caused by a CCTG repeat within the first intron of the *CNBP* gene. Mutant transcripts are retained in cell nuclei and alter the functions of MBNL1 and CUGBP1 splicing factors leading to missplicing of several genes. To understand the molecular mechanisms that play a role in DM2 progression, the evolution of skeletal muscle histopathology and biomolecular findings have been studied in 5 DM2 patients who underwent two successive biopsies at different years of age. All DM2 patients examined show a worsening of muscle histopathology and an increase of MBNL1 sequestration and of CUGBP1 protein expression. The progressive worsening of myotonia in DM2 patients may be due to the decrease of CLCN1 mRNA observed in all patients examined. However, a worsening of alternative splicing alterations has not been evidenced overtime. These data indicate that DM2 is a slow progression disease since histological and biomolecular alterations observed in skeletal muscle are minimal even after 10-year interval. Muscle histopathological alterations evolve more rapidly than the molecular changes indicating that muscle biopsy is a more sensitive tool than biomolecular markers to asses disease progression at muscle level.

Heterogeneity of an Italian family affected with Adult Polyglucosan Body Disease

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Adult Polyglucosan Body Disease is a rare autosomal recessive leukodystrophy, affecting mainly Askenazi Jewish, due to mutations of glycogen branching enzyme gene (*GBE1*), leading to accumulation of polyglucosan bodies (PB) in central/peripheral nervous system.
We describe three affected siblings from a non-Jewish family. The proband presented distal paresthesia at the age of 55 years, followed by gait ataxia and urinary urgency. His nerve conduction study (NCS) demonstrated a sensory-motor demyelinating neuropathy. His sister developed paraparesis at the age of 52 years, complicated by neurogenic bladder. The youngest sister presented a transitory episode of orthostatic vomit and mild ataxia at the age of 53 years. In all cases, MRI showed diffuse hyperintense infra/supra-tentorial white matter abnormalities, with bulbar/spinal cord atrophy. In both sisters NCS was normal, whereas muscle biopsy showed non-specific alterations. In the proband, muscle/nerve biopsies showed PB, which prompted genetic investigation for *GBE1*: all siblings were compound heterozygous for c.1604A > G mutation, previously described, and the novel c.1064G > A.
In conclusion, common clinical signs occurred together with "atypical" ones (demyelinating neuropathy/transient symptoms), featuring a peculiar intrafamilial variability. Indeed, PB detection at muscle/nerve biopsy correlates with NCS alteration.

Prevalence of sub-sarcolemmal mitochondrial aggregates (SSMA) and age at biopsy in paediatric mitochondrial disease

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The diagnosis of mitochondrial disease (MtD) in children is challenging and muscle biopsies often lack ragged red and COX negative fibres. Instead, SSMA are thought to be more prominent and various %SSMA cut-offs (> 2%, ≤ 4%) have been proposed as markers of MtD.
By both by conventional light microscopy and a novel image analysis (IA) tool for precise quantitation, we aimed to assess the prevalence of %SSMA in muscle biopsies of patients with MtD (N = 31) and age-matched controls (CTRL) (N = 39) from 0-16 years and examine their relationship to the age at biopsy and mtDNA copy number.
We found that %SSMA was significantly lower in mtD (4%) versus CTRL (13%), it increased by 4%/year in the first 3 years of life and showed a positive correlation with mtDNA copy number. At multivariate analysis, disease group, age at biopsy and mtDNA copy number were significantly associated with %SSMA.
The lower prevalence of %SSMA in MtD and their correlation mtDNA copy number suggest that SSMA may be a good pathological marker of MtD in pediatric muscle biopsies. However, age-dependent prevalence of SSMA may limit using absolute SSMA cut-off for the diagnosis of MtD in children.

iPSC-derived neural stem cells act via kinase inhibition to exert neuroprotective effects in Spinal Muscular Atrophy with Respiratory Distress Type 1

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The aim of the study was to demonstrate that neural stem cells (NSCs) from human induced pluripotent stem cells (iPSCs) have therapeutic potential in the context of SMARD1 disease. Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an hereditary motor neuron disease caused by mutations in the IGHMBP2 gene, without a cure. We generated iPS cell lines derived from human skin fibroblasts with a

non-viral non integrating method based on the expression of reprogramming factors with episomal vectors. We differentiated iPSCs using a protocol to promote neuronal stem cells fate. The phenotype of these cells was analyzed by morphological, gene expression, and protein analysis. Finally, iPSC-purified NSCs were transplanted by intraspinal cord injection into nmd mice, an animal model of SMARD1. NSCs from iPSCs are self-renewing and multipotent and can differentiate in vitro into the three neuroectodermal lineage as well as in motor neurons. We show that upon transplantation NSCs can appropriately engraft and differentiate into the spinal cord of SMARD1 animals, ameliorating their phenotype, by protecting their endogenous motor neurons. To further evaluate the effect of NSCs in the context of human disease, we generated human SMARD1-iPSCs motor neurons that had a significantly reduced survival and axon length. Notably, the co-culture with NSCs ameliorate these disease features, an effect attributable to the production of neurotrophic factors and their dual inhibition of GSK-3 and HGK kinases. Our data support the role of iPSC as SMARD1 disease model and the translational potential of pluripotent cells for cell-mediated therapies in motor neuron disorders.

Critical illness myoneuropathy complicating akinetic crisis in parkinsonism

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Introduction. Akinetic Crisis (AC) is a life-threatening complication of parkinsonism characterized by an acute severe akinetic-hypertonic state, alterations of mental status, dysphagia, dysphonia, and dysautonomia with transient unresponsiveness to current treatment or to an increment of dopaminergic drug doses. Hyperthermia and muscle enzymes elevation frequently occur. It is frequently complicated by infections, pulmonary embolism, disseminated intravascular coagulation, and cardiac arrhythmias. Critical illness myopathy and/or neuropathy (CIMN), often occurring in intensive care settings, is primarily associated with sepsis, systemic inflammatory response syndrome, multi-organ failure, inactivity and steroid treatment.
Methods. To describe the occurrence of CIMN during the course of AC.
Results. Of 25 patients referred to our Clinic for AC in the last 3 years, three (12%) developed acute disappearance of hypertonia substituted by flaccid quadriparesis. Electrophysiological studies evidenced primary involvement of both muscle and nerve. In one patient myopathy was bioptically demonstrated.
Discussion. Although AC encompasses most of the putative risk factors for CIMN, its occurrence is difficult to recognize and was never reported. In AC patients CIMN should be suspected when hypertonia-rigidity subsides despite persistent akinesia and a bimodal pattern of CK and myoglobin increments occurs.

Pharmacogenetics of hNav1.4 sodium channel mutations causing myotonia

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Gain-of-function mutations of the Nav1.4 sodium channel are responsible for paramyotonia congenita or sodium channel myotonia. The sodium channel blocker mexiletine has received orphan drug designation in myotonia. Yet some patients show limited benefits from mexiletine due to side effects or lack of effects. We previously showed that the G1306E hNav1.4 mutant causing myotonia permanens is less sensitive to the drug in vitro, and that patients carrying G1306E can gain benefits by shifting treatment to flecainide, another sodium channel blocker, thereby opening the way toward a bench-to-bedside pharmacogenetics strategy (Desaphy et al. Neurology 2001; J. Physiol. 2004; Eur. J. Clin. Pharmacol. 2012). Here we report the case of a girl with severe myotonia associated to the new P1158L hNav1.4 mutation. The patient obtained unsatisfactory response to mexiletine and shifted treatment to flecainide with some success. Using patch-clamp, we found alteration of P1158L channel fast inactivation that can explain the myotonic phenotype. The P1158L currents were less sensitive to mexiletine compared to wild-type, while sensitivity to flecainide was not altered. This study supports our hypothesis of pharmacogenetics strategy, which we propose to extend to a larger number of sodium channel myotonias. Supported by Telethon-Italy (GGP10101).

Pilot study of serial casting of ankles in muscular dystrophy patients

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Contractures of Achilles tendons (TAs) deteriorate the performance in daily living activities of patients with neuromuscular diseases. Nocturnal use of ankle-foot orthoses (AFOs) helps to prevent the progression of deformities and to obtain optimal position of the joint. In clinical practice ankle serial casting is used to reduce TA contractures and to allow an improving in AFOs fitting, however only scanty reports focused on these aspects. The aim of this work was to assess the effect of TAs' serial casting on: 1) patient's perspective (using a self reported questionnaire), 2) joint physical examination (range of Motion (ROM)) and 3) functional performances (six minute walking test (6MWT)) in ambulant patients affected by Duchenne muscular dystrophy (DMD) and limb gird muscular dystrophy (LGMD) 2A. The protocol included three casting five days apart and was proposed to ambulant patients with contractures < 35°. After the cast removal on day 5 and 10, the new cast was reapplied with the TA on a stretch. We included 12 patients (10 DMD, age range: 4-12 yrs, 2 LGMD 2A). Our results showed in

all patients a significant improvement of ROM of ankles, in ten out of twelve patients a reported improvement of mobility and autonomy (questionnaire). Only the youngest patients had an improvement at 6MWT. The procedure has been well tolerated by all patients, no adverse events have been reported during the procedures. All patients received indication of daily stretching of TA and use of AFOs after treatment. Although further studies will be required to evaluate the effect of this procedure in a larger cohort, our results suggest that serial casting may be a valid alternative to surgery, avoiding therefore the needed immobilization.

Symptomatic heterozygosity due to definite GAA mutations, in late onset Pompe disease

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Genotype and phenotype are reported of 14 members of the third generation of a late-onset Pompe disease family which counts 36 individuals. Clinic, laboratories and GAA enzymatic and genetic studies disclosed widespread myalgias and low back pain as well as mild weakness of pelvic girdle muscles in 5 individuals (3F, 2M; aged 24 to 30 years) 3 of whom had slight increase of CPK. Symptoms onset was during the second decade of life. GAA enzyme activity ranged 2-4µmol/h/L in all patients. Direct sequencing of the GAA gene carrying the mutations already identified in their parents, disclosed the R40X mutation in the 5 symptomatic individuals whereas the splicing mutation c.2647-7G > A was found in the remaining 9 who did not show any symptom of neuromuscular disease.
Although the Pompe phenotype is by definition due to two mutations in the GAA gene, rare symptomatic heterozygous have been reported. The most relevant findings of our study are the identification of several symptomatic heterozygous in the same family who all share the identical GAA mutation. Thus suggesting that specific deleterious mutations even in heterozygous may address the prognosis. These symptomatic carriers, represent a unique model to identify factors modifying the phenotype.

Lipodistrophy and progeroid features associated with mutation of DNA polymerase δ (POLD1)

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Mandibular hypoplasia, Deafness and Progeroid features with concomitant lipodystrophy and insuline resistance, define a multisystem disorder named MDP syndrome. MDP has been recently associated to heterozygous microdeletion in POLD1 gene affecting the active site of DNA polymerase δ.
Here we report a case of a 13-yr-old girl, second-born of non consanguineous parents, who showed retarded growth, anemia and xerotic skin at age 1. Other early clinical features included sensorineural deafness at age 8, and a notable subcutaneous fat loss from her extremities with accumulation at abdomen level and insuline-resistance. At last neurological examination she presented generalized hypotonia and severe muscular wasting with joint contractures. She also has facial dismorfisms including, micrognathia, low-set and small ears. A muscle biopsy performed to exlude a congenital myopathy was normal. Molecular analysis of genes responsible for laminopathies and lipodystrophy such as LMNA and BSCL2 excluded pathogenetic mutations.
We analyzed the POLD1 gene and identified the heterozygous in frame deletion (c.1812_1814delCTC, p.S605del), previously detected in the three patients with MDP syndrome.
The case underlines the genetic heterogeneity of patients with progeroid features and lipodistrohy and the overallapping clinical presentation with congenital myopathy.

Exploring mitochondrial dysfunction in *CAPN3* related myopathy

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Limb-girdle muscular dystrophy type 2A (LGMD2A) is the most frequent form of recessive LGMD worldwide and it is caused by a defect of calpain-3 gene. Calpain-3 is a muscle specific, calcium dependent, multi-substrate cysteine protease. The exact pathomechanism underlying muscle damage due to calpain-3 deficiency, remains largely unclear. Animal model (CAPN3 KO mice) exhibits morphological and biochemical evidence of mitochondrial abnormalities in muscle, including irregular distribution of mitochondria and reduced in vivo mitochondrial ATP production. These findings have never been reproduced nor quantified in patients' tissue.
In this work we investigated pathological effects of calpain-3 mutations in myoblasts from LGMD2A patients, in terms of a putative mitochondrial dysfunction. In particular we examined the activity and amount of respiratory chain (RC) enzymes, cellular ATP level and ROS production.
Routine histochemical stains for oxidative metabolism in muscle biopsies revealed the typical aspect of subsarcolemmal accumulation of mitochondria. Measurement of RC enzymes revealed reduction of complex I and IV in one case and of complex III in another case, whereas the immunodetection pattern of the RC complexes was within normal values.
Luminometric measurement of ATP in patient's cultured myoblasts showed a specific reduction of ATP content compared to control cells in the presence of 2-deoxyglucose and pyruvate, a condition that supports only mitochondrial ATP synthesis.
We also observed a statistically significant increase of ROS production in patients fibroblasts after a short term H2O2 treatment.

Taken together these data support evidence for a secondary mitochondrial damage with energy production defect and increased oxidative stress also in human calpainopathy.

Novel mutations in CHKB gene in the first Italian case presenting with mental retardation, congenital deafness, seizures and muscular dystrophy

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CHKB gene encodes the choline kinases B, an enzyme that catalyze phosphorylation of choline by ATP, the first step of the enzymatic pathway for biosynthesis of phosphatidylcholine.
Homozygous or compound heterozygous mutations in the CHKB gene have been recently associated with a form of muscular dystrophy characterized by early-onset muscle weakness and mental retardation. Interesting muscle biopsy shows peculiar enlarged mitochondria that are prevalent toward the periphery of the fibres.
We describe a case from Italy presenting at birth with hypotonia and congenital deafness in which we have identified two novel mutations in the CHKB gene (c.140_146del p.Arg47Pro fs*21 in exon 1 and c.1066_1067delTG p.Trp356Val fs*72 in exon 11). The patient now age 9 is mentally retarded with particular impairment of speech. She also suffers of epileptic seizures since age 6. Brain imaging showed no structural change. At last neurological examination she presented proximal weakness and wasting of limb muscles, waddling gait and hyperlordosis. Hypertricosis was also present. Muscle biopsy was consistent with previous CHKB cases, showing necrotic and regenerating fibers, endomysial fibrosis, and abnormal COX staining with reduction at the centre of the fibres and abnormally large mitocondria at the periphery of the fibres.
Our case confirms the effect of CHKB defect in human pathology and extends the clinical phenotype.

