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ACTA MYOLOGICA

(Myopathies, Cardiomyopathies and Neuromyopathies)

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Official Journal of
Mediterranean Society of Myology
and
Associazione Italiana di Miologia

Founders: Giovanni Nigro and Lucia Ines Comi

Four-monthly

G. CONTE ACADEMY - NAPOLI

PACINI EDITORE MEDICINA

Established in 1982 as Cardiomyology

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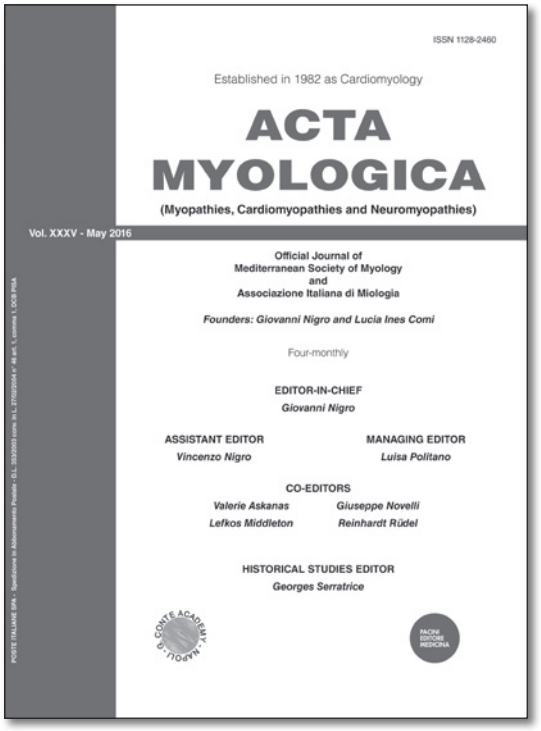
EDITOR-IN-CHIEF

Giovanni Nigro



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EDITORIAL

Acta Myologica Online

Acta Myologica is the oldest international myology journal, with its first issue published in June 1982 with the name of Cardiomyology. At present, it is the official journal of the AIM (Associazione Italiana di Miologia) and of the Mediterranean Society of Myology.

Starting from the next issue (October 2016), Acta Myologica will become fully online with an electronic submission process and a completely free web-based access. Our purpose is to renew this historical printed journal devoted to clinical and molecular myology by disseminating new knowledge through electronic media platforms.

Acta Myologica Online (AMO) will publish readily accessible research and ideas that have clear relevance for diagnosis and treatment of muscle disorders, with a special focus on neuromuscular disorders and cardiomyopathies. AMO will pay attention to the peer review process by accepting articles with consistent new findings in the myology field or clinical reports of patients from countries that have limited presence in the scientific literature.

The renewal process will be ongoing over the next issues and will not be limited to graphic form, but will invest the way to present the scientific contents in order to make them more suitable for computer reading. For example, the text of each section of results will be shortened and always coupled to a figure, without a strict limitation in total article length. This will increase the use of the figures in each paper, by improving the readability.

For review articles or for the discussion in a scientific report, one or more graphical abstracts will be required to illustrate the main points.

A special section will be created to host "sponsored articles". Authors, sponsors, and medical education and communications companies may publish medical information, but only in the form of a peer-reviewed manuscript.

Requirements for author designation will be based on guidelines of the International Committee of Medical Journal Editors (ICMJE).

We are confident that AMO will be a better-suited tool for a new season of exciting myology research.

Vincenzo Nigro
Associate Editor
Seconda Università di Napoli and
Telethon Institute of Genetics and Medicine

www.actamyologica.it

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PROCEEDINGS OF THE XVI CONGRESS OF THE ITALIAN SOCIETY OF MYOLOGY

Lecce, Italy

June 8-11, 2016

Associazione Italiana di Miologia

16° Congresso Nazionale AIM

Lecce, 8 - 11 Giugno 2016

Castello Carlo V

16° Congresso Nazionale AIM — Lecce, 8 - 11 Giugno 2016

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16° Congresso Nazionale AIM Lecce, 8 - 11 Giugno 2016

Programma Scientifico

WEDNESDAY 8TH JUNE

14.00 Registration of participants

15.00 Welcome address

15.40 – 17.00 **WORKSHOP-1: Nutrition and Myopathies**

JOINT AIM-ADI (Associazione Italiana Miologia e Associazione Italiana Dietetica e Nutrizione Clinica):

Chairpersons: A. Caretto, G.Siciliano

15.40 Exercise for skeletal muscle: nutritional strategies *(M. Giampietro)*

16.00 A rationale for a dietary intake in Muscular Dystrophies *(L. Zoni)*

16.20 Nutritional challenges of childhood-adulthood transition in Muscle Disorders *(S. Esposito)*

16.40 The role of nutrition in Metabolic Myopathies *(M. Filosto)*

17.00 – 17.20 Coffee break

17.20 – 18.00 **LECTURE 1: What's new in Muscle Channelopathies** - G. Meola

Introducing:: D. Conte-Camerino

18.00 – 20.00 **Round Table with Patient's Associations:**

- L'alleanza Neuromuscolare Telethon-AIM-ASNP
- Le Reti di Riferimento Europee (ERN) per le Malattie Rare
- La voce dei pazienti e il modello di Telemedicina nelle Malattie Muscolari

Invited Participants:

- UILDM, Duchenne Parent Project, Beta-sarcoglicanopatie, FSHD Italia, Miotonicinassociazione, Mitocon, Associazione Italiana Glicogenosi, ASAMSI-famiglie SMA, CIDP Onlus

- Telethon Foundation

- National Health System Representative

Discussants: M. Moggio, M. Pane, L. Politano

20.30 Opening Ceremony

Associazione Italiana di Miologia

Programma Scientifico

THURSDAY 9TH JUNE

07.30 – 08.30 **BREAKFAST SEMINAR: Respiratory treatment in Muscle Diseases: When, Why and How**
(non accreditato ECM)

Discussants: G. Di Iorio, P. Tonin
Speakers: G. D'Angelo, M. Pedemonte, F. Racca

08.30 – 09.30 **ORAL COMMUNICATIONS**

09.30 – 11.00 **WORKSHOP-2: From epigenomics to phenomics in Muscle Disorders**

Chairpersons: M. Mora, V. Nigro

09.30 Molecular understanding of phenotypic variability in myopathies *(F. M. Santorelli)*
09.50 What do we look for in epigenetic markers in muscle disease? *(L. Migliore)*
10.10 Epigenetics in facioscapulohumeral muscular dystrophy *(F. Magdinière)*
10.30 Epigenetics in myotonic dystrophies *(A. Botta)*

11.00 – 11.20 Coffee break

11.20 – 12.00 **LECTURE-2:** ***In memory of Stefano Di Donato***
Mitochondrial Diseases: the past and the future - M. Zeviani

Introducing: S. Di Mauro

12.00 – 13.30 **ORAL COMMUNICATIONS**

13.30 – 14.30 Lunch

14.30 – 16.00 **PARALLEL POSTER SESSION-1**

16.00 – 17.00 **ORAL COMMUNICATIONS**

17.00 – 17.20 Coffee break

17.20 – 18.50 **WORKSHOP-3: Neuroimaging diagnostics in Muscle Disease: power and limitations**

Chairpersons: R. Massa, M. Scarpelli

17.20 Muscular dystrophies *(G. Tasca)*
17.40 Inflammatory Myopathies *(S. Previtali)*
18.00 Metabolic Myopathies *(O. Musumeci)*
18.20 Brain in muscle diseases *(E. Bertini)*

18.50 – 20.00 **Future Projects and Programs for AIM**

Chairpersons: G. Antonini, G. Siciliano

16° Congresso Nazionale AIM — Lecce, 8 - 11 Giugno 2016

Programma Scientifico

FRIDAY 10TH JUNE

08.15 – 09.30 **Muscle Club**
Discussants: A. Di Muzio, G. Ricci

09.30 – 10.10 **LECTURE-3: New experimental perspectives in Myology** - A. Musarò
Introducing: G. Nicolardi

10.10 – 10.25 Coffee break

10.25 – 12.15 **WORKSHOP-4:**
New concepts in therapy of Muscle Disorders: DMD, SMA, s-IBM and Pompe disease
Chairpersons: C. Minetti, S. Servidei
10.40 Duchenne Muscular Dystrophy *(E. Mercuri)*
11.00 Spinal Muscular Atrophy *(S. Messina)*
11.20 Sporadic Inclusion Body Myositis *(M. Mirabella)*
11.40 Pompe disease *(A. Donati)*
12.00 Adulthood acid maltase deficiency *(T. Mongini)*

12.15 – 13.30 **ORAL COMMUNICATIONS**

13.30 – 14.30 Lunch

14.30 – 16.00 **PARALLEL POSTER SESSION-2**

16.00 – 17.15 **ROUND TABLE: Trial readiness in Muscle Diseases**
Charing: C. Angelini, G. Vita

Principles and methodology for guidelines *(M. Leone)*, Guideline Issues on Myopathies and Myasthenia Gravis in Italy *(A. Toscano and R. Mantegazza)*, Outcome Measures in Mitochondrial Disorders *(M. Mancuso)*, Diseases Registries *(G.C. Logroscino)*

17.15 – 17.30 Coffee break

17.30 – 19.00 **Assemblea dei Soci**

20.30 **Social dinner**

Associazione Italiana di Miologia

Programma Scientifico

SATURDAY 11TH JUNE

08.30 – 10.00 **WORKSHOP-5: Congenital and myofibrillar myopathies**

Chairpersons: A. Berardinelli, M. Sciacco

08.30 Genotype-phenotype correlations in congenital myopathies *(C. Fiorillo)*

08.50 New genes in congenital myopathies *(E. Pegoraro)*

09.10 Myofibrillar myopathies *(G. Comi)*

09.30 Titinopathies *(M. Savarese)*

09.50 – 10.30 **LECTURE-4: Recent steps towards treatment in Laminopathies** - A. Muchir

Introducing: G. Lattanzi

10.30 – 10.45 Coffee break

10.45 – 12.00 **WORKSHOP-6: Disorders of Neuromuscular Junction**

Chairpersons: P. Bernasconi, R. Liguori

10.45 New insights in immunopathogenic mechanisms *(A. Evoli)*

11.05 Paraneoplastic NMJ syndromes *(C. Rodolico)*

11.25 Late onset congenital myasthenic syndromes *(L. Maggi)*

11.45 Assessment of long term efficacy of thymectomy *(R. Ricciardi)*

12.05 – 13.05 **ORAL COMMUNICATIONS**

13.05 **AIM 2016 best contribution awards**

13.10 – 13.30 **ECM questionnaire**

13.30 **Congress Closure**

ABSTRACTS OF INVITED LECTURES

(in alphabetical order of the first Author)

Brain in muscle diseases

Bertini E.

Unit of Neuromuscular and Neurodegenerative Disorders, Laboratory of Molecular Medicine, Bambino Gesù Children's Hospital IRCCS, Rome

It is well known that brain involvement is generally unusual in muscle disease, and should be considered in the algorithm of the differential diagnosis of myopathies. Classification of brain involvement in myopathies, excluding mitochondrial encephalomyopathies, can be classified for convenience in two main subgroups:1) those conditions with mental disabilities that are not associated with brain malformations; and 2) those that are associated with malformations or peculiar brain abnormalities, and can be detected by MRI.

The main neuromuscular disorders that show mental disability without a brain malformations or distinctive MRI abnormalities are dystrophinopathies, particularly occurring in 40-50% of boys affected by Duchenne muscular dystrophy with mutations generally downstream exon 44, which are associated with involvement of dystrophin isoforms expressed at high levels in brain. Moreover, another condition with high prevalence that is associated with mental disability is DM1. Besides typical motor symptoms, DM1 patients also display non-motor symptoms such as particular personality traits. Around 30% of DM1 patients seem to be at high risk of developing a psychiatric disorder. Moreover, psychological traits differ across phenotypes, with the most severe phenotype tending to show more severe psychological symptoms. Some studies have shown that the presence of higher phobic anxiety and lower self-esteem are associated with lower education, a higher number of CTG repeats, more severe muscular impairment, and lower cognitive functioning.

In the subgroup of brain malformations or brain abnormalities, the classical conditions are congenital muscular dystrophies (CMDs) with abnormal glycosylation of alpha-dystroglycan (Fukuyama CMD, Muscle-eye-brain disease, Walker-Warburg syndrome, CMD1C, CMD1D). The spectrum of brain structural defects is wide, ranging from complete lissencephaly in patients with Walker-Warburg syndrome to isolated cerebellar involvement. Cerebellar cysts and/or dysplasia and hypoplasia are also predominant features in patients with FKRP, POMGnT1 mutations, but rarely seen in POMT1 and POMT2. Brainstem and pontine abnormalities are common in patients with POMT2, POMGnT1, and LARGE mutations. Abnormalities of the white matter are characteristically seen in CMD with merosin deficiency (CMD1A), also in the mildest cases with a LGMD phenotype. Moreover progressive brain MRI abnormalities are observed in adult DM1 patients as white matter involvement and brain atrophy, and some studies underline that WM damage is likely to be the major contributor to cognitive impairment in DM1.

Epigenetics in Myotonic Dystrophies

Botta A.

Dept. of Biomedicine and Prevention, Tor Vergata University of Rome, Italy

Myotonic dystrophy type 1 (DM1, Steinert's disease, MIM#160900) is the most common form of adult-onset muscular dystrophy in humans, characterized by myotonia, muscle weakness, cataract, cardiac disease and central nervous system dysfunctions. The molecular defect underlying DM1 consists in the expansion of an unstable CTG repetition located in the 3'UTR of the *DMPK* gene, on chromosome 19q13. Given the emphasis on post-transcriptional mechanisms in DM1, only a few publications discussed the role of epigenetic alterations in the disease, either as a mechanism to explain repeat instability or as the cause of altered expression of the DMPK transcript itself. Additional complexity arises with production of an antisense transcript that initiates within the *SIX5* adjacent promoter, producing a CAG-containing RNA able to induce transcriptional silencing and heterochromatin formation. In a recent study, we showed that in DM1 uninterrupted alleles, hypermethylation occurs only in the upstream region of the CTG repeat, and this modification was present in patients with larger CTG expansion (> 1000), earlier age at onset (< 18 years) and in congenital DM1. The association of hypermethylation with congenital or childhood onset forms, characterized by severe cognitive manifestations, suggest that this epigenetic modification might affect the expression of genes regulating brain development and/or or synaptic plasticity. Conversely, in "atypical" DM1 patients carrying CCG/CTC/CGG interruptions at the 3' end of the CTG array, hypermethylation occurs exclusively in the downstream region of the DM1 expansion. Our results suggest that either the inherited size of the expanded allele and the presence of interruptions in the CTG array are associated with a highly polarized pattern of CpG methylation at the DM1 locus. Additional studies on different DM1 tissues and eventually in DM2 patients will help to understand the functional role of epigenetic modifications in the pathogenesis of myotonic dystrophies.

Myofibrillar myopathies

Comi G.P.
Dino Ferrari Centre, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Neurology Unit, I.R.C.C.S. Foundation Ca' Granda, Ospedale Maggiore Policlinico, Milan

Myofibrillar myopathies (MM) are a heterogeneous group of diseases characterized by progressive muscle weakness and common histological features, including abnormal accumulation of myofibrillar degradation products, the ectopic expression of intrasarcoplasmic proteins, the presence of vacuoles, foci of myofibril dissolution and a disorganization of the intermyofibrillar network beginning at the Z-disk.

The underlying mechanisms of the disease are still unknown and can differ from a type of disorder to another. The main abnormalities observed in pathophysiological studies include aggregation and decreased elimination of abnormal mutant proteins. Severe clinical patterns have been observed and six main genes (DES, CRYAB, LDB3/ZASP, MYOT, FLNC and BAG3) are now classically considered as responsible for Myofibrillar myopathies. Other entities such as FHL1 myopathy or Hereditary myopathy with early respiratory failure linked to mutations of titin can now as well be included in this group. Occasionally MM patterns have been described in laminopathies or selenopathies. Recently two genes, HSPB8 and DNAJB6, have been associated to a myopathy with histologic features of MM with aggregates and rimmed vacuoles. HSPB8 and DNAJB6 are part of the chaperone-assisted selective autophagy (CASA) complex. In these cases the molecular muscle pathology is apparently mediated through impaired CASA functions and possibly other complexes needed for the maintenance of the Z-disk and sarcomeric structures. Next-generation sequencing technology will expand our genetic knowledge.

The diagnosis of MFM is not always easy as histological lesions can be focal, and muscle biopsy may not lead to a focused genetic analysis; this has led to a growing importance of muscle imaging, and the selectivity of muscle involvement has now been described in several disorders. Therefore a precise knowledge of clinical presentation, age of onset, muscle selectivity on muscle imaging and histological abnormalities will help in many cases to find the genetic cause of the disease.

New concepts in therapy of Pompe disease

Donati M.A.
Metabolic and Muscular Unit, Department of Neuroscience, Meyer Children's Hospital, Firenze, Italy.

Pompe disease is a rare disorder due to deficiency of alpha-glucosidase (GAA). Infantile Onset Pompe Disease (IOPD) is characterized by early generalized hypotonia and severe cardiomyopathy with death within the first year of life, infantile variant form shows slower progression and less severe cardiomyopathy but onset is in the first year of life, childhood/juvenile variant is a heterogeneous group presenting later than infancy and usually without cardiomyopathy.

The efficacy of enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) had been shown in two studies demonstrating a clear improvement of cardiomyopathy and prolonged survival in all treated infants but it is clear that the early start yielding better results. ERT became commercially available only in 2006. A critical point became evident a few years later: patients with high titer anti-rhGAA IgG antibodies had a worse outcome than those with low-titers, and those having high titers were mainly CRIM (cross reactive immunologic material)-negative. Aiming to improve response to ERT in CRIM-negative IOPD, several immune tolerance induction strategies have been reported.

Italian data of 29 Italian treated IOPD patients and the long-term outcome are similar to that reported by other national multicenter studies: a consistent number of patients become ventilatory dependent on the long term and many have a secondary loss of the previously reached motor milestones, age and signs and symptoms at start of ERT and CRIM-negative status influenced negatively the outcome.

We underline good outcome in childhood/juvenile patients with early diagnosis and start ERT.

GAA enzyme assay on dried blood spot (DBS) is reliable, non-invasive and specific for early diagnosis in symptomatic patient and in newborn screening programs (NBS). We have started a pilot NBS program for Pompe disease in Tuscany and Umbria since 2014.

Oral chaperone therapy, modified rhGAA, autophagy suppression and gene transfer represent potentially promising novel therapies.

Myasthenia gravis: new insights in immunopathogenic mechanisms

Evoli A.
Institute of Neurology, Catholic University, Roma, Italy

Although myasthenia gravis (MG) is considered a prototype antibody-mediated condition, pathophysiology investigations are complicated by the disease heterogeneity. Three main antigens have been described in MG, so far. In the great majority of patients, antibodies (Abs) against the acetylcholine receptor (AChR) can be detected, 5-8% of MG cases have serum Abs to the muscle-specific tyrosine kinase receptor (MuSK), while a few "double-seronegative" patients harbor Abs against the lipoprotein-related protein 4 (LRP4). Autoantibody profile,

age at the disease onset and weakness pattern identify patient subgroups and are relevant for clinical management.

Like in other autoimmune conditions, immunological, genetic and environmental factors play a role in MG development. In AChR-MG, genome-wide association studies have confirmed the association with HLA genes, with differences between patients with early-onset and late-onset disease. Other associated genes, as PTPN22 (tyrosine phosphatase non-receptor 22), CTLA4 (cytotoxic T-lymphocyte antigen 4), TNFRS11A (TNF receptor superfamily 11a) and BAFF (B cell activating factor), are involved in the immunological response. MuSK-MG association with HLA DR14/16 and DQ5 has been confirmed in several ethnic groups. Few studies have evaluated microRNA profile in MG cohorts, so far with differing results.

Among environmental factors, viral infections could trigger inflammatory changes of the thymus leading to autoimmunization against the AChR, while a defective T cell selection seems to be responsible for tolerance breakdown in thymoma-associated MG. Failure of peripheral tolerance control, as defects of regulatory T and B cells, as well as increased levels of inflammatory cytokines have consistently been reported in MG.

The investigation of Ab specificities has further contributed to the understanding of MG pathogenesis. While complement activation and antigen modulation are the main effects of IgG1/IgG3 anti-AChR Abs, anti-MuSK are mainly IgG4 Abs that interfere with the protein function.

The role of nutrition in metabolic myopathies

Filosto M.
Clinical Neurology, Center for Neuromuscular Diseases and Neuropathies, University Hospital, "Spedali Civili", Brescia, Italy

Metabolic myopathies, i.e. glycogen storage myopathies, lipid storage myopathies and mitochondrial myopathies, are hereditary muscle disorders caused by specific enzyme defects due to defective genes. They are heterogeneous conditions sharing an energy metabolism impairment resulting in skeletal muscle dysfunction.

Although significant progresses have been made in understanding molecular and pathophysiological mechanisms underlying this group of diseases, therapeutic approaches are, except for rare cases, largely unsatisfactory.

In the last years, a considerable interest has grown in exploring the effects of nutritional modifications as a therapeutic intervention in metabolic myopathies. Changes in macronutrient composition or administration of nutritional supplements have been evaluated in order to improve skeletal muscle function, bypass fuel deficiency or substitute fuel sources.

Ketogenic diet, high-protein diet, carbohydrate-rich diet, low-carbohydrate diet have been often proposed as a treatment for some metabolic myopathies with varying results.

Supplemental nutrients have been used in some specific diseases as sucrose taken before exercise in GSDV, riboflavin in multiple acyl-CoA dehydrogenase deficiency and nicotinamide riboside in mitochondrial myopathies.

However, strong experimental evidences supporting both short- and long-term effects and safety of diet modifications or supplementation are often still lacking and many variables can impact the efficacy of treatment, i.e. nutrient type, timing and duration of administration, as well as age of patients and duration of disease. Moreover, only few controlled trials including small numbers of participants have been conducted in some metabolic myopathies. Thus, definitive conclusions about the role of nutritional modifications in metabolic myopathies cannot yet be completely drawn and more detailed studies on larger numbers of patients are needed.

Genotype-phenotype correlations in congenital myopathies

Fiorillo C.
UOC Neurologia Pediatrica e Malattie Muscolari, Istituto Gaslini, Genova

The congenital myopathies (CM) are genetic skeletal muscle diseases, which usually present at birth or in early infancy. They are heterogeneous for genetic background and disease severity. Clinical manifestations range from foetal akinesia, with lethality in the newborn period, to later onset cases with milder muscle impairment. Typically, CM are defined by the presence of one or more characteristic histological features on muscle biopsy; namely cytoplasmic rods structures or nemaline bodies (Nemaline Myopathies), cores and minicores areas (Core Myopathies), central displacement of the nuclei (Centronuclear and Myotubular Myopathies) and disproportion of distribution and diameter of fibre subtypes (Congenital Fibre Type Disproportion).

Over the past decade there have been great advances in defining the genetic basis of CM subtypes, and it is now known that most of the genes involved in the pathogenesis of CM encodes for proteins with a fundamental role in muscle structure and functioning such as contractile proteins, light filaments proteins, microtubular network proteins, sarcolemmal proteins and protein involved in molecule turnover. However the relationship between each form, defined on histological grounds, and the genetic cause is complex. Infact the same CM can be due to defect of distinct genes, whereas mutations in the same gene can cause different muscle pathologies. On a clinical prospective the same genetic defect has been linked to

variable degree of severity and analogously the same histopathological alteration can determine different clinical manifestation. Still some correlations can be made thanks to the increasing availability of studies describing and collecting results from congenital myopathies patients.

This work will summarize the different forms of CM and show some clinical, histopathological and genetic data from our population of 40 affected children. We will also focus on the recent progress in the understanding of the pathogenesis of these disorders, which are fundamental basis for genotype-phenotype correlation and at the same time able to provide diagnostic clues to use in clinical practice.

Physical exercise and muscle tropism: nutritional strategy

Giampietro M.
Coordinatore gruppo tematico "Attività fisica e salute. Alimentazione e sport" dell'A.D.I.

Under physiological conditions muscle increase depends on various factors: sex, age, genetics, training and feeding.

The protein synthesis decreases during and immediately after the training session and it subsequently increases to reach a maximum peak twenty-four hours after.

For muscle tropism it is important to respect recovery times between two training sessions: increasing loads, intensity and number of repetitions and series are all factors that can be very "aggressive" for the muscle, but it is very "aggressiveness" that stimulates its growth..

The building of muscle masses requires very intense training programs that involve an increase in energy and protein requirements, and in micro-nutrients.

In sports and in athletes the primary nutritional objective must be an adequate supply of energy, mainly in the form of carbohydrates, to enable them to maintain good muscle glycogen stores, and an equally adequate protein intake, that can allow repairing tissues that have been "damaged" during exercise and the growth of the of the muscle mass.

The lipid intake does not require special increments.

Only in the case of adult subjects who regularly perform muscle strengthening programs and practice "resistance exercise" at least twice a week feeding acquire specific characteristics, such as taking a larger amount of protein (1,0-2,0 g/kg b.w./day) which allows to support metabolic adaptation, and the repairing and remodeling of muscle cells, as well as turnover.

In addition, a carbohydrate-protein snack, within 20-30 minutes after training session, can ensure optimal post-exercise recovery.

Protein intakes greater than 2g/kg b.w./day seem to have no additional effects on performance, nor are they able to enhance the volume of muscle mass: protein synthesis therefore shows no linear correlation with protein food intake but reaches a plateau at relatively low protein levels.

Increased intakes may prove appropriate for a short period of time during the training period (away from racing), or in the case of energy restriction.

Epigenetics in Facio-Scapulo-Humeral Dystrophy

Magdinier F.
Laboratory of Medical Genetics & Functional Genomics. INSERM UMRS_910

Facio-Scapulo-Humeral Dystrophy is a common muscular dystrophy characterized by weakness of specific muscles. It is linked in 95% of cases (FSHD1) to shortening of an array of macrosatellite elements, *D4Z4*, at the subtelomeric 4q35 locus. In the 2-3% of patients, the pathology is associated to mutation in the *SMCHD1* gene (FSHD2). For the rest of patients, the cause is still undetermined.