Myotonic Dystrophies in a National Italian Registry: IRCCS Policlinico San Donato - start up

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Patients' enrolment in the National Registry for DM began on November 30th, 2013 at the Coordinator Center, IRCCS Policlinico San Donato.
Patients' compliance was adequate, they were highly willing to take part in the project and to undergo clinical evaluations. Website accessibility was good and easy and data entry was simple and clear. To date, we have enrolled a total of 80 patients, 70 of whom completed the data collection sheets with data entry required; all the data were validated. Thirty-one medical sheets were completed and validated too. The data collected so far have been analyzed and processed, highlighting results in line with the literature. The oncoming involvement of several other italian Neuromuscular Centers in data collection will permit to join a greater number of patients.

A *DOK-7* myasthenic syndrome: a treatable disorder?

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Mutations in *DOK-7* gene are responsible for postsynaptic Congenital Myasthenic Syndromes (CMS). In *DOK-7* related CMS cholinesterase inhibitors treatment is usually not effective But salbutamol, a selective β2 adrenergic agonist, has been associated with improvement. We studied a 32 years old male presenting since childhood with diffuse muscle hypotrophy and weakness, ligamentous hyperlaxity, kyphoscoliosis, ptosis, faccial weakness and arched palat. He showed hypotonia and cianosis at birth, and laryngospasm and asphyxia episodes during the first year of life.
Muscle weakness did not progress, but few day-long symptoms worsening was reported, occasionally related to infections. Laboratory findings were normal, including thyroid function test, CK, anti-AchR and anti-Musk antibodies. Brain MR, ECG and echocardiogram were negative. Respiratory function tests showed severe restriction. Electromyography revealed myopathic changes and a decreasing action potential amplitude at repetitive nerve stimulation test. Patient underwent two muscle biopsies: at age13 and 32. Both, showed polydimensional fibres and central hyporeactive cores at oxidative reactions.
Diagnosis of CMS was confirmed by detection of heterozygous *DOK-7* gene mutations: 1124_1127 dupTGCC and c.480C > A (p.Y160X). After diagnosis salbutamol treatment was initiated with significant clinical improvement: patient was able to climb stairs without support and walk unassisted. The exact mode of action of salbutamol in DOK7 CMS is unknown; however skeletal muscle β2-receptor stimulation may results in numerous effects on muscle function including muscle hypertrophy and transition of slow to fast fiber type.

Heart arrythmia in genetically confirmed facioscapulohumeral muscular dystrophy

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Background. Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy with prevalence

for 1:20000. Clinically significant cardiac disease is uncommon in FSHD but the heterogeneous clinical observations reported to date have shown that the heart alterations are possible in FSHD.
Patients and methods. 85 FSHD patients, aged between 14-80 years, attend our Institute. The diagnosis of FSHD was made on a clinical basis and was confirmed by genetic tests. 58 of these undergo annual clinical heart examination, 12-lead electrocardiography, 24 hour Holter monitoring and echocardiography.
Results. 9 patients (15%) had conduction defects or arrhythmia, in the absence of cardiovascular risk factor and with normal left ventricular function (3 cases with juvenile onset disease).
Supraventricular arrhythmias were detected in 5 cases. The AV node conduction was abnormal in 3 patient and infranodal conduction was abnormal in one. These heart abnormalities were symptomatic in 3 patients (palpitations associated with supraventricular arrhythmia). Two cases needed antiarrhythmic drug therapy. In addition, 6 patients (10%) manifested minor ECG abnormalities.
Conclusions. Data confirm the evidence of cardiac involvement in FSHD (in agreement with literature data of prevalence around 5-27%) and suggest regular cardiac follow-up in FSHD. Therefore, results support the hypothesis of possible conduction degeneration in FSHD and suggest a cardiac longitudinal study.

'Dropped head' in a case of recessive Oculopharyngeal Muscular Dystrophy: description of an unusual clinical phenotype

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Recessive cases of OPMD are rarely reported. We report a case of a 69-years-old woman admitted to our Department for progressive head ptosis and dysphagia begun two years before. She and her two sisters received the diagnosis of schizophrenia during youth. No other symptoms had been reported in the other members of her family. Neurological examination, other than severe weakness of neck extension with dropped-head and dysphagia, showed mild weakness of orbicularis oculi and proximal limb muscles. Electrophysiological assessment showed myopathic changes in weak muscles and neuromuscular transmission disorders were ruled out. CK levels were mildly elevated (350 UI/L). Anti-AChR and anti-MuSK antibodies were negative. Neck MRI showed cervical paraspinous muscles myopathic changes. Deltoid muscle biopsy showed increased variability in fibers size with some small angular dark stained fibers on NADH, with predominance of type I fibers and the presence of rimmed vacuoles in many fibers. No inflammatory infiltrates and MHC-I overexpression was found. Molecular study for Oculopharyngeal Muscular Dystrophy (OPMD) was performed and an homozygous expansion of GCN11 repeats in the PAPBN1 gene was found.
The elective involvement of the extensor muscles of the neck with "dropped head" is an unusual phenotype for OPMD. Probably, clinical phenotypes of recessive cases of OPMD are more heterogeneous than dominant ones and muscle biopsy findings. Muscle biopsy can be helpful to identify patients without familiar history who should be genetically tested for OPMD.

'Cap myopathy' in a family with unusual clinical phenotype

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The "Cap Myopathies" are rare congenital myopathies characterized by the presence of structures peripherally located in the muscle fibers, mainly composed of material containing thin and thick filaments, fragments of Z lines and cellular debris. We report the clinical and morphological features of the muscle biopsies from two patients (mother and daughter, P1 and P2 respectively) with an unusual clinical phenotype. Both patients showed a complex clinical syndrome concerning 1) signs of congenital myopathy, characterized by the delay in the motor milestones, exercise intolerance and mild weakness in the lower limbs, 2) dysmorphic features with retractions (short neck, micrognathia, retraction of the fingers) and 3) the pseudo-paralytic episodes, most evident in P2. In addition, the mother (P1) showed cardiac rhythm disturbances requiring a pacemaker implantation at the age of 56.
Muscle biopsies performed at the age of 26 years (P1) and 59 years (P2) showed the presence of areas sousarcolemmali evocative of "Caps" structures, variability of the of the fiber size and a mild predominance of fibers type1. Ultrastructural analysis confirmed the presence of peripheral, well demarked, subsarcolemmal areas, corresponding to the type of "Caps".
None mutation of known genes associated with the "Cap Myopathies" (TPM2, TPM3, ACTA1) and Anderser - Tawil Syndrome (KCNJ2) has been found. For these patients an exome sequencing study is now in progress.

P2X antagonist oxidase-ATP (oATP) treatment in alpha-sarcoglycan null mice

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Limb-girdle muscular dystrophy 2D (LGMD2D), caused by mutations in the gene encoding alpha-sarcoglycan (a-SG), is a rare disorder characterized by progressive weakness and degeneration of skeletal muscle. Pathological features of muscle biopsies from these patients show myofiber degeneration and necrosis, endomysial fibrosis, and reactive inflammatory response. In this scenario, extracellular ATP (eATP) molecules released from the cytosol of dying cells, contribute to the initial phase of the immune response

and later to the amplification of the inflammasome reaction. Intriguingly, a-SG binds eATP and displays an ecto-ATPase activity, thus controlling eATP concentration at the surface of cells expressing P2 receptors, attenuating the magnitude and/or the duration of eATP-induced signals. alpha-SG deficiency leads to membrane instability and decreased ecto-ATPase activity causing increased eATP concentration. Excessive eATP causes protracted P2X7 activation with alteration in muscle intracellular calcium homeostasis as well as recruitment of inflammatory cells.
We performed a systemic treatment with P2X antagonist oxidase-ATP (oATP) in mice deficient in α-sarcoglycan (Sgca-null mice), a model for human LGMD2D, and we analyzed the clinical and histological parameters of muscle disease progression. We observed an improvement in the forelimb muscular strength and in the diaphragm muscle morphology with a reduction of the number and areas of the reactive inflammatory infiltrates.

Pharmacological purinergic antagonism improves muscle dystrophy in *mdx* mice

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Extracellular Adenosine-5'-Triphosphate (eATP) plays a crucial role in the priming of immune response and directly regulates calcium homeostasis in muscle cells.
Primary muscle cells express various eATP-purinergic (P2X) receptor subtypes; significant up-regulation of P2X7 occurs in skeletal muscle from *mdx* mice as well as in Duchenne Muscular Dystrophy patients.
We analyzed the consequences of P2X7 pharmacological inhibition in *mdx* mice through a systemic treatment with the irreversible P2X antagonist periodate oxidase-ATP (oATP).
Blockade of P2X receptors improved muscular function and morphology and enhanced myofiber regeneration in *mdx* mice. The beneficial effect exerted by purinergic blockade was associated with a reduction of the number and area of the inflammatory infiltrate, a decrease of muscle levels of Il1 and Il6, a decrease of muscle infiltrating CD3+ T cells with a parallel 2-fold increase of FOXP3, a marker of regulatory T (Treg) cells.
oATP inhibitory effect on innate and adaptive immunity translated into a decrease of the expression of the pro-fibrotic factors TGFβ and Connective Tissue Growth Factor (CTGF).
In conclusion, purinergic antagonism led to a functional and histological improvement of the dystrophic process bound to dystrophin deficiency. This effect was mediated by a double action on the inflammatory response: down-regulation of the innate inflammasome pathway and induction of Treg cell population.

MRI of scapular muscle involvement in facioscapulohumeral muscular dystrophy (FSHD)

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FSHD is one of most common muscular dystrophies, characterized by extreme variable degree of facial and scapular muscle involvement. Clinical and genetic determination may be sometimes difficult, as genetic analysis is not always definitive and other similar muscle disorders, as scapulo-peroneal syndromes, are still lacking of molecular characterization. Here we evaluated the MRI of scapular muscles in 30 patients with clinical and molecular diagnosis of FSHD, in order to determine any specific pattern of muscle involvement. A Philips 1.5T-scanner was used and two examiners blindly evaluated fourteen muscles per patient. Muscle fatty replacement and atrophy were measured by using a four-point semi-quantitative visual scale. We observed that the most frequently and fatty replaced muscles were trapezius and serratus anterior, followed by teres major and pectoralis major. Instead, sopraspinatus, infraspinatus and scalenus muscle were relatively spared. Asymmetric scapular muscle involvement was observed. Interestingly enlargement of brachial plexus nerve trunks was observed in 33% of patients. Scapular muscle MRI was very sensitive to detect the selective muscle involvement of FSHD patients and useful to assess the involvement of non-clinical testable muscles. MRI imaging may be considered a potential tool to differentiate FSHD from other muscular dystrophies to drive the molecular analysis.

Reference values of fat infiltration and residual muscle volume for morpho-functional predictive behaviour in Duchenne Muscular Dystrophy: a longitudinal MRI study

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Purposes. i) quantitative assessment of fat infiltration (FI) and residual muscle volume (volume of muscle index, VMI) in lower limbs of children with Duchenne Muscular Dystrophy (DMD) by Magnetic Resonance Imaging (MRI); ii) establishment of a morpho-functional relationship between MRI values and functional outcomes.
Materials and Methods. 26 children with DMD were longitudinally assessed by lower limb 3T MRI and functional tests (Gowers, 10-meter time, North Star, 6-minute walking test). 5 age-matched healthy controls were also examined. A total of 85 MRI studies were performed. T1 Signal Intensity Ratio (SIR) of muscle and nearby fat was used to quantify FI. Muscle volume was measured by applying thresholds on T1-weighted images, and results were normalized for the whole muscle volume to obtain a VMI.
Results. SIRs and VMIs were significantly different from control values, except for sartorius and gracilis. Age-related curves with percentile values were calculated for SIRs and VMIs. SIRs and VMIs significantly correlated with all clinical measures, and could reliably predict functional outcomes.
Conclusion. SIRs and VMIs are objective measures that can improve DMD staging. MRI-based curves display the multistep

muscle involvement and provide reference values of FI and residual muscle for both clinical and research settings.

Magnetic Resonance of the lower limbs in Duchenne Muscular Dystrophy: longitudinal semi-quantitative study of muscular degeneration

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Purpose. Semi-quantitative assessment of Duchenne Muscular Dystrophy (DMD) progression in lower limb muscles by Magnetic Resonance Imaging (MRI).
Materials and methods. 26 DMD patients were longitudinally studied with 3T MRI of the lower limbs (72 MRI studies with T1, T2, STIR, 3DTHRIVE sequences). Two neuroradiologists evaluated 44 muscles per study, visually scoring the presence/absence of fat infiltration (FI), atrophy, hypertrophy and oedema. The severity of FI was also scored (0-3).
Results. In 6/7-year-old patients, FI was mainly present on gluteus maximus/medius(100%), adductor magnus(86%), rectus femoris(42%) and biceps(42%). At the age of 8/9, FI worsened and involved vasti(68%), gastrocnemii(72%), soleus(50%), and peronei(54%). In 10-12 year-old patients FI was also present on gluteus minumus (62%), obturators(62%), semitendinosus(62%), sartorius(62%) and gracilis(25%). The most frequently affected muscles were also the most severely scored in terms of FI.
Atrophy was noted since the age of 8/9 on gluteus maximus(18%), quadriceps and biceps(18%), with sparing of gracilis(0%), sartorius(0%) and calf muscles, which were hypertrophic since the age of 6/7(42%). FI, atrophy and hypertrophy were always present and progressing, whereas oedema was inconstant over time and mostly appeared in hypertrophic muscles.
Conclusion. MRI displays a typical proximal-to-distal pattern of muscular involvement with sparing of gracilis and sartorius. This finding can be useful in DMD diagnosis and staging.

Neuromuscular diseases in pregnancy: multidisciplinary management of elective c-section in 7 women

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Background. Neuromuscular diseases can have a tremendous impact on pregnant women ad affect offspring. Patients and Healthcare providers need to be fully aware of the potential complications they and their babies may encounter.
Objective. We describe the multidisciplinary management of 7 women affected by different neuromuscular disorders (NMD) and the outcome through a multi-step and multi-professional organization.
Methods. 7 women (LGMD, n = 3, SMA type II, n = 1, ALS, n = 1, axonal polyneuropathy, n = 1) with variable degrees of disability and skeletal deformities were subjected to complete neurological, respiratory and cardiological assessments. Grandround discussion with gynaecologists and anaesthesiologist occurred in each case. Before the procedure all patients were subjected to respiratory training (air stacking, cough-assist machine and preventive NIV)
Results. Fetal assessment was normal in all but 1 patient, in whom a congenital cardiac problem was found. Elective Caesarean delivery and spinal anesthesia was possible in all but 1. Clinical outcome was positive for all mothers.
Conclusions. Appropriate multidisciplinary management of neuromuscular patients in childbearing age including family counselling, gestation and delivery in an adequate setting is mandatory. A Centre fully dedicated to NMD, such as NEMO, permits to provide individualized care plan, tailored to meet the specific needs of this new growing population.

Isolated camptocormia – a two cases report

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Camptocormia may be a manifestation of disorders of the CNS, such as in Parkinson disease (PD); more rarely it is due to myopathies or motor neuron disease. The morphological changes associated with camptocormia are still under debate and the pathophysiology is unknown also in PD in which myopathic changes have been observed. A muscular pathogenesis of isolated axial myopathy was found in rare cases of DM1, IBM, and nemaline myopathy.
Here, we describe two patients with muscular disease manifesting with isolated camptocormia.
The first patient, a 65 years old man, initially presented weakness of the truncal muscles reaching fully developed camptocormia in about 18 months. CTscans and MR imaging showed progressive para-spinal dorsal-lumbar muscles atrophy and adipose tissue substitution. Paraspinal muscle biopsy revealed neurogenic aspects with central cores like recently observed in bent spine syndrome described in families from North Europe with a defective Ryr gene.
The second patient, a 70 years old man suffering from neoplasm, presented weakness and mialgia of the axial muscles. Paraspinal muscle biopsy revealed a myositis. To date, only few cases of camptocormia secondary to idiopathic inflammatory myopathies have been described.
Our data emphasize the role of primary muscle disorder in the etiology of camptocormia and the need to consider these common myopathies as a cause of the paraspinal muscle weakness.

Stepping gait: when should ankle foot orthosis be prescribed? preliminary data in a cohort of patients with DM1

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Background. Patients with Myotonic Dystrophy type 1 (DM1) often complain of gait instability. This is often related to ankle dorsiflexor weakness and steppage. Ankle foot orthosis are thus prescribed.
Aims. To determine: (i) the frequency and severity of proximal and ankle dorsiflexor weakness in a cohort of DM1 patients complaining of gait instability; (ii) if foot-leg orthosis can improve gait pattern and reduce falls.
Methods. 26 patients with classical DM1 phenotype (mean age: 46.26 ± 12 were subjected to: Manual Muscle Strength Testing (modified MRC scale) and performed the 6 minute walking test (6MWT). Ankle foot orthosis (AFO) were applied if ankle dorsiflexor weakness was ≤ 4 MRC. The 6MWT was repeated and subjective assessment of efficacy was accounted for with self-reported measures. To obtain additional information on gait parameters we performed a quantitative spatio-temporal analysis using BTS Bioengineering (Milan) G-Walk.
Results. Ankle foot orthosis improve walking distance (0-20 meters) in a minority (1/3) of our cohort of patients. G-walk analysis confirms 6MWT results. Despite this, patients who accepted AFO have positive perspective of efficacy.
Conclusions. Detailed analysis of gait including assessment of vestibular and proprioception causes, central and cardiovascular causes is mandatory to target management and tailor physiotherapist treatment and care in patients with DM1.

FOR-DMD: Double-blind randomized trial to optimize steroid regime in Duchenne Muscular Dystrophy (DMD)

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The FOR DMD study ("Find the optimum corticosteroid regime for DMD") is open to recruitment. The study compares benefits and side effect profile of three commonly prescribed corticosteroid regimes: daily prednisolone, daily deflazacort and intermittent prednisolone (10 days on and 10 days off). The study aims to enrol 300 steroid-naïve DMD boys, between the age of 4 and 8 years to be followed according to the current care recommendations over three to five years.
The FOR DMD study will provide information to allow families and clinicians to make informed decision about dosage and regimens of corticosteroids with regard to efficacy and tolerability. By standardizing corticosteroid treatment and prevention of side effects, the FOR DMD study will provide a more stable and uniform baseline which will impact the assessment and success of future studies with new investigational therapies. Additionally, the FOR DMD study is a unique opportunity to further investigate phenotype-genotype correlations, bone health, behavioural issues and the correlation of functional change with quality of life.
The study set up has been a long process with extended delay in study site activation and recruitment. This has been largely due to issues related to funding, approval and contract agreements between different countries and sites. This has underlined the lack of harmonisation in the regulations for international academic studies between US and Europe and among different European countries which need to be urgently addressed to ensure timely development of translational research in this field.
Regardless, the FOR DMD study is currently open with 39 sites actively recruiting in 5 different countries: Italy (6 sites), UK (8 sites), Germany (5 sites), US (15 sites) and Canada (5 sites). 112 boys have been screened for the study and 80 have been already enrolled and are taking study medication. We aim to actively continue recruitment with the aim to reach the enrolment target within the first half on 2015.

Functional characterization of novel CLC-1 mutations associated to myotonia congenita from Italian families

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Myotonia congenita (MC) is caused by loss-of-function mutations in the skeletal muscle CLC-1 chloride channel leading to impaired muscle relaxation after forceful contraction (Desaphy et al. Exp Neurol, 2013). We chose to functionally characterize five novel mutations, selected among the largest cohort of Italian families reported to date, on the basis of the associated clinical phenotype and position in the protein 3D-structure (Brugnoni et al. J Hum Gen, 2013). The dominant mutations (F484L, L198P and L520P) are located outside the common hot-spot for dominant genetic variants, instead residing within the channel pore, whereas the recessive mutations (V640G and L628P) occur in the C-terminal domain close to the proposed ATP binding sites. Whole cell patch-clamp recordings from wild-type and mutant chloride channels expressed in HEK293 cells revealed a huge reduction of current amplitude for F484L, L198P and V640G. In addition the open probability for F484L and L198P is dramatically right shifted compared to wild-type, which likely contributes to impaired muscle repolarization. Further experiments are required to clarify the dominant inheritance for mutations located outside the dimer interface, to address the mechanism of ATP modulation, and to clarify genotype-phenotype correlations. Pharmacogenetics studies are in progress. This work was supported by the Italian Ministry of Health (Grant No. 1580433).

Modulation of neuronal nitric oxide synthase by the isoflavone genistein promotes muscle regeneration in mdx mice

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Genistein has been reported to have pro-proliferative effects, promoting G1/S cell phase transition through the induction of cyclin D1, anti-apoptotic properties and increases nNOS expression. Apoptosis and the exhaustion of muscle regenerative capacity are implicated in Duchenne muscular dystrophy (DMD) pathogenesis and therefore are relevant therapeutic targets. Dystrophin lack in DMD, causes neuronal nitric oxide synthase (nNOS) membrane delocalization which in turn promotes functional muscle ischemia, and exacerbates muscle injury during exercise. We tested whether genistein could have beneficial effects on morphological, biochemical and functional pattern in *mdx* mice. Five-week old *mdx* and wild type mice received for five weeks genistein (2 mg/kg i.p. daily) or vehicle. Genistein treatment: 1) reduced muscle necrosis and enhanced regeneration with an augmented number of myogenin-positive satellite cells and myonuclei; 2) increased cyclin D1 and nNOS expression; 3) showed an antiapoptotic effect, modulating the expression of BAX and Bcl-2; 4) increased forelimb strength (+ 31%, $p < 0.01$) and strength normalized to weight (+ 28%, $p < 0.01$). Our results suggest that this isoflavone might restore the altered nNOS expression and increase regeneration modulating cell-cycle and apoptosis. Further studies with longer time treatment or using different experimental approaches are needed to investigate the underlying mechanisms of such encouraging results.

New mutation in CHKB gene in two Italian patients with congenital muscular dystrophy and enlarged mitochondria

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Congenital Muscular Dystrophies (CMD) are a heterogeneous group of autosomal recessive disorders characterized by infancy onset of muscle weakness, CNS and variable heart involvement with dystrophic changes at the muscle biopsy. Recently CHKB (choline kinase B) gene, which participates to the biosynthesis of the major membrane phospholipid phosphatidylcholine, has been associated to congenital muscular dystrophy and cardiomyopathy in 15 patients. We describe two sisters from unrelated healthy parents presenting since the first month of life with psychomotor delay, cognitive impairment, ichthyosis, muscle weakness and hypotonia. The older sister died at 10 years because of a severe dilatative cardiomyopathy, while the 8 years old sister still do not present any heart involvement. Electron microscopy performed in patients' muscle biopsy and in the cardiac tissue of the died girl showed enlarged, peripheric mitochondria, associated with some dystrophic features; respiratory chain activity was normal. We identified a homozygous deletion delTTTG 565_568, p.L188frx7 in CHKB gene in both patients. We may hypotize that the anomalous biosynthesis of phosphatidylcholine could alterate the mitochondrial membrane potential, producing the peculiar morphological aspect of mitochondria in muscle and heart. In conclusion we describe the first Italian patients carrying a new mutation in CHKB gene.

Genetic analysis of non-dystrophic myotonias in Italian patients

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Non-dystrophic myotonias (NDMs) are a heterogeneous group of skeletal muscle diseases presenting myotonia as a main feature. NMDs are caused by mutations in *CLCN1* and *SCN4A* genes, coding for the chloride (ClC-1) and sodium ($Na_v1.4$) muscle channels respectively. NMDs comprise autosomal dominant (Thomsen) and recessive (Becker) myotonia congenita, autosomal dominant paramyotonia congenita and sodium channel myotonia.
We describe the genetic screening of 194 subjects screened at our laboratory in the last 7 years (2007-2014). We achieved a genetic diagnosis in 106 subjects (54.6%): 73 patients (68.6%) presented with *CLCN1* mutations, whereas 33 patients showed mutations in the *SCN4A* gene (31.4%, of which 33.3% paramyotonia congenita and 66.6% sodium channel myotonia). 34.2% of *CLCN1* patients had Thomsen myotonia, 54.8% Becker, and in 10.2% only one allele was determined. This screening revealed 24 new mutations increasing the knowledge of genetics of NDMs and confirming the need for tight collaboration between clinicians and geneticians in this expanding field.

20% subcutaneous immunoglobulin HIZENTRA® in inflammatory myopathies: the experience of Ancona

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Introduction. Polymyositis and Dermatomyositis are chronic immune-mediated systemic diseases which mainly affect skel-

etal muscle. The best know diagnostic criteria are the Bohan and Peter's criteria. Among immunomodulatory treatment options, the role of intravenous Ig (IVIg) is that of an add-on therapy. However, the use of IVIg is associated with high care cost (need of venous access, hospitalization) and poor quality of live for patients (adverse systemic effects, absence from work). The beneficial effect of SCIg administration (as add on treatment) in these inflammatory myopathies, due to a different way in modulating pathogenic immune response from IVIg, has been previously documented.

Material and Methods. We describe seven patients with a new onset or recently relapsed PM or DM in whom 20%SCIg (Hizentra®) has been add to glucocorticoid therapy using a standardized protocol of infusion consisting of the administration of a weekly dose of 0.2 g/kg/week of SCIg. Mean follow-up, after the treatment start, was 18 ± 3 months.

Results. In all patients an improvement of muscle strength, assessed by MRC score, and of Rankin modified scale, have been documented; a decrease of serum CK levels was also recorded. It had been also possible to decrease the daily glucocorticoid dose. Adverse systemic events or local reactions in the infusion sites have been not observed.

Conclusion. Hizentra ® is an effective and well tolerated add on treatment option in active inflammatory myopathies. It reduces the risk of adverse effects and improve patient's quality of life.

Muscle channelopathies in a large cohort of Italian patients

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Skeletal muscle channelopathies are rare diseases, including non-dystrophic myotonia and periodic paralysis, which are associated with a great inter- and intrafamilial phenotypic variability, making challenging genotype-phenotype correlations. Hence studies on large populations of patients are needed.

We included patients referred to Carlo Besta Neurological Institute molecular laboratory with a clinical diagnosis of periodic paralysis or nondystrophic myotonia and mutated in CLCN1, SCN4A, KCNJ2 or CACNA1S.

We investigated 301 patients, among which 195 (64.3%) patients mutated in CLCN1 gene, 74 (24.6%) in SCN4A, 28 (9.3%) in CACNA1S and 4 (1.3%) in KCNJ2. We found 22 novel mutations: 8 in CLCN1 gene, 13 in SCN4A and 1 in CACNA1S. All the mutations detected in SCN4A, CACNA1S and KCNJ2 genes were missense, except for an unreported 9-nucleotide deletion in SCN4A. On the contrary CLCN1mutations were missense in 131/195 (67.2%) patients and remaining cases showed nonsense, splice site or deletion mutations.

Our study confirms genetic heterogeneity of muscle channelopathies, although a relatively small number of mutations is responsible for most of the cases.

Sport therapy and nutritional supplementation in a case of Facioscapulohumeral dystrophy

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Introduction. We describe the effects of 6mo exercise therapy and nutritional supplementation in a 43-year-old woman severely affected by Facioscapulohumeral dystrophy (FSHD).

Methods. Resting energy expenditure and the VO2peak were calculated by indirect calorimetry and submaximal handbike incremental test. Maximum voluntary contraction (MVC) of elbow flexors, elbow extensors, shoulder abductors groups was calculated with a dynamometer. Body composition was analyzed with Bioelectrical Impedance Analysis (BIA).

Endurance training consisted in 10min up to 20min at 90% VO2peak with assisted handcycling equipment 2 times/wk. Strength training consisted in 2 series of 8 repetition for each district with elastic band (2times/week, green Thera-band®). Respiratory training consisted in isocapnic hyperpnea (2min, 4 times/wk, 50% vital capacity, 20/min). Supplements were: Branched Chain Amino Acid Mixture (BCAAem, BigOne Professional Dietetics, 0.1gr/kg/day) and creatine monohydrate (CreaATP Syform, 0.1gr/Kg/day) plus conjugated linoleic acid (CLA Syform, 2.4gr/day).

Results. An improvement was observed in terms of cardiorespiratory fitness and body composition. Additionally we observed steadiness of respiratory volumes.

Conclusion. A mixed exercise program combined with nutritional supplementation can be safely used with beneficial effects in selected patients with FSHD.