At the molecular level, the pathology is associated with hypomethylation of *D4Z4*. The current model proposes that chromatin decompaction triggers activation of the DUX4 retrogene encoded by the last *D4Z4* repeat and activation of a cascade of genes. If DUX4-fl expression represents an interesting indicator, its direct involvement in the pathology progression and onset remains puzzling and other pathways cannot be excluded.

At the epigenetic level, a key question FSHD is whether hypomethylation is a cause (instability), a consequence of *D4Z4* array shortening or linked to the presence of symptoms. We have evaluated *D4Z4* methylation in clinically affected FSHD patients, asymptomatic carriers, FSHD2 and controls and shown that symptoms correlates with a low methylation level in FSHD1 and FSHD2 compared to controls but also to asymptomatic carriers indicating that *D4Z4* hypomethylation is not directly linked to shortening of the array but suggesting a direct correlation between hypomethylation and clinical signs.

To investigate the regulation of *D4Z4* methylation, we generated human induced pluripotent stem cells (hiPSCs) from FSHD1 patients and FSHD2 patients carrying *SMCHD1* mutation. Besides, we developed a new protocol to generate muscle progenitors and multinucleated fibers from these hiPSCs. We showed that *D4Z4* methylation is dynamically regulated after reprogramming and differentiation with differences between FSHD1 and FSHD2 suggesting a role for SMCHD1 during differentiation. Altogether, our data emphasize hiPSCs as an interesting model to investigate the early events regulated by SMCHD1, D4Z4 array contraction and FSHD pathomechanisms.

Late onset congenital myasthenic syndromes

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Congenital myasthenic syndromes (CMS) are a heterogeneous group of rare genetic disorders affecting the neuromuscular junction (NMJ). Diagnosis is mainly based on clinical history, examination and neurophysiological studies. To date, 21 genes, encoding for presynaptic, synaptic and post-synaptic NMJ proteins, have been implicated in CMS. CMS are recessively inherited, apart from slow-channel syndrome, which is a dominant disease. At present genetic mutations are identified in only about 50% of CMS patients. The molecular mechanisms arising from mutations influence the phenotype, including treatment response and disease course over time. CMS treatment is based on different symptomatic therapies, which could markedly improve symptoms over the time. CMS are clinically characterized by non-progressive fluctuating muscle weakness and fatigability involving limb, trunk, bulbar, respiratory, facial and extra-ocular muscles. Although most of the cases present at birth or within the first year of life, a recent study on a wide cohort of CMS patients in UK showed that about 15% of patients had onset between 3 and 10 years of age, 5% in the second decade and 8% after the age of 20 years. Hence, a relevant amount of patients present after the age of 3 years and also since the second decade of life; among these patients the genetic characterization was reached in a smaller proportion than in the cohort with onset before the age of 3 years. Of note, late-onset CMS, in particular in adult life, should be differentiated from the more common autoimmune form. In contrast to the acquired myasthenic syndromes, antibodies are absent and there is no response to immunomodulation or immunosuppressive treatments. Genes more frequently involved in late-onset CMS are *DOK7*, *RAPSN, GFPT1* and *AGRN*; in addition slow-channel syndrome due to mutations in subunits of the acetylcholine receptor may present in second decade of life or later.

What's new in Muscle Channelopathies

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Skeletal muscle channelopathies are an important group of genetic muscle diseases characterized by dysfunction of ion channels that regulate membrane excitability. The clinical manifestations are often striking, with an inability to relax after voluntary contraction (myotonia) or transient attacks of severe weakness (periodic paralysis). An essential feature of these disorders is fluctuation of symptoms that are strongly impacted by environmental triggers such as exercise, temperature, or serum potassium levels. They are rare disorders characterized by marked genotypic and phenotypic variability. A mutation in voltage-gated muscle sodium (gene: SCN4A) of the skeletal muscle can cause paramyotonia congenita, sodium channel myotonia, hyperkalemic periodic paralysis and hypokalemic periodic paralysis. Ninety percent of cases of hypokalemic periodic paralysis are caused by mutation of the calcium channel (gene: CACNA1S). Myotonic disorders can similarly be caused by sodium channel or chloride channel (gene: CLCN1) defects. Andersen-Tawil syndrome is caused by mutation of the inward rectifier potassium channel (gene: KCNJ2). These disorders can cause lifetime disability and affect quality of life. Recognition and treatment of symptoms might reduce morbidity and improve quality of life. Detailed characterizations of human genetic mutations in voltage-gated muscle sodium, chloride, calcium and inward rectifier potassium channels have resulted in new insights into disease mechanisms. An update of clinical manifestations, diagnostic studies, pathophysiology, and treatment options in skeletal muscle ion channelopathies will be discussed.

New concepts in therapy of muscle disorders: Duchenne muscular dystrophy

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A better understanding of the mechanisms underlying the molecular pathogenesis of Duchenne muscular dystrophy and availability of preclinical models hsve led. In the last edcade, to the development of a number of experimental approaches, some of which already in clinical trial.

Several studies have eneterd the clinical arena in the last few years with different approaches,: many of the recent approaches aim to resteore dystrophyn. One recent exciting development has been that of targeting the mutant RNA in DMD using antisense oligonucleotides (AO) to induce exon skipping and restore the reading frame in boys with eligible deletion. As ~ 70% of DMD boys have out-of-frame deletions, the strategy to target the pre-mRNA and induce exon skipping, restoring the open reading frame and generating internally deleted but in-frame molecules (hence mimicking what happens naturally in the much milder BMD condition) has rapidly moved, to phase III clinical trial. The use of drugs (Ataluren) promoting..ribosomial readthrough of nonsense mutations has led to the approval of the first drug for DMD.

Other approaches incliude pharmacological upregulation of the dystrophin-related protein utrophin is also

being pursued as this is believed to partially compensate for dystrophin deficiency.

More recently other approaches focus on the possibility to reduce the progression of the dystrophic process , by preventing the progression of fibrosis and/or incraesing muscle mass.

New concepts in therapy of Muscle Disorders: spinal muscular atrophy

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In the past decade, improved understanding of spinal muscular atrophy (SMA) aetiopathogenesis has brought us to a relevant turning point towards the development of disease-modifying treatments. The increasingly precise delineation of molecular targets within the survival of motor neuron (SMN) gene locus has led to the development of promising therapeutic strategies. These novel avenues in treatment for SMA include gene therapy, molecular therapy with antisense oligonucleotides, and small molecules that aim to increase expression of SMN protein. Stem cell studies of SMA have provided an in vitro model for SMA, and stem cell transplantation could be used as a complementary strategy with a potential to treat the symptomatic phases of the disease. We will provide an overview of established data from preclinical evidence and ongoing clinical trials. The final remarks will be dedicated to future clinical perspectives in this rapidly evolving field, with a discussion on the comparison between the outlined therapeutic approaches and the remaining open questions.

What do we look for in epigenetic markers in muscle disease?

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Epigenetic marks can be defined as heritable modifications of the genome (DNA sequence or associated proteins, like histones) without change in primary DNA sequence. Epigenetic modifications, that have a fundamental role in the regulation of gene expression, involve DNA methylation, specific histone modifications and other forms of higher-order chromatin remodeling affecting how tightly DNA is packaged in chromatin and, consequently, its accessibility to the transcriptional machinery; as well as post-transcriptional interventions by noncoding RNAs [notably silencing RNA and microRNA (miRNA)].

Both genome and epigenome can contribute to phenotypic variation and disease susceptibility, and the complex outcome of common and rare diseases may be due or be exacerbated by both genetic and epigenetic anomalies as well as the interaction between them.

Aberrant epigenetic regulation is becoming an integral aspect of many monogenic diseases and of an increasing number of complex disorders, including muscle disease. A common pathophysiological pathway caused by epigenetic changes is activated in some forms of congenital neuromuscular disorders, such as ryanodine receptor 1 (RYR1)-related myopathies. Some muscle disease are included in repeat expansion disorders and a role for epigenetic alterations in their pathogenesis has been proposed, as for myotonic dystrophy type 1 characterized by a CTG repeat expansion in *DMPK* gene, near a differentially methylated region (DMR). Facioscapulohumeral muscular dystrophy (FSHD), is caused by disrupted genetic and epigenetic regulation of a macrosatellite repeat.

Also the mitochondrial genome could be epigenetically modified, in a manner similar to the well characterized epigenetic modification of the nuclear genome. Increasing evidence suggests the role of the mitochondrial epigenetic signatures in both physiological and pathophysiological conditions.

It is clear that the emerging field of epigenetics is already generating novel potential therapeutic chance even for this group of diseases.

Sporadic inclusion-body myositis (IBM)

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Treatment of IBM remains a challenge because of its complex pathogenesis and resistance to immunosuppression due to underlying muscle degenerative cascade. Because of muscle inflammation, various combinations of *prednisone* with *other immunosuppressants* have been trialed in IBM with most studies being inconclusive. Immunosuppressive therapy may potentially benefit small subsets of IBM patients, however current evidences do not support the use of immunosuppressive therapy. Improvement of dysphagia was reported with *intravenous immunoglobulin* though this was not replicated in larger cohorts. Based on small case series also *subcutaneous immunoglobulin* are now being proposed. *Lymphocyte depletion therapy* suggested to reduce muscle weakness by decreasing muscle injury related to T-cell response have shown unconvincing results, likewise *TNF-blocking agents* showing no effect on disease progression.

Since IBM muscle is characterized by aggregates containing amyloid-_ and a number of oxidative or cell stress-related proteins**,** *lithium* was proposed as potential treatment; however, a 12-month study observed no significant benefit in muscle strength.

Anakinra, interleukin-1 receptor antagonist, evaluated in IBM to reduce amyloid deposits produced mixed results. *Arimoclomol,* a potential treatment increasing production of heat shock proteins, appeared safe and well tolerated, but no evidence of efficacy was observed in a recent proof-of-concept trial in IBM.

Stem cell transplantation is also being evaluated for IBM. A phase I trial with *hematopoetic stem cells* after high dose immunosuppression is underway. Modulation of factors selectively dysregulated in IBM regulating myogenic differentiation of *mesoangioblasts* may represent an attractive approach to enhance muscle regeneration.

Several strategies currently investigated in IBM patients aim to restore muscle mass (*bimagrumab, follistatin gene transfer, regular exercise programs*). Unfortunately the primary study end-point of the pivotal trial with bimagrumab was not reached. There is an urgent need for trials exploring new strategies to concurrently suppress degenerative and inflammatory changes and counteract muscle atrophy. Implementation of an *individualized exercise regimen and orthotic intervention* may help patients to reduce falls and temporarily reduce disease progression. Further insights into local cues affecting muscle regeneration will also be critical to design strategies to treat IBM, alone or in combination with stem cells therapy.

Adulthood acid maltase deficiency

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Ten years after the formal approval by Regulatory Drug Agencies of the recombinant enzyme therapy for the glycogenosis type 2 (acid maltase deficiency or Pompe disease), the knowledge about this disorders has dramatically expanded, thanks to the strong impulse on the scientific community to understand and to ameliorate patients clinical outcomes and quality of life.

In Italy, the AIM Italian Study Group for Pompe Disease has been active since 2006, collaborating on common clinical protocols and recommendations, and following about one hundred of patients on therapy. The treatment of Pompe disease, a rare lysosomal disorders affecting patients at any age and with variable severity, requires the coordinated effort of a multidisciplinary team of experts in neuromuscular disorders. In fact, ERT has changed the natural history of the diseases, producing in adult patients a stabilization over the years and reducing the mortality rate; however, complementary therapies have also shown an important role, such as exercise, diet, and respiratory support. New therapies are under investigation, including modification to the recombinant GAA to improve the drug entering into muscle cells, and gene therapy mediated by AAV; both approaches are in advanced phase of clinical investigation and will add new options for the knowledge of the disease and for patients improvement.

Recent steps towards treatment in laminopathies

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The nuclear envelope separates the cytoplasm from the nucleus in eukaryotic cells. It is composed of the nuclear membranes, nuclear lamina and nuclear pore complexes. The hypothesis that nucleus compartmentalization plays an important role in human diseases is an old and recurrent story. Mutations in *LMNA*, encoding nuclear A-type lamins, cause a range of clinical disorders that affect different tissues and some that even affect the aging process itself, highlighting the many crucial functions localised to the nuclear envelope. The reasons for this remain a puzzle. Several hypotheses have been proposed attempting to link the pathophysiology of laminopathies to known or emerging functions of A-type lamins. Prominent amongst these hypotheses include those based on functions that A-type likely have in maintaining the mechanical integrity of cells subject to stress.

An emerging body of work supports a new view of the nuclear envelope as a node that integrates and transduces a range of signals during development and in terminally differentiated cells. Studies of laminopathies have provided insights into novel functions of the nuclear envelope. These studies strongly suggest that the nuclear lamina, although serving as a structural support for the nuclear membranes, must have additional functions. As very different disease phenotype can result from alterations in lamins, the nuclear lamina likely has cell-type and tissue-selective properties. Hence, beyond its classical barrier function, studies of the nuclear envelope are increasingly providing insights into basic aspects of cellular organisation and function and providing novel insights into the pathogenesis of human disease. Dissecting these pathways would likely lead to the discovery of new mechanisms that control development and tissue-specific diseases.

In the past decade, there has been an explosion of researches on the nuclear lamina, which to a large extent has been stimulated by attempts to explain the pathogenesis of laminopathies. A main unanswered question is how mutations in genes expressed in virtually all differentiated somatic cells lead to such a wide range of human diseases affecting specific tissues or the aging. In the case of *LMNA*, a second major question is how different mutations in this one gene lead to numerous different disease phenotypes in addition to those involving striated muscle.