The National Registry of Limb Girdle Muscular Dystrophy: clinical and molecular characterization of a sample of 466 Italian patients

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Limb girdle muscular dystrophies (LGMD) are highly heterogeneous disorders characterized by predominant limb girdle weakness. Molecular analysis and clinical-genetic correlations are fundamental for genetic counselling, definition of natural history and insight into pathogenesis.
We collected detailed clinical, biochemical, histological and molecular data of 466 Italian LGMD patients, belonging to 8 neuromuscular Italian centres.
Among them 309 patients are molecularly defined, 111 (24%) are still un-diagnosed and 46 (10%) carry heterozygous mutations in genes determining autosomal recessive forms. Relative frequency was as follows: 5.5% LGMD1B, 11% LGMD1C, 25.2% LGMD2A, 27% LGMD2B, 9.2% LGMD2I, 9.1% LGMD2D, 6% LGMD2E, 4% LGMD2C, 2.1% LGMD2L, 0.3% LGMD2F, LGMD2R (0,3%) and LGMD2S (0,3%). Onset spans from the first decade to adulthood; LGMD2E being the most precocious (6.2 ± 5.3 years) and LGMD2L the latest (36.6 ± 7.1 years). Creatine-kinase values were generally increased, especially in sarcoglycanopathies, LGMD2B, LGMD1C. Cardiomyopathy was more frequent in LGMD1B (100%), LGMD2E (47%) and LGMD2I (50%) and restrictive pulmonary involvement in LGMD2I (53%) and LGMD2E (47%). 30% of patients was wheelchair-bound.
Overall this study defined the relative frequency of Italian LGMD and improved the knowledge about clinical, morphological and molecular spectrum as far as their natural history. Furthermore the study of undiagnosed patients will potentially lead to identification of new LGMD causative genes.

ISPD mutations account for a small proportion of Italian Limb Girdle Muscular Dystrophy cases

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Isoprenoid synthase domain containing (ISPD) gene mutations are a recently described cause of Limb Girdle Muscular Dystrophy (LGMD) associated with defects in α-dystroglycan (α-DG) glycosylation. Since now few cases have been described and the frequency in Italian population is unknown.
We studied α-DG expression and ISPD gene mutations in 44 undiagnosed patients selected from a cohort of 180 Italian LGMD patients, which included 9 LGMD2I subjects showing α-DG reduction.
At immunohistochemical (IHC) analysis, performed in 32/44 undiagnosed probands, 29 subjects had normal α -DG expression, 2 partial deficiency, 1 complete absence. ISPD direct sequencing, performed in patients showing α -DG reduction, revealed in the last subject two novel heterozygous mutations: c.836-5T > G (leading to Exon6 deletion with production of an out-of-frame transcript) and c.676T > C (p.Tyr226His). This 43 years-old man presented sural hypertrophy and tiptoes walking at the age of 6 years and developed proximal progressive weakness at the age of 30 years, associated to severe respiratory insufficiency.
Overall ISPD mutations are a rare cause of LGMD in our population accounting for 0.7% of the entire cohort (FKRP mutations represent 5%). However, considering the increasing number of genes involved in α-DG glycosylation, α-DG IHC should be always performed in un-diagnosed LGMD, in order to detect reduction to be further investigated.

Mitochondrial DNA single deletion and related phenotypes: data of the Italian Network of Mitochondrial Diseases

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Mitochondrial DNA (mtDNA) single deletion is one of the major causes of mitochondrial disease. It is commonly associated with progressive external ophthalmoplegia (PEO), Kearns-Sayre syndrome (PEO associated to pigmentary retinopathy and cardiac conduction block), and Pearson syndrome (pediatric refractory sideroblastic anemia associated with pancreatic insufficiency), but its variability is incompletely understood. Aim of this study is to revise the phenotypic spectrum associated with mtDNA single deletion in more than 150 Italian patients, by a retrospective, database-based study ("Nation-wide Italian collaborative network of mitochondrial diseases"). The great majority of our patients had a PEO phenotype (≈ 70%), whereas KSS (≈ 15%) and Pearson syndrome (≈ 5%) were rarer. The remaining patients had non-specific (encephalo)myopathic clinical pictures. Furthermore, our results showed higher clinical heterogeneity than commonly thought, with the presence, in several cases, of cardiomyopathy, neuropathy, migraine, tremor, dementia, psychiatric disorders and/or other clinical features.

Prevalence of congenital muscular dystrophy in Italy: a population study

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Congenital muscular dystrophies (CMD) are rare diseases and small patient number represents the major impediment to progress in research and care. Classically the term CMD includes a group of genetically, clinically and biochemically distinct entities sharing clinical and pathological features such as early presentation of weakness and hypotonia and dystrophic features on muscle biopsy. In the last years the identification of several new genes responsible for different forms of CMD has not only expanded the spectrum of the known forms of CMD, but has produced exciting progresses in the understanding of the mechanisms underlying this group of disorders.
The really incidence and prevalence of CMD in populations is not sufficiently known.
The aim of this study is to establish the prevalence of CMD in Italy.

Our inclusion criteria were all registered patients with a diagnosis of CMD currently seen in the participating Centers.
The diagnosis of CMD was based on the following criteria: dystrophic or severe myopathic features on muscle biopsy and early presentation of weakness and hypotonia.
Preliminary results show 346 cases of CMD in Italy: 42.77% dystroglycanopathies, 22.83% laminin α2 deficient, 19.36% collagen VI deficient, 5.7% laminopathies, others 6%.
As the study includes all the Italian tertiary care centers for pediatric neuromuscular disorders, we can presume that our findings will provide an estimate of at least all the cases of CMD in Italy.

Neuropsychiatric comorbidities in Duchenne Muscular Dystrophy

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Amongst boys with Duchenne muscular dystrophy (DMD), intelligence quotient (IQ) is on average 1 SD below the general population mean, with working memory impairments being especially prominent. We previously described in smaller cohorts that 23% of DMD boys present with clinically significant traits of Autistic Spectrum Disorder (ASD) and 35% of DMD boys meet the criteria for Attention-Deficit/Hyperactivity Disorder (ADHD). Other comorbid psychopathologies included internalising (27%) and externalising (15%) behavioural problems. The objectives of our study were to describe to what extent neuropsychiatric comorbidities coexist in the same individuals, and to describe the bearing that the genotype has on the neuropsychiatric phenotype in DMD. We recruited 135 DMD boys who underwent standardised neuropsychological assessments including: WISC-IV, 3Di, Conners-3 Questionnaires, and CBCL.
We demonstrated that intellectual disability (ID) and psychopathologies are highly prevalent in DMD, with overall 22% of boys scoring in the ASD range and 29% scoring in the mood disorder range. Different psychiatric symptoms coexist in the same individual: 50% of the boys with ADHD score in the ASD rage, and 75% of boys with ASD manifest ADHD. The presence of internalising and externalising behavioural problems significantly correlated with the diagnosis of ASD and ADHD, but not with ID. We observed a genotype effect: mutations downstream of exon 31 (disrupting Dp260, 140,Dp116 and Dp71) are at a higher risk of psychiatric comorbidities suffering from ID ($p = 0.001$) ASD ($p = 0.03$) and clustering of neuropsychiatric symptoms. These striking findings suggest the need for a systematic assessment of the neurobehavioural problems and targeted support in routine clinical care.

Homozygous IVS1-13T>G mutation in two patients with Late-Onset Pompe Disease (LOPD): a rare genotype

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Pompe disease is an autosomal recessive metabolic myopathy due to mutations in the acid alpha-glucosidase (GAA) gene causing deficiency of lysosomal alpha-glucosidase activity. To date, more than 400 pathogenic mutations havebeen reported in the literature. In the late-onset form (LOPD), the IVS1-13T > G is the most frequently mutation found in the Caucasian population, accounting for approximately 70% of cases. Thisis classified as a "potentially mild mutation", because it results in low levels of normal transcript. In large LOPD population studies, the majority of patients are compound heterozygous for IVS1-13 T > G with a second mutation on the other allele whereas patients homozygous for IVS1-13 T > G have been very rarely reported.
We describe herein two brothers affected by LOPD, carrying a homozygous IVS1-13T > G mutation.
The proband was a 51 years-old man, complaining of exercise-induced muscle cramps since at least 20 years. He showed macroglossia, postural hands and head tremor and a mild proximal muscle weakness. He showed hyperckemia (max 837 UI/L) and a moderate vacuolar myopathy with glycogen accumulation on muscle biopsy. Muscle GAA activity was severely reduced (13% of residual activity); diagnosis of LOPD was made and confirmed by GAA genetic analysis that documented the IVS1-13T > G mutation on both alleles. His brother, 42 years-old, carried an asymptomatic hyperckemia (630 UI/L), a mild postural tremor at the hands. DBS for GAA activity showed a clear reduction (0.179 umol/h/L) and genetic analysis resulted in the same genotype as his brother.
Although GAA low levels, our data showed that homozygous IVS1-13T > G was associated with a mild LOPD phenotype in our patients. Those findings are somewhat in contrast with few other cases described in the literature where this genotype is associated with a more severe phenotype.

Early diagnosis and early treatment in LOPD: when asymptomatic patients should be treated

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Pompe disease is a lysosomal disorder caused by GAA deficiency. LOPD is characterized by progressive muscle weakness and/or respiratory failure but, sometimes, only by an asymptomatic hyperCKemia. Being a muscle degenerative disorder, it has been suggested that an early diagnosis could be more useful for a timely ERT start. According to the current treatment guidelines, ERT is recommended for patients who have symptoms or signs of Pompe diseas and in presymptomatic patients who have detectable proximal weakness or reduction in respiratory parameters. In a recent high risk population study, involving several Neuromuscular Italian Centers, we were able to diagnose 17 new LOPD patients.
Among those patients, 35% showed an asymptomatic hyperCKemia, 59% hyperCKemia and limb girdle muscle weakness (LGMW) whereas 6% manifested only LGMW. In these patients, the median time from the onset of symptoms/signs to the diagnosis was 7.7 years. ERT has been initiated in 11 patients. 8 out of the 11 showed LGMW with hyperckemia and two of them also had severe respiratory involvement. The last 3 only had hyperCKemia without any symptoms. Despite the presymptomatic condition, muscle morphology showed severe muscle damage and the muscle MRI revealed an adipose substitution in proximal muscles at lower limbs.
Conclusions: Of the 17 newly diagnoses Pompe patients, remarkably 35% of patients with only asymptomatic hyperCKemia were early identified but a combination of clinical and morphological data prompted us to start ERT early. To initiate ERT we suggest to consider, apart from the clinical symptoms, different parameters such as muscle MRI or muscle morphology to optimize the treatment efficacy.

RNA therapy for Spinal Muscular Atrophy by SMN increase or modulation of secondary cell death events

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The aim of our research is the development of RNA-based therapeutics to treat Spinal Muscular Atrophy (SMA) using a stem cell in vitro platform. SMA is a genetic motor neuron disorder caused by mutations of the survival motor neuron gene (SMN1). No effective treatment is available so far, but antisense therapy to increase the SMN level is a promising strategy. We obtained induced pluripotent stem cell (iPSC) lines reprogramming SMA/wild-type human fibroblasts with lentiviral constructs and with a non-viral non integrating method based on the expression of reprogramming factors with episomal vectors. We differentiated iPSCs using a protocol to promote motor neuronal commitment. The phenotype of these cells was analyzed by morphological, functional, gene expression, and protein analysis. RNA strategy based on antisense morpholino and shRNA aiming at increasing SMN level or inhibiting Fas activation were investigated. We demonstrate that SMA iPSC-motor neurons recapitulate the disease phenotype with a significantly fewer and smaller motor neurons at later time periods in culture compared to wild-type subject iPSC lines. These features were rescued in SMA motor neurons treated with antisense morpholino or U1 shRNA that increase SMN level. During motor neuron development, SMA lines showed an up-regulation of Fas ligand-mediated apoptosis and increased caspase-8 activation. Importantly, this could be mitigated by Fas silencing. Our data support the utility of SMA iPSCs as in vitro disease model, suggesting that RNA therapy can be a possible therapeutic strategy for SMA trough SMN up-regulation and modulation of disease pathways that can be achieved with different therapeutic tools.

Infantile Bilateral Striatal Necrosis: if not a mitochondrial disorder, what else?

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Infantile bilateral striatal necrosis (IBSN) is a unique and very distinctive neuroradiological phenotype characterized by a specific involvement of the striata, with initial swelling of the putamina and caudates followed by degeneration, leading ultimately to cellular necrosis.
IBSN has been associated with different genetic conditions, including mitochondrial disorders.
We report a child with neuroradiological findings compatible with IBSN referred to us with a suspect of mitochondrial disorder. Reduction in the activity of the respiratory chain complexes (in particular complex III) was documented on muscle biopsy.
A diagnosis of Aicardi–Goutières syndrome was made in this patient, subsequently confirmed with identification of two mutations in the ADAR1 (AGS6) gene.
We speculate that in our case mitochondrial dysfunction could be secondary to interferonopathy, the pathogenetic process of AGS and that the mitochondrial dysfunction could contribute to selective involvement of basal ganglia in AGS.
This research has received funding from the European Union's Seventh Framework Program (FP7/2007–2013) under grant agreement number 241779.

36 month longitudinal data in ambulant boys with Duchenne muscular dystrophy

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The 6 minute walk test (6MWT) has been recently chosen as the primary outcome functional mobility, endurance, and ability to walk. Its use has been reported in international multicenter clinical trials in DMD ambulant patients.
The aim of the study was: to assess the spectrum of changes over 36 months in ambulant boys affected by Duchenne muscular dystrophy, to establish the difference between the first the second and the third year results, and to identify possible early markers of loss of ambulation.
Three children (3%) lost the ability to perform the test within 1 year, another 13 between year 1 and year 2 (14%) and other 11 between year 2 and year 3 (12%). The 6MWD showed an average overall decline of −15.8 meters in the first, and of −58.9 in the second year and -104.22 in the third year.
The changes were significantly different in the two baseline age groups: children below 7 remained on the average stable. Children above 7 had a decrease during the first, the second and the third year.
The changes were also significantly different according to steroid treatment.
These results can be of help at the time of using inclusion criteria for a study in ambulant patients in order to minimize the risk of patients who may lose ambulation within the time of the trial.

Assessment of Upper Limb function in DMD patients:12 month changes

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As a result of an international effort, a new tool, the Performance of Upper Limb (PUL) has recently designed to assess upper limb function in DMD boys. The purpose of the PUL is to assess changes that occurs in motor performance of the upper limb over time from when a boy is still ambulant to the time he loses all arm function when non-ambulant.
The test has proved to be reliable, suitable for multicentric studies with excellent inter and intra-observer reliability.
Cross-sectional results in 322 DMD patients (mean age 12.7; range 4.1-35.1), showed a progressive deterioration of scores with age, with early involvement of the proximal muscles that was more obvious after the age of 10 years. Even the oldest and weakest DMD patients were still able to perform some of the distal items, suggesting that the scale is capable of measuring small distal movements (lifting small weights, tracing a diagram) that are important as they relate to activities of daily living such as using a mobile or using a computer mouse.
Results. The results showed some variability I the 12 month changes.
Conclusion. The PUL Scale demonstrated to be a useful tool for upper limb motor disease assessment in DMD ambulant and non-ambulant patients, both for evaluation in clinical trials and for therapy follow-up.

Juvenile dermatomyositis: two case report

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The idiopathic inflammatory myopathies, including juvenile dermatomyositis (JDM) are rare systemic autoimmune diseases characterized by proximal muscle inflammation and weakness. JDM affects children younger than 18 years with skin and muscle features similar to adult DM and is characterized by histopathologic findings as perifascicular and perivascular inflammatory infiltrates. Diagnostic criteria include characteristic skin findings, muscle weakness, elevated muscle enzyme levels, and evidence of an endomisial, mononuclear inflammatory infiltrate on muscle biopsy. Those criteria are not ever obvious. Actually, we propose two children with increased CPK, muscle weakness, cramps, faticability, no serum inflammatory signs and skin involvement. Histopathology shows minimal perifascicular atrophy and scant endomisial mononuclear infiltrate. Electron Microscopy played a key role in the differential diagnosis showing Tubular Reticular Structures (TRI) in endothelial cells of muscular small vessels. TRI are considered an ultrastructural pathognomonic sign of DM and its presence could even anticipate skin involvement in these myopathies.

Serum sclerostin in myasthenia gravis: an index of bone fractures risk in glucocorticoids-induced osteoporosis. A protective role of pyridostigmine on bone metabolism

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Myasthenia gravis (MG) is a neuromuscular junction disease which has been associated to an increased risk of glucocorticoid-induced osteoporosis. Recently it has been reported that, in MG, the use of oral glucocorticoids is not associated with an increased risk of fractures, suggesting that pyridostigmine may have an anabolic effect on bone, mediated by its interaction with osteoblasts receptors. Sclerostin is a glycoprotein which inhibits osteoblasts and bone formation, binding to the co-receptor LRP5/6. We have compared DEXA scan, serum sclerostin expression and other bone remodeling markers (CTX, osteocalcin, alkaline phosphatase, calcium, phosphorus, vitamin D, parathyroid hormone, creatinine) in 24 AChR-related MG patients, exposed to a prolonged steroids and pyridostigmine treatment, versus 16 MuSK-reletaed MG patients exposed only to steroids. We have found that markers of osteoporosis are more evident in patients affected by MuSK-related MG treated with the same dosage of glucocorticoids, compared to those affected by AChR-related MG, who don't disclose the same severe pattern of osteoporosis. Furthermore, we found lower sclerostin levels in patients on pyridostigmine treatment, suggesting its protective role on bone metabolism. Further investigations must be performed to confirm, on

a large scale, the possible protective role of pyridostigmine against glucocorticoids-induced osteoporosis.

Bone mineral density and body composition in 39 duchenne muscular dystrophy patients: a two-years follow-up

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Individuals affected with Duchenne muscular dystrophy (DMD) show significantly altered whole-body composition compared with normal control population. We assessed for at least two years 39 DMD-patients to evaluate how their bone mass and body composition change during time.
All patients underwent clinical evaluation and dual-energy-X-ray-absorptiometry (DXA) assessment every six months. By DXA we obtained subtotal total body (without head) and spine bone mineral density (BMD), lean tissue mass (LTM) and body fat percentage (PFAT). We compared data using Wilcoxon tests. At baseline visit the average age was 7 ± 1.62 (range 4-11) years, 11 patients were already treated with steroids since a mean of 3 years, 17 started therapy after that visit and 4 about 1 year later. At baseline we found significant Spearman correlations between all four DXA parameters each other and with weight, height and BMI. We observed an increasing trend (with statistically significant differences) in all DXA parameters during the two-years follow-up, especially in PFAT. Comparing boys over 10 years old with others we found significant differences in all parameters. These results confirm usefulness of DXA as tool both for DMD follow up and for treatment adjustment. Correlation with clinical outcome measures will be presented.

Phenotypic characterization of DM1 individuals carrying premutation alleles

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Myotonic dystrophy type 1 (DM1) is caused by the expansion of an unstable CTG repeat. Larger alleles are associated with a more severe phenotype and an increase in length from one generation to the next, accounting for the clinical anticipation. DM1 premutation alleles – giving rise to new DM1 families – can be identified in distant relatives of DM1 probands, often asymptomatic.
To investigate the phenotype of DM1 individuals carrying premutation alleles, the clinical records of 218 individuals from 50 DM1 families identified at our Service, during the period 2002-2013 were re-evaluated and the following parameters analysed: CTG repeat length, presence of myotonic phenomenon, heart conduction defects, cataract and serum CK values.
A total of 27 subjects (16M;11F, current age range 23-73 years) were identified as premutated patients, sharing a CGT expansion ≤ 100 (mean 76 ± 12,7; range 45-100).
Cataract before 45 years was the most frequent observed symptom (59.2%), occurring more frequently in premutated DM1 males (62.5%) than in females (25%). Atrio-ventricular blocks were the second most frequent (37%) feature observed in these patients, followed by myotonic phenomenon (18.5%).
Ophthalmologists and cardiologists should explore the aetiology of cataract or the presence of AV block in their patients, as an early identification of DM1 premutation alleles, could avoid the transmission of larger alleles and more severe phenotypes in subsequent generations.

Oculopharyngeal muscular dystrophy genotypic and phenotypic features of 13 patients

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Oculopharyngeal muscular dystrophy (OPMD) is a late onset muscular disease, caused by a short GCG expansion in the PABP2 gene, usually dominantly inherited. The mean symptoms onset is after the fourty years, with progressive eyelids ptosis, dysphagia and proximal limbs weakness. We report the clinical and genetic characteristics of 13 patients (3 families), followed in our neuromuscular center since 1990. 6/13 patients showed ptosis as first symptom, mean age 47.5 years; 4/13 complained dysphagia, mean age 45.7; only one patient reported silmutaneous onset of ptosis and dysphagia at the age of 55 years and two patients, aged 49 and 42 years, were asymptomatic. Six patients showed mild proximal limbs weakness, four patients had progressive external ophthalmoparesis, one patient suffered from dysphonia. Four patients underwent to blepharoplasty, with stable improved vision in three of them. Undernourishment was reported in six patients, at a marked degree in two. In one of these patients upper esophageal sphincter myotomy resulted in increase of body weight. Muscle biopsy, performed in four patients, showed myopathic findings with rimmed vacuoles. This is the second larger serie of Italian OPMD patients (Mirabella M. et al, 2001), documenting: GCG6/GCG9 repeat mutation is the most frequent in the Italian population, it shows a meiotic stability within the same family, with a moderate phenotypic associated to this genotype.

sEMG descriptors of central and peripheral fatigue in a case of Facioscapulohumeral dystrophy

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Introduction. In Facioscapulohumeral muscular dystrophy (FSHD) fatigue is a precocious symptom but it is unknown the relative contribution of central and peripheral mechanisms to perceived fatigue and whether it may temporally precede the

appearance of detectable alterations of skeletal muscle by magnetic resonance imaging (MRI).
Aim. To examine the myoelectric manifestations of central (FD, fractal dimension) and peripheral (CV, conduction velocity) fatigue of a FSHD patient (Score 1, Lamperti 2010 *Muscle Nerve* 42, 213); to assess whether fatigue precedes or follows detectable muscle alterations by MRI.
Methods. sEMG signals were recorded in biceps brachii (BB) during isometric contractions at 20% and 60% of the maximum voluntary contraction (MVC) for 1 min and 20s respectively at 90 degrees knee joint angle. Initial values and rate of change (slope) of Mean Power Frequency (MNF), CV and FD were calculated. Total body muscle MRI was also obtained.
Results. MRI revealed no abnormalities in the BB bilaterally. In the right biceps brachii a change in CV slope was observed at 20% MVC (0.0014 vs 0.0021). This change was associated with higher initial value and positive MNF slope and equal initial value and unchanged FD slope. All estimated parameters displayed a significant change in the slope at 60% MVC.
Conclusion. Peripheral fatigue was observed during contraction at 20% MVC whereas peripheral and central fatigue and a progressive reduction of fibers recruitment were observed at higher output only in the right BB. Importantly these changes were observed in absence of phonotypical alterations detectable with MRI.

Muscle magnetic resonance imaging findings in two sisters carrying two new CLCN1 gene mutations

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Few data are available on muscle MRI findings in patients with non-dystrophyc myotonia (NDM). A recent study (Morrow JM et al, 2013) has shown MRI abnormalities in all out of 21 NDM patients, almost half of them showing at least mild extensive T1w changes and a "central stripe" of STIR hyperintensity within the medial gastrocnemius muscle, more frequently in patients (10/11) with CLCN1 myotonia (myotonia congenita).
We report two sisters aged 53 and 56 respectively, who developed myotonic symptoms in early infancy. Both presented with widespread muscle myotonia with limb muscle hypertrophy, the older one with slight proximal muscle weakness. Molecular genetic analysis of CLCN1 gene revealed two new mutations (p.Gly233Ser; p.Thr328Ala). Segregation studies confirmed that these mutations were in trans.
Muscle MRI study showed selective T1w changes at the level of the inferior limb, different at the level of the thigh from those described in literature so far, being the sartorius muscle the most selectively involved. The "central stripe" of STIR hyperintensity within the medial gastrocnemius muscle was also evident in one of them.
This report widens the spectrum of MRI appearances in myotonia congenita, so helping differentiating it form other myotonic dysorders.

New RYR1 mutations discovered by Next Generation Sequencing in Congenital Myopathy patients with different phenotypes

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Congenital Myopathies (CM) are clinical and genetic heterogeneous disorders of skeletal muscle. The clinical spectrum ranges from severe forms with congenital hypotonia, severe muscle weakness and early mortality, to milder forms characterized by slowly progressive weakness. There are different subtypes based on the pathological features: nemaline myopathies, myopathies with cores, myopathies with central nuclei, myopathies with fiber type disproportion. More than half of CM can be attributed to mutations in RYR1 gene that encodes the skeletal muscle ryanodine receptor. The use of Next Generation Sequencing (NGS) allowed us to diagnose in a faster way mutations in RYR1 gene in three CM families, presenting with a bioptical pattern suggestive for Core Myopathy and Fiber Type Disproportion. We identified 6 variants, 4 of them are new. In addition the analysis of a family with four affected members revealed a different clinical course associated to the same haplotype, characterized by the presence of two mutations, one linked to central core and the other associated to malignant hyperthermia. Overall NGS appears promising for the analysis of complex and heterogeneous pathologies like CMs.

Late onset ophtalmoplegia in a typical neonatal congenital myopathy

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Introduction. Congenital myopathies (CM) are a group of muscle disorders clinically characterized by hypotonia and weakness, usually from birth, a static or slowly progressive clinical course, with genetic and phenotype heterogeneity.
Case report. We report a case of a 31 years old female, suffering from a typical congenital myopathy. The father and the younger sister suffer respectively from an adult onset CM and a benign / asymptomatic CM. She presented at birth: generalized hypotonia, facial weakness, cleft palate, breathing difficulties requiring intubation and feeding difficulties requiring nasogastric tube. Only at 5 months of age, she could breath and feed autonomously. Ambulation was acquired at 2 yrs; scoliosis and pectus excavatum were observed early in childhood. Normal CK levels, myogenic signs at EMG, fiber type variability with type I fibers predominance and no irregularity of the oxidative enzymes stain on muscle biopsy completed the clinical feature. At 31 yrs she presents long face, mild facial and proximal lower limb weakness, hypotonia,

and external ophtalmoparesis. This last sign was firstly observed when she was around 22 yrs, and mild progression has been recorded during the years, with no other bulbar signs.
Conclusion. Ophtalmoplegia is frequently associated to severe form of CM and mostly observed since birth, even in cases with a mild skeletal myopathy; it is very rarely reported in adult onset CM. To our knowledges, our case is one of the few in which a mildly progressive ophtalmoparesis appears in young adulthood in a typical benign congenital myopathy.

Psychological benefits of caregiving in relatives of young people with muscular dystrophy

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This paper focuses on the psychological benefits of caregiving in key-relatives of patients with Duchenne, Becker, or Limb-Girdle Muscular Dystrophies (MD). The sample included 502 key-relatives of patients 4-25 years-old in charge for at least six months to one of the 8 participating centers, living with at least one relative 18-80 years-old. 88% of key-relatives stated that they had gotten something positive out of the situation, 96% considered their patients to be sensitive, and 94% viewed their patients as talented. Positive aspects of caregiving were more recognized by key-relatives more convinced that the patient was sensitive and who perceived they received higher level of professional help and psychological social support. These results suggest that most key-relatives consider their caregiving experience as a positive impact on their lives, despite the practical difficulties. Professionals should help relatives to identify the benefits of the caregiving without denying its difficulties.

Sodium channel related mytotonia: different phenotypes and revision of the literature

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Non-dystrophic myotonias are rare diseases caused by mutations in skeletal muscle chloride and sodium ion channels. Mutations of SCN4A encoding the skeletal muscle sodium channel Nav1.4 cause several types of disease, the sodium channel myotonias, including paramyotonia congenita, potassium aggravated myotonia, myotonia fluctuans. Common symptoms are muscle stiffness, transitory weakness, fatigue and pain. Herein, we describe clinical features, neurophysiological and molecular data of five families affected by sodium channel myotonias followed by our Centre: three patients, belonging to two different families, are affected by paramyotonia congenita (c.4367G > A; c.2095G > A); two patients, belonging to the same family, are affected by potassium aggravated myotonia (p. Ala699Thr); two patients, belonging to the same family, are affected by myotonia fluctuans (p. Gly1306Ala); one patient is affected by paramyotonia congenita, with the very rare neonatal onset form, which include the severe neonatal episodic laryngospasm (SNEL) (p. Gly1306Glu). We confirm the clinical variability of these patients and the importance of a correct neurophysiological approach, according to Fournier's guidelines, to guide the molecular analysis. Furthermore, we found a novel mutation responsible for paramyotonia congenita, which need to be biophysically studied, considering that it is responsible for very different clinical phenotypes within the same family. Recognizing patients with channelopathies and confirming this diagnosis is important, as treatment and management strategies differ based on mutation and clinical phenotype.