Within the next several years, we will likely have more clues to answer these questions and these answers will hopefully lead to new ways to treat or prevent laminopathies.

New experimental perspectives in Myology

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The prolongation of skeletal muscle strength in neuromuscular diseases, including muscular dystrophy, has been the objective of numerous studies employing a variety of approaches. Stem cell therapy represents a promising tool. However, this approach is not definitive yet and several hurdles limit the immediate translation of this strategy into clinic. One of the crucial parameters is the microenvironment in which the stem cell populations should operate. Among critical parameters, the activation and persistence of inflammatory pathways, associated with several neuromuscular diseases, may render the muscle incapable to sustain and complete an efficient muscle regeneration. The anti-inflammatory agents glucocorticoids (GC) are the only available treatment for certain genetic diseases, including Duchenne muscular dystrophy (DMD). However, long-term GC treatment causes muscle atrophy and wasting. Thus, targeting specific mediator of inflammatory response may be more specific, more efficacious, and with fewer side effects. The pro-inflammatory cytokine interleukin (IL) 6 is overproduced in patients with DMD and we recently demonstrated that IL-6 is causally linked to the pathogenesis of muscular dystrophy. We also demonstrated that blockade of endogenous IL6R conferred on dystrophic muscle resistance to degeneration and alleviated both morphological and functional consequences of the primary genetic defect. Pharmacological inhibition of IL6 activity leaded to changes in the dystrophic muscle environment, favoring anti-inflammatory responses and improvement in muscle repair. This resulted in a functional homeostatic maintenance of dystrophic muscle. Similarly, we demonstrated that up-regulation of the growth factor mIGF-1, promotes a qualitative environment, modifying the expression of some of the active players of muscle homeostasis. This results in the activation of circuitries that confer robustness to dystrophic muscle. This study promises to significantly advance our understanding of the pathogenic mechanisms that lead to DMD disease in humans and offers novel approaches for treatment of this fatal disease.

Neuroimaging diagnostics in metabolic myopathies

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Metabolic myopathies are a group of rare inherited neuromuscular disorders due to a dysfunction of muscle bioenergetic machinery at different levels. Clinically are heterogeneous and may manifest with premature muscle fatigue, myalgia, episodic rhabdomyolysis, and myoglobinuria, typically occur with exercise or with proximal and axial muscle weakness. Some of them may have a multisystemic presentation. The diagnosis is the result of clinical, morphological and biochemical studies but in the last years neuroimaging is increasingly becoming an important diagnostic tool. In particular muscle MRI has been established as a very accurate technique to detecting muscular involvement as well as describing the degree and pattern of involvement thanks to modern protocols that enable to define the grade the severity of the disease process with greater sensitivity than other clinical scores. Thanks to its high sensitivity in the detection of muscular abnormalities and a good inter-observer and intra-observer agreements, muscle MRI seems very useful in the early stages of the disease but also in some metabolic myopathies as distal glycogenoses or lipid storage myopathies were muscle damage is not often clinically detectable. Recent studies provide evidences that muscle MRI can be use a good biomarker to evaluated disease progression and to apply in clinical and therapeutic trials.

Neuroimaging is becoming important also to study the no-muscle manifestations of some metabolic myopathies. In particular recent reports have focused on evaluation of cardiomyopathy through cardiac neuroimaging as MRI or CT in patients with lipid storage myopathies. In addition brain MRI seems to be very useful to evaluated cerebrovascular abnormalities in some muscle glycogenoses as Pompe disease were the identification of undiagnosed cerebrovascular events or vascular abnormalities have important implications on the management of these disorders.

New genes in congenital myopathies

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The congenital myopathies comprises a heterogeneous group of genetic skeletal muscle disorders with autosomal dominant (AD) or autosomal recessive (AR) inheritance, proximal or distal distribution of muscle weakness and wasting, and onset often, but not only, in the newborn period. In the molecular era identification of new genes

and their protein product, and genotype-phenotype correlation studies have shown extensive genetic (multiple genes responsible of similar phenotype) and clinical heterogeneity (different phenotypes due to mutations in the same gene) adding to complexity.,,

As opposed to classical classification of congenital myopathies based on the presence on muscle biopsy of one or more characteristic histological or ultrastructural features, the nosography has recently changed and it is based more on a genetic bases than histopathological criteria blurring the boundaries between each single entity. Thus, the nosography and nosology in this field is rapidly evolving and pose the challenge of interpreting the pathogenetic significance of multiple identified genetic variants.

Inflammatory myopathies

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Idiopathic inflammatory myositis (IIM) is a group of acquired conditions characterized by inflammation of skeletal muscles. The most common clinical forms include polymyositis (PM), dermatomyositis (DM), myositis associated with neoplasia, myositis associated with collagen vascular disease, and inclusion body myositis (IBM). Although are divided in different subgroups, IIM share some common features including proximal muscle weakness, histological evidence of endomysial inflammation and activation of the immune response. As their potential response to immunosuppressive/immunomodulatory treatments, the early diagnosis of IIM and the exclusion of non-myositis conditions are of great importance. Muscle MRI has been revealing as a useful tool for the diagnosis and follow up of these disorders. In fact, MRI can visualize muscle edema, fatty replacement and atrophy, as well as subcutaneous pathology and fasciitis. MRI may also provide information about the distribution of muscle involvement and help in guiding of muscle biopsy. Finally, MRI may help to identify disease relapsing, especially during treatment tapering, and thus revealing MRI as valid biomarker to be used in experimental trials

Assessment of long term efficacy of thymectomy

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Thymectomy in Myasthenia Gravis (MG) is a widely recommended treatment in patients with anti-acetilcoline receptor antibodies to improve myasthenic symptoms and to achieve the complete remission of the disease.

Since 1993 thymectomy in myasthenia gravis (MG) patients is performed in our Centre in Pisa thanks to a multidisciplinary approach (neurologist, thoracic surgeon, anaesthetist). Its efficacy is consolidated by an accurate and long term neurological therapy (both pre and post operative) to achieve the complete remission of MG or its stable improvement. Aim of our study is to analyze retrospectively the neurological outcomes of thymectomized MG patients, both for thymoma and thymic hyperplasia with a follow-up of 3 years or more. At the end of follow-up, most of the patients (around 60%) of the patients achieved the remission of disease considering both complete stable remission and pharmacological remission. The best neurological outcomes are associated with variables such as serotype, MG class, thymic histology and surgical technique. In our Centre, thymectomy is indicated for patients (1) with chest CT scan detection of a thymoma and (2) with generalized and ocular forms of MG and positivity of AchRAb generally aged less than 50 years. Our study confirms that an appropriate long term neurological treatment associated with radical thymectomy is the paramount factor for the achievement of complete stable remission or for the clinical improvement of myasthenic patients.

Paraneoplastic Neuromuscular Junction (NMJ) Syndromes

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Paraneoplastic neurological syndromes (PNS) are remote effects of cancer. Lambert-Eaton myasthenic syndrome (LEMS) is a well-defined PNS, affecting NMJ. It is characterised by proximal muscle weakness and autonomic symptoms, caused by antibodies interacting with the voltage gated calcium channels (VGCCs) of P/Q-, N- and R-type, in over 50% of the cases associated to an underlying tumour, most often small-cell lung cancer (SCLC). However the molecular mechanisms of LEMS remains largely unknown. Almost all patients will benefit initially from symptomatic treatment with 3,4-diaminopyridine (3,4-DAP), a potassium channel blocking agent. Often, additional treatment is required in the form of immunosuppression

Voltage-gated potassium channels (VGKC), or Kv channels, are membrane channels able to open selectively for potassium ions in response to changes in membrane polarity. Antibodies against VGKC have been associated to peripheral nerve hyperexcitability disorders, characterized by constant muscle fiber activity, linked in some cases, to an underlying tumor, most frequently thy-

moma and haematological malignancies. Autoantibodies against -VGKC were described in 1995 in patients with neuromyotonia (NMT), an acquired peripheral nerve hyperexcitability syndrome; later, similar antibodies were found in patients with encephalopathies and called "autoimmune limbic encephalitis" and in individuals with the rarer Morvan's syndrome (MoS). However, different studies based on immunoprecipitation using patients' antibodies coupled to mass spectrometry allowed to identify other proteins, namely contactin-associated protein-like 2 (Caspr2) and leucin-rich glioma inactivated 1 (Lgi1), as the main antigenic targets of the previously called "VGKC Ab". Further studies are needed to improve our knowledge about the precise pathophysiological mechanisms of PNS; the patients' autoantibodies themselves are promising tools for functional studies of the ion channels involved in neural signaling.

Molecular understanding of phenotypic variability in myopathies

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Hereditary muscular dystrophies and congenital myopathies are chronic, genetic diseases posing a significant burden on patients and the health care system. Despite tremendous research and clinical efforts, the molecular causes remain undefined in about 50% of the cases, due to genetic heterogeneity and the limitation of current molecular diagnostic strategies. Like in complex disorders, monogenetic muscular diseases are often modulated in their phenotype by further genetic, epigenetic or extrinsic factors, that pose by itself a further level of complexity in understanding the molecular basis of clinical manifestations. This gives rise to extensive phenotypic variability, genetic pleiotropy and potentially protection from disease manifestations, known as incomplete penetrance, both in and within families.

The "next-gen" technology has already returned many potential benefits especially for large-scale projects in several fields including inherited myopathies. The use of similar strategies based on massive phenotyping and system biology has the likelihood to overcome the limitations in our understating of phenotypic variability and might offer models for others, even more complex classes of inherited neurological disorders such as motor neuronopathies.

We will summarize current knowledge on the complexity of modifying factors in neuromuscular research, will present a personal view on advantages and limitations of next-gen analyses and profile new skills to be met in clinical adaptation to the new era of myogenomics.

Increasing role of TITIN mutations in neuromuscular disorders

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Mutations in the *TTN* gene cause various cardiomyopathies and a wide range of skeletal muscle diseases and will probably prove to be the cause of many additional phenotypes of muscular disorders in the coming years.

Congenital centronuclear myopathy, early-onset myopathy with fatal cardiomyopathy (EOMFC), multiminicore disease with heart disease (MmDHD) and childhood-juvenile onset Emery-Dreifuss-like phenotype without cardiomyopathy represent an emerging group of *TTN*-related recessive disorders characterized by an early onset.

Next generation sequencing (NGS) has enabled a rapid investigation of *TTN* gene, overcoming issues related to its size and resulting in the identification of a huge number of rare or unique variants. Novel challenges are related to the clinical interpretation of these variants. However, NGS data, together with further functional studies, is and will be useful to get a more detailed genotype-phenotype correlation.

The formation of clinical and research consortiums represents a straightforward answer to these novel issues. A recent ENMC meeting lays the foundation for an international database of *TTN* mutations, variations and their clinical phenotypes, which could help all the researchers to assess the pathogenicity of found NGS variants and would promote significant advances in the understanding of titinopathies.

Neuroimaging diagnostics in Muscle Disease: power and limitations

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Muscular dystrophies

In the last years, muscle imaging and in particular magnetic resonance (MRI) has emerged as a useful tool in the diagnostic workup of neuromuscular patients both to target muscle biopsy and to guide to the final diagnosis, since combinations of muscles that are selectively affected or spared have been reported in a number of inherited myopathies.

Imaging is thus able to integrate clinical examination,

laboratory data and pathological findings to correctly address genetic testing or to help to interpret information from Next Generation Sequencing analyses. More imaging data on several muscular dystrophies, such as Facioscapulohumeral muscular dystrophy, have been recently acquired and international consortia are currently collecting imaging studies on other, less common forms, as well as on large cohorts to cover the entire clinical spectrum of the most common myopathies. Additionally, MRI can be used to identify subclinically affected individuals in segregation studies. In specific disorders, diagnostic imaging has also been able to provide clues to better understand pathophysiology. Finally, MRI is also developing as an extremely valuable instrument to provide biomarkers in clinical trials, both with standard and quantitative techniques. Limitations are currently due to the manual processes in segmentation and the possibility to apply quantitative analyses only to targeted body segments.

A rationale for a dietary intake in muscular dystrophies

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Unlike other diseases, the relationship between diet and muscular dystrophy has not been studied in the past, but the development of research and knowledge has allowed the improvement of service life, both some degree of quality of life in these patients and food and nutrition come inevitably at stake at this point.

Malnutrition, in deficiency or excess, disrupt the health and response to treatment, i.e. steroids. In children limits statural development, steroid therapy promotes obesity and dismetabolisms, in all conditions reduces respiratory and cardiac function, in certain forms it catches on swallowing and digestion.

Food is the source of substances (nutrients) active at various levels in the body, for all people and in Muscular Dystrophies: isolated amino acids or protein in whole or their derivatives active on muscle, antioxidants, vitamins, herbal anti-inflammatory nutrients. These isolated and purified substances are proposed as dietary supplements, but the studies are not always adequate, with mixed results and sometimes deceptive.

In addition, upstream, it is unclear whether there are special dietary pattern in the sick or not; and also if the regular feeding affects or not the disease.

Importantly though, we have to define the importance of evaluation of the nutritional status in these subjects, from diagnosis onwards, across the span of life (maximum growth potential in the sick; preventing or limiting metabolic complications and weight changes; avoid damage from inadequate nutrition support).