Familial disto-proximal mitochondrial myopathy with inflammation: a new phenotype in search for a gene

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Patients. Three brothers (two females and one male) were affected by adult-onset myopathy with disto-proximal progression characterized by severe involvement of distal muscles of lower and upper limbs and less pronounced weakness of limb girdle muscles; they had also cardiac arrhythmia and long QT syndrome (LGTS) that required Implantable Cardioverter Defibrillator in one patient. Congenital strabismus was present in all three brothers.
Methods and results. Muscle biopsies were performed in two patients showing a chronic myopathy with evident mitochondrial proliferation and presence of necrosis, myophagia, regenerating fibers, small lympho-mononuclerar perivascular infiltrates and strong expression of MHC1 in 80% of the fibers. Immunocytochemistry, beside a diffuse immunopositive desmin reaction in regenerating fibers, didn't revealed other proteins aggregates. Interestingly, heart biopsy also showed both mitochondria and inflammation. A therapy with IVIg and steroids induced a partial, but clear improvement of muscle weakness. Mitochondrial enzymes activities and analysis for mitochondrial DNA and LQTS genes were normal. Whole exome sequencing is underway, but all nuclear-encoding genes for mitochondrial proteins were excluded.
Conclusions. We report a family with a distinctive autosomal recessive myopathy and LQTS; the association of mitochondrial abnormalities and inflammation is puzzlingly but consistent, bolstering the emerging debate on the role of mitochondria as a player in the innate immune response in specific disorders.

Novel SPG11 mutation in a case of HSP/ALS overlap phenotype

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Autosomal recessive hereditary spastic paraplegias with thin corpus callosum (AR-HSP-TCC) represent the most common form of complicated hereditary spastic paraplegias (HSP). Autosomal recessive juvenile ALS is a rare form of genetic ALS occurring before age of 25. Although such diseases are genetically heterogeneous, mutations in SPG11 have been reported in both of them. We report the case of a female patient, which presented at the age of 18 years with progressive weakness and spasticity affecting the lower limbs. The neurological examination revealed paraparetic gait associated with bilateral steppage and pyramidal signs.
EMG showed active and chronic partial denervation in all limb muscles and a axonal/demyelinating sensorimotor peripheral neuropathy. Muscle biopsy confirmed a pattern of chronic neurogenic denervation. Motor evoked potentials pointed to an increased central conduction time. Brain MRI showed diffuse white matter abnormalities and thinning of the corpus callosum. Direct sequencing of SPG11 gene identified a novel frameshift mutation, a c.7081insT variant in compound heterozygosis with an already known c.733_734delAT mutation.
Even though a diagnosis of AR-HSP-TCC was initially made, some clinical findings could suggest a juvenile ALS. It thus appears that the lines between ALS and HSP phenotypes are blurred. Given the need for a correct nosologic categorization, strong criteria to differentiate ALS from HSP phenotype linked to SPG11 are warranted.

Reliability and validity of the nine hole peg test in myotonic dystrophy type 1

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Background. Myotonic dystrophy type 1 (DM1) is a multisystemic inherited disorder. Distal weakness is one of the main features of the disease, leading to a manual skill impairment. The Nine Hole Peg Test (NHPT) is a timed test of fine manual dexterity validated in other neurological diseases. The objective of this study is to evaluate the reliability and validity of NPHT in DM1 patients.
Methods. NPHT was administered to 42 DM1 patients and 28 age-matched healthy controls. Both DM1 and controls were retested within one week from the first test. Handgrip and pinch strength were measured in DM1 using a Handheld Dynamometer. Differences in NHPT values between DM1 and controls were evaluated by unpaired t-test. Pearson's correlation coefficient was used to demonstrate NHPT test-retest reliability, and to analyze the relationship between NHPT performances and manual strength.
Results. In both DM1 patients and controls, NHPT in dominant and non-dominant hand showed test-retest reliability ($r > 0.7$, p value < 0.05). Time of NHPT execution was significantly increased in DM1 group compared to controls ($p < 0.001$). Handgrip and pinch strength values inversely correlated with NHPT ($p < 0.05$) in both dominant and non-dominant hand.
Conclusions. NHPT is a simple, rapid, inexpensive and repeatable test to assess manual dexterity in DM1 patients. NHPT could represent a valid instrument to monitor the natural history of the disease or the therapeutic effect in clinical trials.

Exercise Test in skeletal muscle channelopathies: potential applications as learned from 26 patients

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Skeletal muscle channelopathies are rare diseases with overlapping phenotypes. Distinguishing clinical features have been searched for, with unconclusive results. By showing the electrical correlates of myotonia and paralysis, and evaluating the effect of stressors on membrane excitability, the exercise test (ET) may help drive the molecular diagnosis.
We adopted a standarized EMG protocol (Fournier et al, 2004-2006) evaluating 18 controls and 26 mutated patients (44.2 ± 12.2 years, 10 females), among which 19 in SCN4A, 6 in CLCN1, and 1 in CACNA1S. Among SCN4A-mutated patients we include 10 Paramyotonia Congenita-PC, 6 Sodium Channel Myotonia-SCM, and 3 Hyperkaliemic Periodic Paralysis-HPP. The Short-ET was normal in 100% SCM and HPP, abnormal in 100% PC and most CLCN1-Recessive patients, with distinct pattern on exercise repetition and decreased cMAP persistence. Cold sensitivity, though relevant in PCs only, allowed the detection of other, but still not all, CLCN1 patients. On Long-ET, abnormal in all PC and in 2/3 HPP, we found one false negative (HPP with mild phenotype) and one false positive.
In conclusion, the ET narrows the diagnosis, but is not always precise in predicting the abnormal ion channel, being normal ET found in SCM and in some patient with dominant myotonia congenita. The ET is a particularly attractive tool to understand the pathological consequences of new mutations, and for quantitative evaluation of pharmacological interventions.

An updated comprehensive clinical evaluation form for detailed characterization of genetic and phenotypic features associated with D4Z4 reduced allele

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Wide phenotypic variability has been observed in D4Z4 reduced allele (DRA) carriers, sometimes with unexpected mode of inheritance. In some cases the high frequency of FSHD molecular signature might have generated a biased evaluation of families in which a myopathy and a DRA were detected influencing diagnosis and interpretation of clinical and genetic data. Starting from the Italian National Registry for FSHD, in order to capitalize on the rich repository of material from FSHD subjects, we have designed a Comprehensive Clinical Evaluation Form (CCEF) that defines phenotypic subgroups by combination of different clinical features. We believe that the precise phenotypic and genetic classification of patients and families will be central to define the natural history of disease, to propose suitable measure of outcome and to identify new susceptibility/causative factors contributing to FSHD.

Clinical characterization of carriers of borderline D4Z4 alleles from the Italian National Registry of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) has been associated with reduced numbers (≤ 8) of D4Z4 repeats at 4q35. Since 3% healthy individuals carry D4Z4 reduced alleles (DRA) with 4-8 repeats molecular diagnostic in FSHD can be troublesome especially in presence of atypical clinical presentations. Particular attention has to be paid to cases carrying alleles with 9-10 D4Z4 repeats, considered borderline. To define the clinical significance of these alleles, 228 subjects carrying 9-10 DRA (123 index cases and 105 relatives) have been selected from the INRF. We discuss the ongoing evaluation protocol that include: -detailed clinical characterization of probands and relatives; -analysis of disease expression severity; -study of mode of inheritance (familial/sporadic cases); -study of disease penetrance among relatives. Results are discussed, including alternative diagnosis for the atypical/overlapping forms.

Clinical and pathological features of a case with vacuolar myopathy

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A 44-years-old man came to our attention because of a ten months history of exercise-induced muscle pain and early fatigue in daily activities. His family history was inconsistent for neuromuscular diseases. The neurological examination revealed a moderate proximal muscle weakness at lower limb. Serum creatine kinase resulted repeatedly increased (1000-1500 U/L). Needle electromyography recorded a myopathic pattern. Cardiac evaluation was normal. The morphological study of muscle biopsy showed several fibers with clear vacuoles in sections stained with haematoxylin and eosin and Gomori-modified trichrome. Vacuoles had an oval shape or lobulated border. Some vacuoles contained fine amorphous material. Vacuoles did not stain with acid phosphatase, but some of them showed a rim of PAS positive material. Ultrastructural analysis revealed the presence of highly electrondense inclusions, mostly polygonal with rectangular or quadrangular shape, and, sometimes, irregular contour. They were located among myofibrils and were often associated with glycogen particles. The pathological features were suggestive of a surplus protein myopathy. Further details will be added on in site discussion.

ASAH1-related spinal muscular atrophy with no myoclonic epilepsy: expanding phenotypic and mutational features

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ASAH1 gene encodes for the N-acylsphingosine amidohydrolase that is involved in the degradation of ceramide into sphingosine and free fatty acids within lysosomes. Homozygous mutations in ASAH1 are responsible for rare cases of spinal muscular atrophy (SMA) with progressive myoclonic epilepsy (SMAPME) which is phenotypically characterized by childhood onset of proximal muscle weakness and atrophy due to spinal motor neuron degeneration followed by occurrence of myoclonic seizures. The disorder is progressive and patients usually died in the teenage years.

We report the case of a 29-year-old pregnant woman affected with very slowly progressive proximal muscle weakness and atrophy since age of 3 yrs. EMG and muscle biopsy analysis suggested a chronic neurogenic process as usually seen in SMA

but the search for deletions or point mutations in the SMN gene resulted negative. No history of seizures or myoclonus has been reported and EEG was unremarkable.
The molecular study of ASAH1 gene showed the presence of the homozygous nucleotide variation c.124A > G that causes the amino acid substitution p.T42A. This variation has not yet been described and affects the same base involved in the disease-causing mutation described in association with the classical *ASAH1* phenotype (p. T42M). This change is likely to have a pathogenic effect by inserting an alternative splice acceptor site. A similarly affected sister harbored the same mutation.
Our case describes for the first time the association between *ASAH1* mutations and an adult SMA phenotype with no myoclonic epilepsy, thus expanding phenotypic spectrum of *ASAH1*-related SMA.

Muscle mitochondrial dysfunction due to defective mitochondrial biogenesis in SMA patients

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Spinal muscular atrophy (SMA) is characterized by degeneration of motor neurons with skeletal muscle weakness. Depletion of mitochondrial DNA (mtDNA) had been reported in SMA patients as a consequence of the fiber atrophy. We studied muscle samples from 24 genetically SMA patients In all subjects, skeletal muscle biopsy showed a chronic neurogenic pattern. We observed a severe COX deficiency in most samples, in particular in SMA I and SMA II subjects. The enzyme defect was evident in both atrophic and normal/hypertrophic fibers. All respiratory chain complexes were severely impaired. Using custom array gene expression studies, we linked these alterations to the down-regulation of PGC1-alpha and of its downstream targets, including transcription factors NRF1, NRF2 and TFAM. In conclusion, PGC-1α expression and mitochondrial content are significantly reduced in SMA muscles. Therapeutic strategies aiming at counteracting these changes may reveal beneficial for SMA patients.

Myasthenia gravis: diagnostic pitfalls for the atypical phenotypes

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Myasthenia gravis (MG) is an autoimmune disorder characterized by weakness and fatigability, associated to different profiles of antibodies directed against several neuromuscular junction components (AChR, MuSK, LRP4). Although MG has long been considered a well-established autoimmune disease associated with autoantibodies, which are convincingly pathogenic, accumulating data indicate that clinical heterogeneity is very wide. Clinical presentation is distinct from patient to patient, the gravity of symptoms does not correlate to antibodies title and sometimes the clinical phenotype is uncommon and this lead to time consume and misdiagnoses. Herein, we describe various atypical MG phenotypes: three patients with distal upper limbs muscle weakness; a case of cervico-inflammatory myositis complicating by MG involving the arms; a case with acute facial dyplegia; three cases with post-exertional axial muscles weakness; one case with isolated bilateral weakness of the triceps brachii; one case of isolated peroneal muscle weakness. MG should be suspected in patients with "fatigable muscle weakness", even if it is isolated and restricted to "unusual" muscle groups.

A family with epilepsy, movement disorders, mental retardation and exercise-induced myoglobinuria: a complex phenotype caused by two different rare disorders

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We report a 44-years-old man, who presented, since the age of 20, recurrent episodes of rhabdomyolysis after exercise or prolonged fasting; he also showed a mild mental retardation and sporadic choreo-athetoid movements. His 14-years-old son had a psychomotor developmental delay with episodes of drowsiness and head drops, occurring mainly at fasting, and exercise-induced choreo-athetoid movements but no history of pigmenturia. Neurological examination revealed microcephaly, mild spastic ataxia and mental retardation.
EEG was normal in the proband but in the son showed, during fasting, diffuse spike-wave discharges disappearing after food intake. Brain MRI was normal in both. CSF analysis in the son revealed hypoglycorrhachia (40 mg/dl). Clinical and laboratory findings suggested a search for mutations in SCL2A1 (GLUT-1) gene that revealed in both subjects, an already reported pathogenic heterozygous mutation (R333W). GLUT-1 Deficiency Syndrome (DS) is a rare encephalopathy, caused by impaired glucose transport into the brain, presenting with early-onset epilepsy, movement disorders, developmental delay and microcephaly but rhabdomyolysis has never been reported in similar cases. To better define the origin of recurrent exercise-induced rhabdomyolysis in the father, he underwent forearm ischemic test (normal), EMG (myopathic pattern) and muscle biopsy that evidenced unspecific changes. Muscle biochemical studies excluded the most common metabolic causes of recurrent rhabdomyolysis, but VLCAD gene analysis in the father showed two known heterozygous mutations (p.G185S and p.R385W) whereas his son carried only the p.G185S.

Nowadays, it is evident that cases of "double trouble" are increasing and, when a known phenotype is accompanied by some atypical features, we should think of an alternative explanation of unusual presentations.

Reversible acute dropped head syndrome as a presenting feature of mitochondrial myopathy

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Dropped head secondary to weakness of neck extensor muscles has been frequently reported in a wide range of neuromuscular disorders. However, it has been rarely described as a presenting feature of a mitochondrial myopathy.
We here report a 72-year-old man who complained of sudden onset of neck pain followed, after a 15 day period, by weakness of neck extensor muscles and dysphagia.
Since many years he presented a nuanced gait disturbance and diffuse muscle wasting.
Neurological examination showed the presence of dropped head and weakness/atrophy of the upper limb proximal muscles (MRC 4-/5). EMG analysis showed a mixed neurogenic and myogenic pattern at both the upper and lower limbs.
Deltoid muscle biopsy showed severe myopathic changes with prominent features of mitochondrial respiratory chain dysfunction (over 80 COX-negative fibers, many fibers with subsarcolemmal accumulation of mitochondria and ragged blue fibers). Rare small inflammatory infiltrates, scattered fiber atrophy and increase in lipid droplets were also observed. No MHC I expression in muscle fiber was present.
The sequence analysis of the 22 tRNAs in mtDNA did not show any variation. The remaining mtDNA sequencing is ongoing.
In the next two months, the patient experienced a gradual spontaneous improvement until he was able to hold his head upright again and no longer complained of dysphagia.
The role of mitochondrial abnormalities and the mechanisms underlying spontaneous recovery in this patient remain to be explained.