This type of approach is crossed with multi-professional knowledge creation and with the sharing of a common language, aimed at making nutrient recommendations for these categories of sick, scientifically tested and with proposals so attractive to dock a users also often recalcitrant as the pediatric one toward certain foods.

ABSTRACTS OF ORAL COMMUNICATIONS

(in alphabetical order of the first Author)

Biomarkers of muscle and cardiac damage in patients with mitochondrial disease

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Mitochondrial disease (MD) is a neuromuscular disorder affecting both children and adults. Muscle damage is almost constant. Myocardial fibrosis, demonstrated by late gadolinium enhancement (LGE) at cardiac magnetic resonance (CMR), is also a recurrent finding. We aimed to detect reliable biomarkers of muscle and cardiac damage.

Eighteen adult patients with MD and skeletal myopathy, aged 47 ± 3 years, were screened for cardiac involvement. During such screening, they underwent CMR, the dosage of norepinephrine (NE), galectin-3 (Gal-3), creatine phosphokinase (CPK), lactic dehydrogenase (LDH), and myoglobin.

Eight patients had LGE; they had higher NE and Gal-3 levels (both $p < 0.05$). The optimal cut-offs for LGE prediction were 692 pg/ml (area under the curve [AUC] 0.800; 90% sensitivity; 75% specificity) for NE and 13.8 ng/ml (AUC 0.794; 80% sensitivity; 75% specificity) for Gal-3.

High sensitivity troponin I was negative in all cases, while high-sensitivity troponin T (hs-TnT) was > 14 ng/L in 33% of patients, and correlated with three biomarkers of muscle damage: myoglobin (Spearman's Rho = 0.754), LDH (Rho = 0.523), CPK (Rho = 0.876) (all $p < 0.001$).

Myoglobin was > 80 ng/ml in 6% of patients, LDH > 500 IU/L in 17%, CPK > 175 IU/L in 28%; as stated above, hs-TnT was increased in 33% of patients.

NE and Gal-3 were associated with myocardial fibrosis. The ongoing cardiac damage did not result in troponin I release, while hs-TnT levels were frequently increased. Hs-TnT release might be ascribed to muscle depletion, as recently proposed in other myopathies. Hs-TnT could represent the most sensitive indicator of muscle involvement in MD patients.

Genetic and functional implication of muscle-related coiled-coil/cavin-4 gene in asymptomatic hyperckemia

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Caveolar morphogenesis is a highly sophisticated process which requires the interplay of two families of proteins:caveolins and cavins. In muscle, caveolin-3 interacts with a complex formed by cavin-1/-2 and -4. Cavin-4/MURC is selectively expressed in skeletal and cardiac myoblasts and it regulates their differentiation toward mature myotubes. Although Cavin-4 genomic variants were associated with heart phenotypes, its involvement in skeletal muscle disorders has not been assessed. Conversely, mutations in caveolin-3 are a well-established cause of muscle disease such as limb girdle muscle dystrophy, rippling disease and asymptomatic hyperCKemia. On the basis of the functional relationship with caveolin-3, we analyzed Cavin-4 gene in a population of paediatric patients affected by undiagnosed asymptomatic hyperCKemia to uncover the role of rare coding variants in a muscular phenotype. The average patients' age was 8,6 ± 4,3 years (mean ± SD) and serum CK increase was 1.5to5-folds. Our survey identified 5 MURC heterozygous rare missense variants in 5 unrelated probands. Nonsynonymous coding variants were overrepresented in the patients by 10to16-fold when compared to controls with normal CK levels and to the general population database and four of them affected codons of Cavin-4 helical-region1. Although the sarcolemmal Cavin-4 signal was not affected in the available Patients' muscle biopsy, primary myoblasts isolated from the bioptic tissue of a single patient displayed loss-of function effects of the mutant protein on markers of myoblast differentiation/function and on activation of Extracellular-Related Kinases. We cannot exclude whether our patients will develop muscle or cardiac symptoms as they become adults. Our data indicate that rare heterozygous Cavin-4 coding variants represent susceptibility alleles involved in the aetiology of hyperCKemia.

The genetic spectrum of a large cohort of putative CMD

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Congenital muscular dystrophy (CMD) are heterogeneous muscle disorders classified according to the involved gene/protein functions.

We present the genetic breakdown in a large cohort of Italian patients genetically undefined, presenting with a CMD-like phenotype.

Patients with a possible CMD were referred to us for genetic analyses as part of a multicenter study supported by the Telethon Foundation. For genetic tests we used a targeted resequencing method in NGS that included 95 genes linked to CMD, LGMD or related diseases. Detailed clinical, morphological, and neuroradiological data were collected.

One hundred and eight patients were included in the study. Twenty-one patients have not yet been analysed. A diagnosis was genetically confirmed in 36/87 of the cases. Within this group, a diagnosis of alpha-dystroglycanopathy was the most common (50%), followed by merosin-deficiency (31%). Mutations in *GMPPB* represented the most common form of alpha-dystroglycanopathy (found in 5/18 cases). The remaining 19% included patients harbouring mutations in *LMNA*, *COL6A3*, *SEPN1*, *SYNE1*, and *PLEC*.

Nine out of 108 patients did not disclose mutations and their disease is likely due to mutations in genes not analyzed in this study or may represent phenocopies. The study of the remaining 43 patients has revealed either a single pathogenic variant or mutations of unknown significance and data analyses have as yet unfinished.

In our network, a genetic diagnosis of CMD was reached in about 40% of cases, with *GMPPB* being the most common form. The high numbers of *LAMA2*-associated cases reflect scarce routine analysis of mutation analysis in merosin-deficient patients.

Neural correlates of neuropsychological dysfunction in DM1: voxel-based morphometry and tract based spatial statistical analysis

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Myotonic dystrophy type 1 (DM1) has a wide phenotypic spectrum and potentially may affect central nervous system with mild to severe involvement. Our aim was to investigate grey matter (GM) and white matter (WM) structural alterations in a sample of adult-onset DM1 patients and to evaluate relationship with clinical and cognitive variables.

Thirty DM1 patients underwent neuropsychological investigation and 3T-MRI protocol. GM and WM changes were evaluated calculating brain parenchymal fraction (BPF), voxel-based morphometry (VBM), white matter lesion load (LL% and Fazekas scale) and tract based spatial statistical (TBSS).

Patients showed main impairment in tests exploring executive and mnesic domains with visuospatial involvement, significantly related to BPF. VBM revealed clusters of widespread GM reduction and TBSS revealed areas of decreased fractional anisotropy (FA) and increased radial diffusivity (RD), mean diffusivity (MD) and axial diffusivity (AD) in patients compared to a group of matched healthy controls. Multiple regression analysis showed areas of significant negative relationship between atrophy in the left temporal lobe and verbal memory, and between RD and mnesic and visuo-spatial cognitive domains.

Our data indicated presence of extensive atrophy in DM1 over both cerebral hemispheres. Global atrophy, expressed with BPF, correlated with impaired executive and visuo-spatial abilities. TBSS results indicate that the involvement of normal appearance WM beyond the signal changes detected with conventional MR imaging (Fazekas scale and LL%), was associated to neuropsychological deficit. These data suggest that disrupted complex neuronal networks can underlie cognitive-behavioural dysfunctions in DM1.

Inhibition of muscle innate and adaptive immune response and improvement of muscular dystrophic process in a-sarcoglycan null mice by blockade of extracellular ATP/P2X axis

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Limb-girdle muscular dystrophy 2D (LGMD2D), caused by mutations in the gene encoding alpha-sarcoglycan (α-SG), is a rare disorder characterized by progressive weakness and degeneration of skeletal muscle. Pathological features of muscle biopsies show myofiber degeneration/necrosis, inflammatory response and endomysial fibrosis. In this scenario, extracellular-ATP (eATP), once released from the cytosol of dying cells, contributes to the triggering and amplification of the innate and adaptive immune response. Moreover, under physiological conditions, α-SG binds eATP and displays an ecto-ATPase activity, thus controlling eATP concentration at the surface of cells expressing P2 receptors and attenuating the magnitude and/or the duration of eATP-induced signals. Therfore, a-SG deficiency leads to a decreased ecto-ATPase activity causing increased eATP concentration at the cell surface.

Here, we addressed whether the inhibition of P2X receptors could beneficially impinge on the in vivo inflammatory response associated to a-sarcoglycanopathies. For this purpose, we treated Sgca-null mice with systemic periodate oxidase ATP (oATP), a compound that irreversibly antagonizes P2X receptors through the selective modification of lysine residues in the vicinity of the ATP-binding site. Blockade of purinergic signaling led to an improvement of muscular strength and morphology associated with a significative reduction of inflammatory infiltrates, a decrease of IL-6 and inflammasome components and an inhibition of pro-fibrotic growth factors in muscles. Our results point to a role of eATP in contributing to the immunopathological damage of dystrophic muscle and to a possible beneficial effect of pharmacological purinergic antagonism in LGMD2D.

Association study of exome variants in the NF-kappa-B and TGF-beta Pathways identifies *CD40* As a modifier of duchenne muscular dystrophy

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The expressivity of Mendelian diseases can be influenced by factors independent from the pathogenic mutation: in Duchenne muscular dystrophy (DMD), for instance, age at loss of ambulation (LoA) varies between patients whose *DMD* mutations completely abolish dystrophin expression. This suggests the existence of trans-acting variants in modifier genes. Common single nucleotide polymorphisms (SNPs) in candidate genes (*SPP1*, encoding osteopontin, and *LTBP4*, encoding latent transforming growth factor beta [TGF-beta]-binding protein 4) have been established as DMD modifiers. We performed a genome-wide association study (GWAS) of age at LoA in a sub-cohort of European/European-American ancestry (n = 109) from the Cooperative International Research Group Duchenne Natural History Study (CINRG-DNHS). We focused on protein-altering variants (Exome Chip), and included glucocorticoid treatment as a covariate. As expected, due to the small population size, no SNPs displayed an exome-wide significant p value ($< 1.8*10^{-6}$). Subsequently, we prioritized 438 SNPs in the vicinities of 384 genes implicated in DMD-related pathways, i.e. the nuclear-factor-kappa-B [NF-kappaB] and TGF-beta pathways. The minor allele at rs 1883832, in the 5' untranslated region of *CD40*, was associated to earlier LoA ($p = 3.5*10^{-5}$). This allele diminishes the expression of CD40, a co-stimulatory molecule for T-cell polarization. We validated this association in multiple independent DMD cohorts (United Dystrophinopathy Project, Bio-NMD, and Padova, total n = 660), establishing this locus as a novel DMD modifier. This finding highlights the relevance of cell-mediated immunity in DMD, and points to a novel therapeutic target.

Molecular diagnosis of spinal muscular atrophy with lower extremity predominance by NGS: report of a cohort of nine patients

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Spinal muscular atrophy with lower extremity predominance (SMA-LED) is characterized by congenital or early childhood onset motor neuron degeneration with lower limbs predominant weakness. Patients with a phenotype suggestive for motor neuronopathy predominantly affecting the lower limbs were identified and referred for molecular diagnosis by Next-Generation Sequencing (NGS) panel. We report on 9 patients (6 males, 3 females) from 9 different families and characterized by congenital or childhood-onset lower limb wasting and weakness. All patients were sporadic cases. Clinical severity ranged from lower legs arthrogryposis to mild and non-progressive lower limb weakness. One patient had also epilepsy. Four patients underwent brain MRI 4/9 that revealed in one case a polymicrogyric pattern. ENG/EMG showed in all patients a neurogenic pattern of chronic denervation with reduced CMAP. Muscle biopsy was performed in 3/9 and showed neurogenic features. Six patients underwent muscle MRI that showed in 4/6 a common pattern of involvement of the thigh and the lower leg muscles. Molecular analysis showed that 2/9 patients had 2 novel mutations in *DYHC1H1*, 1/9 a mutation in *BICD2*, 1/9 a mutation in *SETX*, 1/9 a mutation in *GARS*, 1/9 was compound heterozygote for 2 different IGHMBP2 mutations, in 1/9 no mutations were identified. For 2 patients the molecular analysis is ongoing. Our findings further expands clinical and mutational spectra of SMA-LED. Although the cohort is not large, our data suggest that in patients with a suggestive phenotype, the diagnostic yield of our molecular test is over 80%.

Altered TDP43-dependent splicing in protein aggregate myopathies

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Background. Abnormal cytoplasmic accumulations of TDP43 (TARDBP gene, OMIM: 605078), a prevalently nuclear RNA binding protein involved in numerous aspects of RNA metabolism, along with rimmed vacuoles (RV), have been consistently described in muscle biopsy of patients with Sporadic inclusion body myositis (sIBM) and other protein aggregate myopathies, including oculopharyngeal muscular dystrophy (OPMD). However, it is not known whether aggregation and mislocalization of TDP43 are associated with changes in TDP43 function, bearing possible pathogenic relevance to muscle fibre degeneration.

Objective. To study the role of TDP43, in terms of quantitative and functional changes, in muscle biopsies of patients with myopathies with RV and protein aggregates, including sIBM and OPMD, in order to assess their tissue-specificity.

Methods. RNA was extracted from muscle biopsies of 10 sIBM, 3 OPMD 4, polymyositis (PM) and 9 healthy controls (CTRL). *TDP43* mRNA expression and splicing analysis of a highly specific TDP43-dependent target (POLDIP3 exon 3) was measured by qPCR and RT-PCR, respectively.

Results. *TDP43* expression from muscle biopsies of sIBM patients was significantly lower compared to OPMD, PM and CTRL. Accordingly, we observed a significant decrease of inclusion of *POLDIP3* exon 3 in 4/8 (50%) sIBM patients and in 3/3 (100%) of OPMD samples, but in none of PM patients and CTRL.

Discussion. to our knowledge, our study provides the first experimental evidence of an altered TDP43 functionality in sIBM and OPDM. RNA sequencing is ongoing in order to identify other differently expressed or misspliced genes as a result of TDP43 depletion and misfunction and possibly involved in viability of muscle fibres.

Diagnosis of Duchenne Muscular Dystrophy in Italy: critical issues and areas for improvements

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The mean age at diagnosis of Duchenne Muscular Dystrophy (MD) is around 4.3-5 years all over the world. Early diagnosis has several implications including a timely access to Standards of Care and a prompt genetic counselling. Moreover, if novel treatments will be approved they could be started in the early phase of the disease.

Here we report findings on a retrospective multicenter study that explores the age at DMD diagnosis in Italy in the past 10 years. Results: We identified 384 Italian boys who were diagnosed with DMD from 2005 to 2014.The mean age of first suspect was 31 months and the mean age at diagnosis was 41 months. The main finding that brought to suspect a DMD was raised CK or transaminases (53% of cases) followed by motor delay (16%), and muscle weakness (14%), Initial concerns about DMD were raised by general pediatricians (30%), by specialists of tertiary centers and first level hospitals (22% and 38% respectively) or parents (10%). Children with an incidental finding of high CK reached the diagnosis first whereas the most delayed diagnosis occurred in those patients who did not manifest any developmental delay.

Conclusions: the mean age at diagnosis, over the last decade, in Italy is about 10-12 months less than other country had reported. The detection of raised CK is the factor that mostly reduces the time for diagnosis. We believe that all male children should be screened in early infancy by a CK assessment even in absence of a neurodevelopmental delay.

A multicenter, randomized, double-blind, placebo-controlled (RCT) clinical trial on the clinical efficacy of non-invasive ventilation and modafinil on excessive daytime sleepiness in myotonic dystrophy type 1 (DM1): protocol outline

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Background. Excessive Daytime Sleepiness (EDS) is amongst the most frequent complaints of patients with Myotonic Dystrophy type 1 (DM1). It may related in part to nocturnal hypoventilation and sleep apneas, which can be corrected by nocturnal non–invasive ventilation (NIV), but is mostly of central origin. Compliance to NIV is limited and symptoms are often overlooked. On the other hand, results with psychostimulants used successfully to treat EDS in narcolepsia, are controversial in DM1.

Aims. The aim of our study is to outline the RCT clinical trial designed to investigate the clinical efficacy of NIV and of modafinil on EDS in 80 patients with DM1. The primary outcome measure will be the reduction of EDS assessed by improvement on the Maintenance Wakefulness Test. Secondary outcomes will be: i) improvement of EDS by subjective scales and actigraphy; ii) maintenance of respiratory function; iii) improved cognition and mood; iv) improved QoL; v) improved motor function; vi) improved compliance to NIV.

Expected results. We expect that modafinil will improve EDS in DM1 and that, if patients with EDS are on NIV, modafinil will correct residual central related EDS. We also expect that the maintenance of respiratory function by NIV, combined with the psychostimulating and antidepressive actions of modafinil will improve compliance to NIV.

Conclusions. The results of the trial will provide information on the impact of NIV on EDS and will represent the phase III clinical data required to confirm or refute the preliminary data that modafinil improves EDS in DM1.

Muscle mri in neutral lipid storage disease (NLSD)

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We present the muscle imaging data of 12 patients from the Italian Registry of Neutral Lipid Storage Disease (NLSD): ten patients presenting NLSD with myopathy (NLSD-M) carrying recessive mutations in *PNPLA2* gene, and two patients presenting the NLSD with ichthyosis (NLSD-I) mutated in *ABHD5* gene. In NLSD-M gluteus minimus, semimembranosus, soleus and gastrocnemius medialis in the lower limbs and infraspinatus, trapezius, deltoid and paraspinous muscles in the upper limbs were the most affected muscles. Gracilis, sartorius, subscapularis, pectoralis, triceps brachii and sternocleidomastoideus were spared. Muscle involvement was not homogenous and characteristic "patchy" replacement was observed in at least one muscle in all the patients. Half of the patients showed one or more STIR positive muscles. In both NLSD-I cases muscle involvement was not observed by T1-TSE sequences but one of them showed positive STIR images in more than one muscle in the leg. Our data provides evidence that muscle imaging can identify characteristic alterations in NLSD-M, characterized by a specific pattern of muscle involvement with "patchy" areas of muscle sparing/replacement. Larger cohorts are needed to assess if a distinct pattern of muscle involvement exists also for NLSD-I.

First italian mutation in *ISCU* associated with an autosomal dominant mitochondrial myopathy

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Myopathy with deficiency of succinate dehydrogenase (SDH) and aconitase is a recessively inherited disorder (MIM255125) characterized by childhood-onset early fatigue, dyspnea and palpitations on trivial exercise. The disease is non-progressive, but life-threatening episodes of widespread weakness, severe metabolic acidosis and rhabdomyolysis may occur. So far, this disease has only been identified in northern Sweden.Quite all Scandinavian patients are homozygous for a deep intronic IVS5+382G > C splicing affecting mutation in *ISCU*; only a single family, compound heterozygous for the common intronic mutation and a second missense mutation in exon 3, has been identified.

We describe a 23 year-old Italian male who presented, in childhood, with ptosis but not ophtalmoparesis, severe muscle weakness in distal harms and legs and important exercise intolerance. The disease was slowly progressive: he showed tachycardia and events of profound exercise intolerance associated with weakness, with partial recovery of muscle weakness between episodes. Muscle biopsy showed a severe SDH (complex II) deficiency at histochemical approach and a reduction in respiratory chain complex I-II-III activities at the biochemistry. Using an NGS approach we identified a heterozygous mutation p.Gly96Val in *ISCU*. The absence of the mutation in DNA from patient's parents indicated a possible de novo dominant mutation. Yeast studies confirmed the dominant effect of theGly96Val mutation.

In conclusion we described the first Italian patient with a mutation in *ISCU;* notably, despite he harbors a single heterozygous mutation, he presentswith a phenotype resembling the recessive diseasereported in Sweden cases.

PEO. The experience of the Italian Network of Mitochondrial Diseases

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Ocular myopathy, with eyelid ptosis and/or ophthalmoparesis, is a frequent manifestation of mitochondrial diseases (MD). Among the patients with detailed clinical picture included in the database of the Italian Network of MD, it was the most common feature, present in 471/1100 (42.8%). Excluding patients with Leber optic neuropathy, it was present in 470/965 (48.7%). It is a possible sign of both mitochondrial (mtDNA) and nuclear (nDNA) mutations. I.e. ptosis/ophthalmoparesis was observed in 25.6% of patients with A8344G mutation and in 27.8% of A3243G carriers, whereas it was much more frequent in subjects with a mtDNA single deletion (90.7%). Ocular myopathy may be a manifestation of a multisystem MD, when associated with central neurological features such as myoclonus (MERRF), stroke-like episodes (MELAS) and others; however, when it represents the major complain it is commonly denoted as "progressive external ophthalmoplegia" (PEO). For a detailed phenotype-genotype analysis we considered the 727 patients with a definitive genetic diagnosis, excluding Leber patients. 326 of them had not ptosis/ophthalmoparesis (44.8%), 134 had ocular myopathy in the frame of a complex encephalomyopathy (18.4%), the remaining 267 had a "PEO" phenotype (36.7%). Of note, 31 patients of the second group had a "PEO" onset, underscoring the need of a better understanding of the factors able to predict the future development of a multisystem disease. Aims of this study are to better characterize ocular myopathy in MD, including genotype-phenotype correlations, to identify clinical and molecular predictive factors, and to revisit the nomenclature in the light of the results obtained.

Central Nervous System involvement in Late Onset Pompe Disease (LOPD): clues from neuropsycological, morphological and functional MRI studies

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Late-onset Pompe disease (LOPD) is a rare, multisystem disorder, mainly affecting limb and respiratory muscles,butother organscould be involved as Central Nervous System- Aim was to assessneuropsycological, and morphologicaland functional brain alterations in LOPD.

We studied 16 LOPD patients (7M, 9 F, mean age 49 ± 16). Clinical aspects were characterized by pre-symptomatic hyperCKemia (3 patients), and proximal/axial muscle and respiratory weakness (13 patients). All patients underwent a complete neuropsychological assessment. We also performed 3T magnetic resonance imaging (MRI),obtaining normalized cortical brain volumeand resting-state functional MRI (Rs-fMRI) for network functional connectivity. Patients were divided in two groups: 20-40 (group I) and over 40 years (group II) and compared with controls. Morphological and angiographic evaluation were performed by 3D-FLAIR and 3D-TOF sequences. In order to quantify white matter gliotic lesions, we applied the Fazekas scale whereas Smoker's criteria were consideredto detectdolichoectasiaat the basilar artery. 10/16 subjects showed a mild cognitive impairment. Memory areas were preserved whereas 5/16 subjects showed abnormal executive functions. At MRI observation, it was shownthatFazekas score was greatly abnormal in about 40% of patients According to Smoker's criteria, 14/16 patients had the dolichoectasia of vertebrobasilar system. Resting-state Rs-fMRI showed significant decreased connectivity in DMN networks, in both groups,even if group II showed a decreased connectivity in the bilateral middle and superior frontal gyrus. Significant gray matter atrophy was found in group II.

In this group of patients with LOPD, results of the studies have revealed in the great majority of cases, neuropsychologicalchanges as well as morphological and functional brain alterations. The pathogenesis of these rather new aspects seems to berelated to cerebrovascular abnormalitiesdue to an hypoxic-ischemic mechanismsomehow linked to the enzyme deficiency

Epigentic investigations of FSHD families from the Italian National Registry of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is causally related to reduced number (≤ 10) of tandemly arrayed D4Z4 repeats on 4q35. The current model explaining FSHD pathogenesis favours the possibility that patients carrying 4q allele with a D4Z4 array less than 11 repeats (FSHD1) and contraction-independent patients (FSHD2), who carry D4Z4 alleles of normal size on both chromosomes 4q, display D4Z4 decreased level of methylation (≤ 25%). In order to test this possibility we performed a systematic study on a cohort of 82 FSHD families (85 FSHD1 patients, 24 non-manifesting relatives and 35 FSHD2 patients) and 10 subjects affected with other muscular diseases. We focused our attention on the FSHD1 families with the reduced penetrance to test if differential clinical expression might correlate with the different degree of D4Z4 methylation between FSHD patients and their healthy relatives carrying the same D4Z4 reduced allele and FSHD2 patients which are sporadic and with milder phenotype. To this purpose we analyzed the level of the D4Z4 methylation and tested DNA for the presence of SMCHD1 mutation. Our study revealed that the D4Z4 methylation status does not strictly correlate with the presence

and severity of a FSHD clinical phenotype as we detected hypomethylation (≤ 25%) and normal level of methylation (≥ 35%) in all analyzed subgroups. Interestingly, we observed FSHD patients carrying hypomethylated D4Z4 alleles of normal size with no SMCHD1 mutation suggesting the presence of additional epigenetic factors determining D4Z4 methylation status. Studies on well clinically characterized families will foster the dissection of epigenetic mechanisms involved in developing FSHD.

The role of transmission electron microscopy in vacuole-associated myopathies

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Vacuoles, membrane-bound area, containing various cellular components or enzymes, can occur in several myopathies and are heterogeneous. Some are filled up by detectable material (glycogen, protein aggregates, ceroid-lipofuscins), others are autophagic vacuoles. Myopathies in which vacuoles, together with additional pathological features, can be useful diagnostic clues are: sporadic Inclusion Body Myositis (s-IBM), glycogenosis and lysosomal diseases, Danon's disease, polymyositis (PM) and dermatomyositis (DM), myofibrillar myopathy (MM) and vacuolar myopathy as caused by a CASQ1 gene mutation. So, clarifying a vacuole's nature and function through the use of TEM could be essential. This study aims to delineate cases in which, focusing on vacuoles, TEM data describe pathognomonic signs or solve differential diagnosis. Patients with a detailed clinical history and a suspicion of neuromuscular disorder have been recruited. Results: in muscle fibers of Type II Glycogenosis, glycogen is detected in membrane-bound areas, without basal lamina and in large lakes of freely dispersed granules. In Danon's disease, basal lamina is seen on inner surface of authophagic vacuoles containing amounts of granular and osmiophilic debris. In CASQ1 myopathy inclusions with polygonal appearance are described. In MM, PM and DM autophagic vacuoles are described, as in s-IBM together with nuclear and sarcoplasmic peculiar filaments. Finally, Ceroid-lipofuscinosis shows membrane-bound vacuoles with curved lipid lamellae detectable in muscle fibers.

In conclusion, TEM analysis of muscle biopsy may be essential to better identify the content morphology and origin of the vacuole allowing pathologist to make a differential diagnosis among vacuole-associated myopathies, thus improving knowledge of different pathogenetic mechanisms.