Sporadic PEO caused by a novel *POLG1* variation and a *Twinkle* mutation: digenic inheritance?

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Progressive external ophthalmoplegia (PEO) with multiple deletions of mitochondrial DNA (mtDNA) is associated with several mutations in nuclear genes. They include *POLG1*, *POLG2*, *ANT1* and *Twinkle*. However, digenic inheritance in mitochondrial disorders has been documented in a few cases over the years.
Here we describe an 80-year-old man with sporadic PEO, dysphagia and diffuse muscle weakness. Muscle biopsy showed mild variation in fiber size, about 5% of fibers negative for cytochrome c oxidase (COX) staining while modified trichrome staining did not reveal ragged red fibers. Long range polymerase chain reaction (PCR) analysis of muscle DNA showed multiple mtDNA deletions. Sequencing the *POLG1* revealed a novel heterozygous mutation (c.2831A > G; p.Glu944Gly), predicted as damaging, in the patient who also carried a heterozygous mutation in *Twinkle* (c.1142T > C; p.Leu381Pro).
This case provides a second report of a digenic PEO caused by different mutations in the *POLG1* and *Twinkle* genes. These data support the hypothesis that the PEO phenotype can be determined by the co-existence of two abnormalities in separate genes, both involved in the maintenance and stability of mtDNA. Finally, this study expands the spectrum of *POLG1* mutations and highlights the need to sequence the whole set of nuclear genes associated with PEO and multiple mtDNA deletions.

A new mutation in MYH7 gene occurs with complex fenotype

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Background. Mutations in the beta-myosin heavy chain gene (MYH7) cause different muscle disorders: Laing distal myopathy (LDM), myosin storage myopathy (MSM), hypertrophic familial cardiomyopathy (FHCM) and fiber type dispropotion (CFTD).
We describe three members of a family with a new mutation in the MYH7 gene (c.5401G > A) and a complex phenotype. A 65 year old man and his two sons, 37 and 35 years old, presented in the third decade a non-compact cardiomyopathy, followed in time by heart rhythm abnormalities; the proband and his eldest son underwent pacemakers implants. All our patients since the age of about 30 years showed a moderate weakness of both proximal and distal muscles of lower limbs, while at upper limbs there was a mild proximal involvement whit sparing of the wrist and finger extensor muscles. At last neurological examination all patients showed an anserine gait with bilateral steppage; the proband needs a cane and all of them need help to stand up from chair. The proband and his eldest son underwent to muscle biopsy, which showed a CFTD picture.
Conclusion. To the best our knowledge the association of non-compact cardiomyopathy, LDM like phenotype and CFTD picture at muscle biopsy have never been described in MYH7 gene mutations.

Facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy and platelet storage pool disease: "triple trouble" overlapping syndrome?

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Introduction. Facioscapulohumeral muscular dystrophy is a common hereditary myopathy characterized by an autosomal dominant inheritance, usually associated with a contraction of variable size of 3.3 kb tandem repeated units (D4Z4) on chromosome 4q35. Limb-girdle muscular dystrophies (LGMD) represent a genetically heterogeneous group of myopathies characterized by a variable phenotype. LGMD 2A is the most common type of recessive LGMD (14) and is caused by mutations in the calpain-3 gene.
Case report. We report the case of a patient with concomitant detection of a heterozygous mutation of the calpain3 gene and a contracted D4Z4 fragment presenting with limb-girdle and facioscapulohumeral muscular dystrophy-like phenotype and platelet storage pool disease.
Discussion. We suggest that our case could represent another example of "double" or even "triple" trouble overlapping syndrome. Therefore, we think that patients with atypical phenotypes should undergo more extensive genetic testing, which may provide further useful information for prognosis and genetic counseling.

Blood vessels depletion in MNGIE patients

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Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder characterized by ptosis and progressive external ophthalmoplegia, peripheral neuropathy, severe gastrointestinal dysmotility, cachexia, leukoencephalopathy and mitochondrial DNA depletion, multiple deletions, or both. This disorder is caused by loss-of-function mutations in the gene encoding thymidine phosphorylase (TP) a cytosolic enzyme that catalyzes phosphorolysis of thymidine to thymine and deoxyribose 1-phosphate. In MNGIE patients. TP activity is very low or absent resulting in dramatically elevated levels of plasma thymidine and deoxyuridine. TP is expressed in most human tissues but is not expressed in skeletal muscle usually affected in MNGIE suggesting that TP deficiency causes the disease through a toxic intermediate. In addition, TP is associated with angiogenesis and high concentrations of thymidine inhibit microvessels formation. In our preliminarly study vessels number between two MINGIE patients and eleven controls was compared. Histologic slides were stained with Alkaline Phosphatase and ratio between blood vessels and fibres number was calculated for each sample. Even if cases and controls numbers are low and they have to be increased, a significative differerence between MINGIE and control patients was revealed suggesting that angiogenesis inhibition could be involved in MINGIE pathogenesis.

Frequency of cerebrovascular abnormalities in patients with Late Onset Pompe disease (LOPD): our experience

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Although muscle skeletal dysfunction is generally the prominent manifestation of LOPD, the disease can present with a broad spectrum of clinical manifestations reflecting multisystem involvement with glycogen accumulation in several tissues including smooth muscle and blood vessels. In recent years cerebrovascular abnormalities have been also described. We report data from our population of 10 LOPD patients which underwent magnetic resonance or TC angiography. Three patients revealed to have significant brain vascular abnormalities (including basilar artery dolichoectasia, ectasia of intra and extra cranial arteries, vascular development abnormalities). Moreover, one patient presented three different aneurysms both on intra and extra cranial cerebral vessels, and has been proposed for surgical intervention. None of patients reported clinical symptoms related to the arteriopathy.
Our data confirm that LOPD patients have a predisposition to dilative arteriopathy, in particular of cerebral vessel, which often can be completely asymptomatic. According to this, rupture of a cerebral aneurysm has recently been described as presenting symptom in a LOPD patient.
We would like to highlight the importance of cerebrovascular investigation for the early recognition of such abnormalities in order to consider surgical intervention and prevent potentially fatal cerebrovascular complications.
Real incidence of vascular abnormalities in LOPD, their pathogenesis and the effect on them of enzyme replacement therapy are issues needing more investigations.

Dichlorphenamide in Hypokalemic Periodic Paralysis: effects on attack frequency and quality of life

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Background. Dichlorophenamide (DCP) reduces attack frequency in HypoPP but its effect on quality of life and interictal strength is not known.
Objective. To assess the efficacy of (DCP) in the prevention of attacks in hypokalemic periodic paralysis.
Trial design. Multicenter, randomized, double-blind, placebo-controlled trial of DCP in which participants were followed for 9 weeks. The primary outcome was the average attack rate over Weeks 2-9. Secondary outcomes included acute worsening, severity-weighted attack rate, and changes from baseline in muscle strength, muscle mass, and quality of life as measured by the SF-36.
Results. Participants on DCP (n = 24) had reduced attack frequency and severity relative to those on placebo (n = 20). Five

participants, all taking placebo, reached the endpoint of acute worsening. The most common adverse event were paresthesia (5% placebo vs 38% DCP) and confusion (10% placebo vs 21% on DCP). Despite these effects on cognition, participants on DCP showed significant improvements in physical and social functioning aspects of quality of life compared to placebo. There were no significant effects of DCP on muscle strength or muscle mass.

Conclusions. DCP is safe and effective in the prevention of episodic weakness and improves quality of life in hypokalemic periodic paralysis.

Myopathy with rimmed vacuoles in a girl with juvenile neuronal ceroid lipofuscinosis (CLN3)

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The neuronal ceroid lipofuscinosis (CLNs) are clinically and genetically heterogeneous neurodegenerative diseases characterized by intracellular accumulation of autofluorescent lipopigment and a clinical picture of progressive dementia, vision loss and epilepsy.

The CLNs were originally classified according the onset age, with CLN3 as the juvenile-onset form (JNCL), occurring between 4 and 10 years of age; now CLNs are classified according to the underlying gene defect. CLN3 refers to CLN caused by mutation in the CLN3 gene, whose hallmarks are the ultrastructural pattern of lipopigment with a 'fingerprint' profile and lysosomal vacuoles in blood lymphocytes.

We have carried out a study on a patient with the homozygote deletion (1.0 kb) of the CLN3 gene, who presented with dementia, epilepsy, retinal dystrophy and – in the late stages of the disease – creatine kinase increase. Muscle biopsy showed a myopathy with degenerative aspects and various fibers with rimmed vacuoles.

Even though there are numerous electron microscopy studies on CLNs, the literature lacks in the muscular histopathological aspects of the disease. The presence of a myopathy with rimmed vacuoles in our patient supports the pathogenetic hypothesis of an impairment of autophagy in CLN3. Moreover, if confirmed, the CLNs, mainly the CLN3, may be included in myopathies with rimmed vacuoles.

Clinical and molecular characteristics of dysferlinopathy

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Dysferlinopathy is a muscular dystrophy due to mutations in dysferlin gene. Muscular weakness can be prominent in lower limbs distal muscles (Miyoshi myopathy) or in proximal muscles (limb girdle muscular dystrophy 2B). Cardiac involvement is rare and respiratory involvement usually mild and occurs later in the disease.

The objective was to determine the clinical characteristics dysferlinopathies, and to investigate its genetic background. To identify the most relevant outcome measures to describe the progression of the disease.

The past medical history was collected, disease course was evaluated by specific questionnaires. Molecular analysis of *DYSF* gene was reviewed, as well as immunoblot analysis of the protein. A specific evaluation protocol was used, including clinical-instrumental quantitative evaluation of motor, respiratory and cardiac function.

Seventeen patients (12M, 5 F) from 15 families were included in the study, the age ranging from 19 to 55 years. Four patients were non-ambulant. All patients presented muscular weakness, variably expressed in severity and distribution. No patients referred signs and symptoms of respiratory insufficiency or cardiac involvement. Both mutations were identified in 16/17 patients, and eighteen different mutations were recorded. On western blot analysis dysferlin was absent in 9/14 patients and markedly reduced (< 10%) in 5. No clear correlations between genetic background and clinical features were observed.

The rarity of the disease and its clinical and molecular heterogeneity impose a multicenter longitudinal study. The choice of correct outcome measures is crucial to be prepared to the advent of possible new therapies for rare diseases.

The challenge of phenotypic heterogeneity in facioscapulohumeral muscular dystrophy

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Introduction. Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of hereditary myopathy. In the majority of cases FSHD is associated with a contraction of variable size of 3.3 kb tandem repeated units on chromosome 4q35.

Case report. We report the case of a family with a history of FSHD with the same findings at the after molecular diagnosis but with significant variability in clinical expression among the members affected.

Discussion. Our case corroborate that FSHD presents important inter- and intrafamilial clinical variability that makes it difficult to formulate clinical prognosis and to do genetic counseling. Other interesting evidence is the concurrence of FSHD and autoimmune disease in some of the members affected, which may be coincidental or maybe related with the pathogenesis of FSHD. Further work is needed to understand what the genetic, epigenetic and environmental factors that may influence phenotypic expression are to help genetic counseling and future therapeutic approaches.

Very late-onset ataxia with eyelid ptosis due to *POLG* mutation

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Genetic ataxias are especially suspected in early-onset cases. Here we report the case of an 82-year-old man who complained of progressively worsening dizziness since age 80 years. Neurological examination showed marked gait ataxia with central nystagmus and mild limb ataxia and eyelid ptosis. At last, mitochondrial polymerase γ gene (*POLG*) sequencing revealed the point mutation 1550G > T leading to the substitution p.517G > V. Most likely, this mutation is an incompletely penetrant dominant mutation with variable clinical features; other genetic variations (in *POLG* itself and/or in other loci) can elicit or modify the pathogenic effect, but further studies are needed. *POLG* is a very complex gene associated with incomplete penetrance and variable expressivity, which must be considered in ataxic patients, even in late-onset cases, especially when other signs of mitochondrial dysfunction (such as eyelid ptosis) are observed.

Myopathy in a carrier of *SDHD* (succinate dehydrogenase, subunit D) gene mutation. Report of a case

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The succinate dehydrogenase, subunit D (*SDHD*) gene encodes a subunit of the complex II of the respiratory chain, responsible for the oxidation of succinate. The encoded protein is one of the two integral membrane proteins anchoring the complex to the mitochondrial inner membrane. Mutations in this gene are associated with inherited tumors, namely hereditary paragangliomas. The transmission of hereditary paragangliomas occurs almost exclusively through the paternal allele, suggesting that this locus may be maternally imprinted.

We describe here the case of a 20-years-old man carrier of the c.242 C > T mutation in heterozygosity, in the *SDHD* gene. His mother is affected by multiple paragangliomatosis. He came to our attention because of exercise intolerance with muscle cramps in the upper limbs. Neurological examination was normal. Creatine kinase and lactate levels were normal. Electromyography revealed a myopathic pattern. Muscle biopsy showed mild changes suggestive of mitochondrial myopathy. The patient started therapy with reduced coenzyme Q10 (ubiquinol) with marked benefit.

Our case suggests the need of further, systematic studies to better elucidate the consequences of *SDHD* gene mutations on mitochondrial function in skeletal muscle.

Safe anaesthesia table and undiagnosed myopathy: a three year's experience

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Because of possible severe general anaesthesia complications, myopathic patients represent a challenge for anaesthesiologists. Review of medical literature indicates that currently the great majority of critical side-effects concerns surgery of subjects with clinically-unapparent and thus undiagnosed myopathy (C.U.Myopathy). To help anaesthesiologists in prevention of anaesthesia complications in such patients, we recently outlined [1] a "Safe Anaesthesia Table" (S.A.T.), listing anaesthetic drugs to be avoided and those considered harmless for myopathic patients. Our data concerning methods aimed to identify possible C.U.Myopathy before surgery and the ensuing S.A.T. appliance during their surgery, are presented. Throughout a three-year period, at pre-surgical anaesthesiological examination, 1500 subjects were searched for C.U.Myopathy applying one of the following: a Questionnaire self-administered by patients or, as alternative, a "Correlation-Table" [1] about signs/symptoms suggesting myopathy, derived from Questionnaire and managed by anaesthesiologist. Table and Questionnaire appeared equally useful to the purpose. C.U.Myopathy was recognized in 49 subjects (41: hyperCKemia; 3: clubfoot; 3: dystrophic patients' siblings; 1: ptosis; 1: myotonia). Consequently, the same patients (3.2% from 1,500) underwent surgery with S.A.T. Altogether, recognition of patients with possible C.U.Myopathy and related surgery with S.A.T. appliance enabled anaesthesiologists to avoid anaesthesia complications without delaying surgery.