Congenital myasthenic syndromes: molecular and phenotypic correlations and long term follow-up in a cohort of 20 Italian patients

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Congenital myasthenic syndromes (CMS) are a group of heterogeneous inherited disorders caused by mutations in genes encoding proteins, essential for the integrity of neuromuscular transmission. CMS are characterized by fatigable muscle weakness (e.g. ocular, bulbar, limb muscles) with onset at birth or in early childhood; rarely, symptoms may present later. Clinical, electrophysiological and morphological studies are essential for the molecular diagnosis, counseling and therapy. In the last 21 years, 20 patients with CMS were clinically and genetically defined at our Department. The onset of symptoms ranged from birth to 38 years of age. In some patients, in the last year, the mutations were identified using Next Generation Sequencing (NGS). In our series, CMS subtypes are: 9 CHRNE, 2 CHRND, 4 GFPT1, 1 DOK-7, 2 RYR, 1 RAPS, 1 CHAT. In two sisters CHRND mutations resulted in a lethal phenotype. We confirm that CMS linked to CHRNE mutations is the most frequent phenotype and that, in these patients, pyridostigmine is the best treatment. Salbutamol resulted efficacious in DOK-7 and CHRND forms. Ephedrine was used in one CHRNE patient with a clear benefit. NGS is an essential tool to characterize CMS.

Peptide-conjugated morpholino oligomers for treatment of spinal muscolar atrophy

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The use of Antisense oligonucleotide (ASO) represents a promising treatment for Spinal Muscular Atrophy (SMA) by its ability to increase the production of a functional SMN protein and rescue the phenotype in SMA animal models. However, there are several hurdles to overcome. To increase the cellular and tissue uptake and pharmacological profile of Morpholino Oligomers (MO), an ASO variants, one possible strategy is the conjugation with cell-penetrating peptides (CPPs). In this study we investigated the efficacy of different CPPs linked to our validated MO sequence (Tat, R6, r6 and $(RXRRBR)_2XB$) and of novel MOs sequences in *in vitro* and *in vivo* SMA models.

I*n vitro*, we nucleofected induced pluripotent stem cells (iPSCs) derived from SMA patients with the four MOs. The treatment with MO B and D, and in particular their combination, showed a consistent increase of SMN protein levels and a significant upregulation of SMN GEMS in the cell nuclei. The same increment was obtained *in vivo* in the SMAΔ7 mouse model.

Moreover, we administered our alreadyvalidated MO sequence (MO-10-34) conjugated with four CPPsin a small pilot group of pre-symptomatic SMA mice, using the protocol already established for unconjugated MO (Nizzardo et al. 2014). The best conjugated was selected for next studies in presymptomatic and symptomatic SMA mice to assess its therapeutic potential.

We will assess the feasibility of this strategy to: 1) cross the

blood brain barrier, allowing MO non-invasive systemic delivery, and 2) treat the disease in a symptomatic phase, expanding the therapeutic window.

Comparison amongst detection of PAS-positive granules in lymphocytes on blood-smear and GAA enzyme activity assessment on Dried Blood Spots as screening methods for Late Onset Pompe Disease

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Measurement of alpha-acid glucosidase (GAA) enzyme activity on dried blood spots (DBS) as well as detecting PAS-positive granules in lymphocytes on blood smears, have both been proposed as screening methods for LOPD. This study aims to compare these procedures in detecting carriers of *GAA* mutations.

Ninety-four individuals (51M-43F; age ranging 11-72 year) were consecutively observed at our Clinic because of symptoms of muscle disease and hyperCKemia. Blood samples were collected to measure GAA activity on DBS (Center for Neonatal Screening and Metabolism of the University Clinic of Hamburg) and to obtain blood smears. These latter, after haematoxylin-eosin-PAS staining, were double-blind assessed for PAS-positive granules on lymphocytes (Laboratory of Neuropathology of the Second University of Naples), by qualitative and quantitative light microscopy. PAS-positive lymphocytes/total lymphocytes ratio as well as granules density in lymphocytes were evaluated. Blood samples from 12 healthy volunteers served as controls.

LOPD compatible reduction of GAA activity was found in 10/13 *GAA* homozygous mutations, 2/12 heterozygous mutations, 1/69 non-LOPD patients and 0/12 healthy subjects. PAS-positive lymphocytes were observed in 13/13 *GAA* homozygous and 12/12 heterozygous mutations, 5/65 non-LOPD patients who suffered of glycogenosis other than LOPD and in 4/65 patients who harbored multiple polymorphisms of *GAA* in homozygous. Density and caliber of PAS-positive granules allowed distinguishing GAA-mutated patients from other glycogenosis and heterozygous from homozygous LOPD.

Assessing PAS-positive granules in peripheral blood lymphocytes appear to be a highly sensitive and specific tool to identifying *GAA*-mutated individuals who have a nearby normal residual GAA activity on DBS.

Brain magnetic resonance in mitochondrial disorders: can an integrated approach help to diagnosis?

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To determine the spectrum of Magnetic Resonance Imaging (MRI) findings in patients with mitochondrial disease (MD) we performed a retrospective evaluation in 93 patients with morphological, biochemical and genetic diagnosis of MD (15 A3243G-PEO/MELAS, 14 MERRF, 3 MNGIE, 53 PEO with single or multiple deletions of mitochondrial DNA, 8 Other).

Abnormal MR findings consisted of cerebral cortical atrophy (35 patients), cerebellar atrophy (24 patients), white matter cerebral abnormalities (45 patient of which 35 with aspecific gliosis and only 10 with confluent diffuse alterations), signal changes in deep grey matter (25 patients), brain stem atrophy (11 patients) and stroke-like episodes (7 patients with MELAS-A3243G and one with MERRF). Moreover, Proton Magnetic Resonance Spectroscopy (H1-MRS) has been performed in 61 patients (14 MERRF, 15 MELAS, 3 MNGIE, 23 PEO and 6 Other). Normal spectra were found in all patients with single or multiple deletions at any stage of the disease, independently from severity of the phenotype and CNS involvement. In patients carrying A8344G or the A3243G non-MELAS patients we found increased peak of choline-containing compounds (Cho), variable reduction of N-acetyl-L-aspartate (NAA) and absence of Lactate (LA) peak in brain, while in a minority of these patients LA peak was present in CSF. In MELAS, a LA peak was instead found in both affected and non-affected brain areas and in CSF.

In conclusion MRI can identify common pattern in patients with MD but only the integration with MRS can provide useful information to guide genetic studies and follow-up in this heterogeneus group of disorders.

Autonomic nervous system involvement in spinal muscular atrophy

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Although in clinical practice we are aware of patients affected by spinal muscular atrophy (SMA) reporting symptoms of autonomic dysfunction, like palpitations and excessive sweating, scanty literature investigated these aspects.

Autonomic nervous system (ANS) involvement has firstly been reported in three SMA1 children and in some patients with SMA and respiratory distress, however detailed studies evaluating ANS function in SMA2 and 3 patients have never been performed.

We assessed autonomic dysfunction in patients with a genetically confirmed SMA, using the following specific tests: plasma levels of catecholamines (supine and tilted), head-up tilting (HUT), skin sympathetic reflex, cold pressure test, Valsalva maneuver and deep breathing tests.

Overall 10 patients (3 SMA2 and 7 SMA3) (age range 7-48 years, mean: 25 ± 11 SD), were prospectively included. All were able to perform HUT test, despite 2/10 patients that were evaluated on a sitting position due to severe lower limbs contractures. None experienced orthostatic intolerance symptoms. All patients showed normal vasoconstrictor sympathetic response at the cold pressure test and normal skin sympathetic reflex.

Interestingly, catecholamines measurements showed high supine level of epinephrine in 6/10 patients with values 2-to 5-fold higher than standard values, and 3 of them did not show rise on tilt. Furthermore, one patient had high supine norepinephrine too and he showed hypertension.

In conclusion, we showed for the first time abnormal epinephrine levels in SMA although overall normal autonomic

function on test. Albeit a larger cohort is required to confirm this evidence, our preliminary data indicate an hyperadrenergic status in patients with SMA.

ERT in late-onset Pompe disease: long-term follow-up and IgG anti rh-GAA assessment in 9 patients

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Long-term efficacy of enzyme replacement therapy (ERT) with alglucosidase alfa in late-onset Pompe Disease (LOPD) has been evaluated only in a limited number of patients.

We report clinical and functional findings from 9 LOPD patients treated with ERT for a time ranging between 3 and 9.5 years. Serial measurements of IgG anti rh-GAA antibodies were performed in 7 of them.

At the end of observation, respiratory function tests improved or were stable in 66% of cases; the walked distance at 6MWT improved in 88% of the patients up to 24 months, while in the subsequent follow-up (up to 108 months), 63% of them slowly reduced the walked meters.

Overall, in 71% of the patients (5/7) we observed a correlation between antibody titer (AT) and clinical conditions. Particularly, in 42% of the patients (3/7), worsening of clinical conditions correlated with increase in AT over time. In 15% of them (1/7), stability of clinical conditions was associated with stable AT and in another 15% (1/7) a reduction in AT was associated with clinical improvement. Only in 28% of the cases (2/7), clinical conditions remain stable despite the increase of AT over time.

Our results confirm that ERT maintains its effectiveness in the long term, though it appears to be reduced over time compared to the first two years of therapy.

A correlation between functional deterioration and production of anti rh-GAA antibodies is apparently present in most patients but these findings need to be extended across multiple cohorts to be confirmed.

Longitudinal study of disease progression in 232 FSHD patients from the Italian registry

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common muscular dystrophies. The disorder is clinically defined by a classical distribution of muscular weakness involving face, shoulder girdle, scapular stabilizer and peroneal muscles. The degree of muscular involvement is variable, from asymptomatic subjects to a severe generalized myopathy; the molecular hallmark consists in a deletion of repetitive elements on 4q35 region, named D4Z4.

Despite a great number of clinical studies, little is known about the real progression over the years and the natural history of the disease; longitudinal studies are poor and limited to a few patients.

The Italian Network for FSHD designed a retrospective study to assess rate and distribution of disease progression on a long-term period. The study was performed by collecting data from Italian FSHD Registry, based on a nationwide collaboration. We selected patients with a documented clinical history and follow up. MRC testing and FSHD score were compared at baseline and after 5 years.

The patients were 232, aged 15-85 years, with a D4Z4 allele < _38kb. No changes were observed in 41%; in 10% a severe worsening was documented, and 3% lost walking abilities. Disease progression rate on a five years interval seems to be independent from age and functional status.

FSHD has a slow progression over the years: the average decline is about 1 point on the FSHD score. These results stress the importance to rely on more sensitive outcome measures, specifically designed to monitor disease progression, in view of future therapeutic trials.

MUSCLE CLUB SESSION

(in alphabetical order of the first Author)

Sporadic late-onset nemaline myopathy successfully treated with intravenous immunoglobulin and immunosuppressive therapy

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A 40-year old man manifested subacute painful proximal weakness with difficulty in abducting his arms, climbing stairs and rising from a chair since one year. Neurological exam revealed hyperlordosis, waddling gait, severe limb-girdle and axial muscle weakness and atrophy. CK were slightly elevated and immunoelectrophoresis revealed IgG-kappa monoclonal gammopathy. EMG showed spontaneous activity and myopathic changes. Muscles MRI demonstrated fatty infiltration of scapular and pelvic girdle muscles, paraspinal muscles, posterior compartment of the thigh and quadriceps bilaterally and diffuse increased T2 signal. Muscle biopsy documented numerous angulated atrophic fibers with small nemaline rods, which stained positive for alpha-actinin and myotilin at immunohistochemistry. Clinical, laboratory and histological findings led to the diagnosis of sporadic late-onset nemaline myopathy (SLONM) and the patient was treated with intravenous immunoglobulin (IVIg) with improvement of muscle strength. From then on IVIg was administered monthly. After two months he also started prednisone and azathioprine. He continued to improve over 2-3 years and now he is completely autonomous in daily activities, monoclonal protein is undetectable and MRI demonstrates resolution of muscle oedema.

SLONM is a severe disorder of dysimmune etiology, frequently associated with monoclonal gammopathy. Autologous peripheral blood stem cell transplantation has been reported to be a promising treatment but has many serious side effects. Few patients have been treated with IVIg alone or in association with various therapies with heterogeneous results and frequent recurrences. The favourable response in our patient warrants treatment with IVIg and chronic immunosuppressive agents as the first line therapy for this severe disease.

Distal upper limbs muscle weakness in a 55-years-old woman

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We report a 55- years- old woman who came to our department because of a ten years history of upper distal muscles weakness. She was also affected by autoimmune thyroiditis. Neurophysiological assessment ruled out a peripheral neuropathy. Brain and cervical spine MRI did not reveal any abnormalities…

Inflammatory myopathy in collagen VI mutation carrier

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Inflammatory myopathies are a heterogeneous group of muscle diseases, traceable in adult and children. They are characterized by proximal muscle weakness, usually sparing distal and ocular muscles. Diagnosis is based on clinical manifestation, serological tests, and pathological examination.

We describe a case in which clinical and pathological features suggestive for myositis coexist with a mutation of Collagen VI.

A 14-year-old female developed diffuse hyposthenia and myalgia two weeks after a febrile pharyngitis. Laboratories tests showed high CK (1150 U/I) and positive serology for Enterovirus. Suspecting post-infective myositis, steroids were administered, with a positive response after one month.