Late-onset myopathy with undefined features: mitochondrial or lipid storage myopathy?

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We report the case of a 62 year old woman presenting with ptosis, dysphagia, respiratory distress and muscular fatigue after minimal physical exercise. She reported a history of dyslipidemia, high blood pressure, diabetes and hypothyroidism. Neurological examination showed unilateral ptosis, hypophonia, and normal muscle strength. Needle EMG evidenced myopathic changes and nerve conduction studies showed a sensory-motor polyneuropathy. Serum lactic acid level was elevated. Muscle biopsy showed small lipid droplets in type 1 fibers and the presence of COX-negative fibers. Molecular analysis helped in refining the diagnosis.

Late-onset dystrophinopathy due to a novel intronic mutation resulting in skipping of the exon 11: an exception to the "reading frame rule"

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The dystrophinopathies are characterized by a wide spectrum of disease ranging from asymptomatic hyperCKemia to more severe forms including Duchenne/Becker muscular dystrophy and cardiomyopathy. The currently most used rule to predict whether a mutation will result in a severe or mild phenotype is the "reading frame rule". We here report on a 29-year-old patient complaining of left thigh muscle weakness since his 23. Previously, he was a semi-professional football player. When he was 27, he started complaining of moderate proximal weakness at both lower limbs with difficulty in climbing stairs and running. No winging of the scapulae and hypertrophy of calf muscles were observed. Deltoid muscle biopsy showed few cell necrosis and degeneration, marked fiber size variability with atrophic and hypertrophic fibers, rare fiber splitting and only mild focal endomysial fibrosis. The immunohistochemical study by using anti dystrophin antibodies showed globally reduced staining with focal loss of staining in some muscle fibers.

MLPA (multiplex ligation-dependent probe amplification) analysis did not detect deletions, duplications or complex rearrangements in the dystrophin gene. Direct sequence analysis of the dystrophin gene exons and flanking intronic regions revealed a novel c.1150-3C > G substitution in intron 10. Reverse transcription analysis showed the absence of incorporation of exon 11 in the dystrophin RNA.

To date, isolated deletion of exon 11 was not reported in the databases. Mutation alters the reading frame of the gene and is predicted to result in a severe DMD phenotype.

Despite these considerations, our patient presents with a mild and late-onset clinical picture. Therefore, this seems to be a singular rare exception to the "reading frame rule".

Our report expands the clinical and allelic heterogeneity of dystrophinopathies.

Sodium channel gene as modyfing factor of DM2 phenotype

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Myotonic dystrophy type 2 (DM2) is an adult onset muscular dystrophy generally characterized by mild and inconsistent myotonia. New evidences have shown how co-occurrence mutation in chloride channel (CLCN1) may influence the phenotype. To date a possible effect of SCN4A mutation on DM2 phenotype has never been evaluated. In our study we investigated two DM2 patients with severe and early onset myotonia without mutation in CLCN1 gene. In one patient we identified a variant c.215C > T (p.Pro72Leu) in SCN4A gene suspected to be pathogenetic. Whole-cell voltage-clamp analysis showed a hyperpolarizing shift (-5mV) of the voltage dependence of activation that may increase cell excitability. In the other patient we found on SCN4A gene a S906T polymorphism that has been reported to influence channel biophysical properties (Kuzmenkin et al. 2003). In both cases SCN4A variants seem to determine the atypical phenotype of the patients. A SCN4A gene screening is suggested in DM2 patients with early and severe myotonia.

Paternal germline mosaicism in colvi related myopathies: a case report

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Ullrich congenital muscular dystrophy (UCMD) can be due to autosomal recessive mutations in one of the three genes of collagen VI with a related 25% recurrence risk. In the majority of UCMD cases nevertheless, the underlying mutation is thought to arise de novo and the recurrence risk is considered as low. Here we report a family with recurrence of UCMD in two sibs only by father's site. In both, the molecular analysis revealed heterozygosity for the c.896G > A mutation in COL6A1 exon 10 (Gly299Glu) and for the COL6A1 c.1823-8G > A variation within COL6A1 intron 29. The Gly299Glu mutation, despite not previously reported, is likely to be pathogenic, leading to the disruption of the Gly-Xaa-Yaa motif in the triple-helix domain of collagen VI alfa(1)-chain. The intronic variation was inherited from the father and RNA analysis in skin fibroblasts allowed to exclude its role in affecting COL6A1 transcript processing. The Gly299Glu mutation occurred apparently de novo in the two sibs. The described mutational segregation strongly suggests the occurrence of paternal germline mosaicism. The reported family represents the first observation of gonadal mosaicism in collagen-VI related myopathies and, similarly to other collagen-related diseases as osteogenesis imperfecta, this possibility deserve to be considered in genetic counseling and recurrence risk estimation.

Air stacking treatment improves cough efficiency in neuromuscular diseases

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In neuromuscular diseases (NMD), respiratory muscle weakness or glottis dysfunction impairs cough leading to ineffective clearance of airway secretions from lungs.

Air stacking (AS) technique with manual resuscitation bag provides lungs expansion optimizing lung recoil pressure and so increasing peak cough flow (PCF) as previously showed in Duchenne Muscular Dystrophy (DMD).

We have evaluated cough efficiency, by means of PCF and peak expiratory flow (PEF), in 30 NMD patients (16 DMD, 6 SMA type II, 3 Congenital Myopathies, 3 Congenital Muscular Dystrophies, 2 Myotonic Dystrophy) (mean age 17.1; 8.6-31.9 years) with restrictive lung disease (mean FVC 37.1% pred.) before and after six months of daily AS treatment.

FVC and PEF were collected by standard spirometry. PCF was measured by standard peak flow meter connected to an anesthesia mask. Data values were compared by a paired t-test.
In all patients, after a single AS treatment we observed a significant increase in PCF ($p < 0.0001$), in line with what already observed in DMD patients. In addition, at six months of regular daily AS treatment, a significant increase of PCF and PEF ($p < 0.005$ and $p < 0.03$, respectively) was observed in all cases.
Our data show that AS treatment improves the cough efficiency by increasing PCF and PEF, after a single treatment, and particularly after a six months of regular daily treatment not only in DMD, but also in other patients with different neuromuscular diseases.

Improving the diagnosis of Duchenne muscular dystrophy

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Despite the recent advances in the care and management of Duchenne muscular dystrophy (DMD) there has not been a significant improvement in the age of diagnosis over the last 30 years. Recent data suggest that early initiation of steroid treatment could be associated with better long-term outcomes. In addition there are potential promising therapeutic interventions on the horizon and these are likely to be of greater benefit in younger children with less damaged muscles. Early diagnosis of DMD is therefore crucial.
We reviewed the diagnostic process for DMD in boys without a family history at our tertiary centre for Neuromuscular Diseases in England over the last 10 years. The mean age of first reported symptoms of DMD was 2.7 years (32.5 months). First engagement of a health care professional occurred at a mean age of 42.9 months (presentational delay = 10.4 months). Diagnosis of DMD was confirmed at a mean age of 51.7 months (4.3 years). The diagnostic delay (8.8 months) was almost entirely due to delay in testing Creatine Kinase (CK) levels (7 months). The total delay from parental concern to genetic diagnosis was 19.2 months (1.6 years).
Our study showed an improvement in the age of diagnosis in DMD in the UK, however there continues to be a presentational delay, in addition to a delay in obtaining a CK test. To address this and further lower the age of diagnosis of DMD we need to raise awareness of DMD in primary care. We propose screening for DMD with a simple mnemonic as part of the Child Health Care programme in the UK. Any child who presents in primary care with hallmark features of DMD (including unexplained motor or speech development delay) should be offered a CK test as soon as possible. Comparing the diagnostic process for DMD in different countries would also help to identify where and why the delay in diagnosis is still occurring and to identify areas of interventions to improve the care for boys with DMD worldwide.

cPEO and Huntington Disease with reduced penetrance: a singular double trouble

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We report on a patient presenting with the unusual association of two different clinical pictures: a mild movement disorder consistent with HD and a mitochondrial myopathy suggestive for a Chronic Progressive External Ophthalmoplegia (cPEO).
A 70-year-old woman presented with progressive eyelid ptosis, bilateral ophthalmoparesis, dysphagia, dysphonia, mild proximal limb weakness, numbness and fatigue since age of 55. By the age of 64 she noticed some abnormal involuntary movements involving head and limbs, imbalance and gait instability. Mini Mental State Examination was rated 20/30. Family history was unremarkable.
Molecular analysis showed expansion of CAG repeats in the range of "reduced penetrance" (repeat count 38) on HTT gene. Muscle biopsy showed presence of ragged red fibers, fibers with subsarcolemmal accumulations of mitochondria and several cytochrome-C-oxidase negative fibers. No major rearrangements in mitochondrial DNA were detected by Southern Blot analysis. The 22 mitochondrial tRNA genes were directly sequenced and a novel m.5613T>C heteroplasmic mutation was identified in the tRNA Alanine gene, which disrupts a strongly conserved site and fulfills the accepted criteria of pathogenicity.
This is the first reported case of mitochondrial myopathy/HD "double trouble".
Mitochondrial involvement is an emerging key determinant in the pathogenesis of HD and mutant huntingtin influences mitochondrial complex II/III function also in non-neuronal tissue as skeletal muscle. Significant abnormalities on muscle histochemistry are usually not observed in HD patients but few cases displaying an excess of SDH positive and COX negative fibers have been reported.
The supposition of an additive effect of the HD mutation on muscle mitochondrial abnormalities and cPEO phenotype in this patient is intriguing and deserves further studies.

Natural history of muscle pathology in 40 DMD patients aged 1 to 10 years: morphologic and morphometric analysis

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We performed a morphologic/morphometric analysis of muscle biopsies in 40 DMD patients aged 1 to 10 years. We considered the following parameters: fibrotic tissue, necrosis, regeneration, hypercontracted fibers, internal nuclei, fiber size variability, inflammatory reaction.

We found that the increase in connective tissue, already present at birth and stable until the age of six years, rapidly increases from 18.5% to 29.7% at seven years of age. The number of necrotic fibers decreases from 2.12/ light microscopy grid area (0.24 sqmm) at five years of age to 0.81 at the age of six years. Regenerating fibers remain stable throughout the years.
These data allowed us to both clarify the natural evolution of muscle alteration and to define the turning point (around 6 years of age) at which fibrotic degeneration exponentially increases.
These data are extremely useful when deciding the starting age of a clinical trial: indeed, the efficacy of any treatment also depends on the initial degree of muscle alterations, thus, it is possible to speculate that when connective tissue affects a large percentage of muscle fibers, muscle tissue restoration will be difficult. Moreover, establishing the morphologic natural history of the disease evolution is useful to figure out if a particular treatment has any effect.

Features in muscle biopsies of late-onset Pompe Disease patients before and after ERT.

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Glycogenosis type II is an autosomal recessive disorder caused by a deficiency in the glucosidase alpha acid (GAA) enzyme leading to deposition of glycogen in heart, skeletal, and respiratory muscles.
The histopathological hallmarks are muscle fiber vacuolization and autophagy.
GSDII is clinically classified into infantile, juvenile, and late-onset. Recombinant human GAA is the only approved enzyme replacement therapy (ERT). It is effective in most infantile patients whereas the outcome is variable in adults.
We analyzed muscle biopsies from 14 late-onset patients.
All patients clinically improved or remained stable after ERT; morphologically, seven patients improved, two patients worsened and all other subject remained unchanged.
Immunohistochemical results show a variable binding of the autophagic antibodies: EEA1 (early endosome antigen 1), LC3 (microtubule-associated protein 1 light chain 3), and LAMP2 (lysosome associated membrane protein 2).
Five patients show a mild increase in GAA by both biochemical and WB analysis in skeletal muscle.

Validation of the Pediatric Quality of Life Inventory™ Neuromuscular Module and correlation with functional assessments over 12 month follow-up in the Italian DMD population

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The main aim of the study was to translate and validate the Pediatric Quality of Life Inventory™ 3.0 Neuromuscular Module (PedsQL NM) in combination with other selected outcome measures, PedsQL TM 4.0 Generic Core Scales (PedsQL GCS), 6–minute walk test (6MWT), North Star Ambulatory Assessment (NSAA) and timed items, in a large cohort of Italian patients with Duchenne muscular dystrophy (DMD). We also aimed to collect longitudinal data over 12 month interval of all the measurements and to verify possible correlations among them.
So far no systematic study has been planned to correlate QoL results with the outputs of the "gold standard" outcome measures for DMD. We enrolled 98 ambulant DMD patients (age range 5-12,8 yrs) with assessment at baseline and 12 months thereafter. A moderate direct correlation was obtained between PedsQL score (General and NM modules) and NSAA and between PedsQL score (General and NM modules) and 6MWT. The analysis of 12-month changes showed a significant correlation among QoL measures and NSAA and 6MWT results. Interestingly the correlations were stronger in patients' older group (> 7 yrs) and in parents' versions. These results suggest that these tools reflect functional changes and are valid to explore parents' and patients' perspectives in the cohort above the age of 7. Further studies are needed to validate new appropriate tools for younger children.

Acute statin-induced neuromyopathy: a rare condition with severe prognosis

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The clinical spectrum of statin-induced myopathy includes asymptomatic hyperCKemia, myalgia, myositis and rhabdomyolysis. Although statins may increase the risk of developing nerve damage, no clear-cut correlation between their use and peripheral neuropathy has been definitely demonstrated.

We here describe a 74-year-old woman who began to complain of muscle pain and rapidly progressive lower and upper limb weakness one week after starting hypolipidemic therapy with simvastatin 20 mg daily.

Neurological examination revealed proximal and distal limb weakness with diffuse loss of deep tendon reflexes and inability to walk. CK was more than 8000 U/L whereas anti-synthetase and anti-signal recognition particle (anti-SRP) antibodies were absent. Antibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) are still pending. EMG-ENG study showed a marked axonal sensorimotor polyneuropathy associated with muscular spontaneous activity and early recruitment, short duration, low amplitude and polyphasic shape of motor-unit potentials. The quadriceps muscle biopsy showed severe myopathic changes characterized by multiple necrotic and regenerating fibers with no inflammatory infiltrates.

Simvastatin was stopped but, because of the rhabdomyolysis, hemodyalisis was necessary. A three day cycle of therapy with IVIg at 0,4 g/kg daily was started and prednisone 1 mg/kg daily was added. Three weeks later, only a slight improvement in upper limb strength was observed.

Our report illustrates the rare concomitant acute involvement of peripheral nerves and skeletal muscles following treatment with statins. The mechanisms underlying this "full peripheral involvement", being presumably autoimmune, deserve further studies to be clarified.