However two years later, the clinical and laboratory picture worsened, without history of recent infection. The patient could hardly jump and not walk on the heels. Biopsy of the vastus lateralis showed perifascicular atrophy confirming an inflammatory myositis. High-dose steroids and Ig ev were started with clinical benefit but not full recovery.

After three years from onset, patient was still dependent from immunosuppressive therapy. Examination revealed persistent limb girdle weakness, mild distal laxity, joint contractures of the elbows and cigarette paper scar from the biopsy site. Muscle MRI of the lower limbs showed fatty infiltration at the periphery of muscles.

Because of the poor disease control, the absence of skin manifestation and the pattern of muscle MRI, genetic analysis for congenital myopathies was performed and revealed the already described c.1688A > G p.D563G mutation in *COL6A3* gene. Skin biopsy confirmed the pathogenicity of the mutation showing defective production of collagen VI in patient's fibroblasts.

A SIGMAR1 mutation causes distal hereditary motor neuronopathy

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We describe clinical, genetic and functional studies performed in an Italian family affected by distal Hereditary Motor Neuronopathy (dHMN). The index case, male aged 27 yrs at the first visit, complained ambulatory and fine hands movements difficulty from the infancy, with very slowly worsening. Neu-

rological examination revealed: bilateral stepping gait, wasting and weakness of hands and legs muscles, bilateral pes cavus and hammertoes, ROT hyperactives, with bilateral Babinski. ENG/EMG documented diffuse, marked cMAPs amplitude reduction, motor conduction velocities slightly reduced, sensory conduction preserved; chronic denervation in four limbs distal muscles. Routine biochemical analyses, vit B12, thyroid hormones and brain/spinal MRI were normal. His brother, visited at 35 yrs old, reported same symptoms, disclosing similar findings. No others family members resulted affected. Diagnosis of dHMN was made and a molecular study of HSPB1, HSPB8, GARS, BSCL2,VAPB genes performed, showing absence of mutations. To identify the disease gene a whole-exome sequencing was performed, identifying the missense mutation c.448G > A, p.Glu150Lys in the *SIGMAR1* gene. The mutation co-segregates in homozygosis with the disease in the family with autosomal recessive pattern. Functional studies in neuronal cell lines documented a reduced cell viability and the formation of abnormal aggregate-like structures. SIGMAR1 gene mutations have been to date reported in a form of juvenile amyotrophic lateral sclerosis [1] and in Chinese family affected by dHMN [2]. Our data strengthen the role of SIGMAR1 gene in motor neuron maintenance and survival.

References

1 Al-Saif A, Al-Mohanna F, Bohlega S. A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis. Ann Neurol 2011;70:913-9.

2 Li X, Hu Z, Liu L, et al. A SIGMAR1 splice-site mutation causes distal hereditary motor neuropathy. Neurology 2015;84:2430-7.

Recessive myosin myopathy associated with a new MYH2 mutation and an atypical muscle biopsy pattern with a two decades follow up

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Hereditary myosin myopathies are a group of muscle diseases with variable age of onset and heterogeneous clinical features, caused by mutations in the skeletal muscle myosin heavy chain (MyHC) genes. Dominant as well as recessive mutations in type IIa MHC (MYH2) have been identified. So far three different mutations associated with autosomal dominant phenotype and fourteen mutations associated with recessive one were reported. Homozygous or compound heterozygous truncating or missense MYH2 mutations have been demonstrated to cause recessive myopathy with ophthalmoplegia, mild-to-moderate muscle weakness and complete lack of type 2A muscle fibres. Here we present a patient with a compound heterozygous truncating mutation in MYH2 (p.R793X / p.E1461X), one of them never reported previously (p.E1461X). Clinically he showed ocular ptosis, severe ophthalmoparesis and disto-proximal muscular weakness. High Creatine Kinase blood ratio and diffuse fibro-fatty substitution at muscle MRI were found.

One muscle biopsy, performed at age of 17, showed a neurogenic pattern with some scattered atrophic fibres. A second muscle biopsy, performed 20 years later, showed a dystrophic pattern with diffused rimmed vacuoles, observed only in autosomal dominant MYH2 disease, and never reported in recessive form. This report expands the genotype and the histological phenotype of the MYH2 disease.

DNAJB6 myopathy: not only a vacuolar myopathy

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LGMD1E caused by mutated DNAJB6 is a dominant inherited muscular dystrophy presenting myopathic o dystrophic findings with rimmed vacuoles as histopathological characteristic.

We describe two adult patients with limb girdle muscular dystrophy, caused by the heterozygous mutation in DNAJB6 gene (c.279C > A, p. Phe93Leu). Clinically and pathological features were reported.

The cohort of patients comprised two related males (father and son). Age at onset was at about 30 years old. All patients developed a slowly progressive muscle weakness involving pelvic girdle. CK levels were > 700 U/I for both patients. The father (58 yrs. old) was wheelchair bound since he was 55 years; the son had just minimal strength deficit in lower girdle. Electromyography founded evidence of myopathic pattern. Patients underwent needle muscle biopsy of quadriceps. Morphological examination by light microscopy showed normal muscle in the earlier and minimal myopathic findings in the older patient. No rimmed vacuoles were found. Molecular analysis showed the same heterozygous mutation in DNAJB6 gene in all patients. Muscle MRI showed a non-symmetric, marked degeneration of adductors, posterior leg compartment muscles, soleus and gastrocnemius.

Our data increase literature cases of DNAJB6 mutation, contribute to a better definition of this disease and expand histopathological phenotypic presentation consisting in myopathic changes without vacuoles.

Novel *GYG1* mutation causing late-onset polyglucosan body myopathy with nemaline rods

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Introduction. Polyglucosan body myopathies are a clinically and genetically heterogeneous group of muscle disorders pathologically characterized by the presence of accumulations of alpha-amylase-resistant glycogen. One recently identified form of polyglucosan body myopathy is caused by deficiency of glycogenin-1. Here we present a patient with a novel homozygous mutation causing complete loss of function of glycogenin-1.

Methods. We studied the patient by clinical examination, muscle biopsy, muscle MRI, gene analyses and expression of glycogenin-1 transcripts and protein in muscle.

Results. The patient presented with a very late-onset (after age 80 years) myopathy with involvement of both distal and proximal muscles. An asymmetric involvement was clinically evident and confirmed by muscle imaging. Muscle pathology showed peculiar findings with presence of both polyglucosan and nemaline bodies. Direct sequencing showed a novel homozygous mutation in *GYG1* causing complete absence of the protein. A targeted next generation sequencing excluded concomitant mutations in other myopathy genes including those known to be associated with nemaline myopathy.

Conclusion. Our case further broadens the spectrum of glycogenosis type XV, which can present with a mild, late-onset myopathy with peculiar features on muscle histology. It also raises questions about the possible reasons of coexistence of structural abnormalities together with polyglucosan bodies, and about the relatively mild phenotype despite the protein loss in a recessive disorder.

ABSTRACTS OF POSTER COMMUNICATIONS

(in alphabetical order of the first Author)

Ergoreflex overactivity in mitochondrial disease: linking skeletal myopathy to exercise intolerance and dysautonomia

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Patients with mitochondrial disease (MD) often display exercise limitation and autonomic imbalance with sympathetic predominance. We aimed to clarify how muscle depletion can lead to these manifestations.

Twenty-five adult MD patients with skeletal myopathy, aged 48 ± 3 years, underwent cardiac magnetic resonance (CMR), proton muscle spectroscopy, cardiopulmonary exercise testing (CPET), Holter monitoring, norepinephrine (NE) dosage, ergoreflex and baroreflex assessment. Thirteen patients were matched for age and sex to healthy, sedentary controls.

Two patients had cardiac hypertrophy, and eight had myocardial fibrosis. At CPET, maximal workload and peak oxygen consumption/kg were significantly lower in patients than controls (both $p < 0.001$).

The ergoreflex is a neural mechanism regulating ventilation and autonomic function during exercise. Ergoreflex sensitivity was markedly higher in MD patients than controls ($p < 0.001$). It correlated with muscle fat-to-water ratio (Spearman's Rho = 0.779, $p = 0.042$), suggesting a link between muscle damage and ergoreflex overactivity. Ergoreflex sensitivity was inversely correlation with peak workload (Rho = 0.711, $p < 0.001$) and peak oxygen consumption/kg (Rho = 0.648, $p < 0.001$); ergoreflex overactivity could then reduce exercise tolerance.

Ergoreflex overactivity could also contribute to autonomic imbalance. Indeed, ergoreflex sensitivity correlated directly with NE (Rho = 0.322, $p = 0.020$), and inversely with the standard deviation of RR intervals (Rho = 0.530, $p = 0.013$).

At univariate analysis, NE was the only predictor of myocardial fibrosis. NE was higher in patients with fibrosis ($p = 0.034$); a 692 ng/L cut-off was selected (area under the curve 0.800, sensitivity 90%, specitivity 75%).

In MD, skeletal myopathy can increase ergoreflex sensitivity, causing exercise intolerance and sympatho-vagal imbalance. Sustained sympathetic overactivity may contribute to cardiac damage.

Analysis of 9-AC binding site on CLC-1 channels and potential chaperone activity in myotonia congenita

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Myotonia congenita (MC) is a skeletal muscle hyperexcitability disorder, caused by loss-of-function mutations in the chloride channel ClC-1, affecting its gating or membrane density. To date, therapy is only symptomatic. One potential approach to promote surface expression for trafficking-defective MC mutants may exploit low concentration of selective and reversible ClC-1 blockers, such as 9-anthracene-carboxylic acid (9-AC).

We first provided an in-depth characterization of 9-AC binding pocket in ClC-1 by testing the sensitivity to 300microM 9-AC of MC ClC-1 mutations residing near the pore and C-terminal region. Second, we tested 9-AC ability to act as a pharmacological chaperone on one trafficking-defective MC mutant, A531V. WT and MC mutant ClC-1 channels were expressed in HEK293 cells and whole-cell currents were recorded with patch-clamp, before and after external application or incubation with 9-AC. Docking studies were performed to substantiate electrophysiological data.

F484L channels completely loose sensitivity to 9-AC whereas G190S, L198P and G270V channels block by 9-AC is reduced compared to WT. Conversely, the C-terminal mutation, L628P, does not affect 9-AC inhibition. Furthermore, docking studies propose that K231, an important residue for Cl- selectivity, plays a central role in 9-AC binding. Interestingly, incubation of cells expressing A531V channels for 24 hours with 30microM 9-AC increases mutant currents by about 2 fold.

In conclusion, we identified pore residues crucial for 9-AC binding and block of ClC-1 channels. Moreover, we provided a proof of concept that small ClC-1 ligands can function as pharmacological chaperones of trafficking-defective ClC-1 mutants and may prove useful in myotonic patients.

RNA profiling discloses a link between circadian genes and muscle damage in Duchenne Muscular Dystrophy

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The muscular dystrophies are inherited genetic conditions that cause progressive weakness and loss of muscle mass. Mutations occurring in structural proteins such as dystrophin, cause muscle fibers' changes in mechanical stability that interfere with contraction.

Circadian rhythm coordinates biological processes with the predictable 24h cycle of day and night.

Circadian rhythm genes' role in maintaining the regular muscle functions is known, both in animal models and in humans. However, the role in muscular dystrophy is still undefined.

The aim of this study was to define the circadian transcriptional profile in *mdx*, unexercised *mdx* and exercised *mdx* mice treated with different types of drugs: resveratrol, apocynin, taurine, nandrolone, prednisolone, enalapril, calpain inhibitor, pentoxifylline and antisense oligoribonucleotides.

We designed an ad-hoc Micro Fluidic Card TaqMan based assay, including 30 genes (FLUID-CIRC) related to circadian rhythms and muscle regeneration. We tested gastrocnemius (GC) and tibialis anterior (TA) muscles from both unexercised and exercised *mdx* mice.

The majority of analyzed genes is strongly upregulated in both exercised and unexercised *mdx* mice. Statistical analysis prioritized seven most deregulated genes (CSNK1E, SIRT1, MYOG, MYOD1, CRY1, CRY2 and ARNTL) in both tissues.

We further evaluated these selected genes in exercised *mdx* mice treated with different drugs, able to ameliorate the dystrophic phenotype. We demonstrated that drug exposures induce modification of circadian genes' expression profile.

Our data demonstrate that circadian genes are affected in both DMD patients and *mdx* mice supporting a correlation between circadian circuit and DMD pathology, open the way to new interesting therapeutic options.

The ubiquitin ligase tripartite-motif-protein 32 (TRIM32) is induced in Duchenne Muscular Dystrophy

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Activation of the proteasome pathway is part of the secondary processes of cell damage which ultimately lead to muscle degeneration and necrosis in Duchenne Muscular Dystrophy (DMD). In *mdx* mice the proteasome inhibitor Bortezomib upregulates the membrane expression of members of the dystrophin complex and reduces the inflammatory reaction. However, chronic inhibition of the 26S proteasome may be toxic as indicated by the systemic side-effects caused by this drug. Therefore, we sought to determine the components of the ubiquitin-proteasome pathway that are specifically activated in human dystrophin-deficient muscles. The analysis of a cohort of patients with genetically determined DMD or Becker Muscular Dystrophy (BMD) unveiled a selective up-regulation of the ubiquitin ligase Tripartite motif-containing protein 32 (TRIM32). The induction of TRIM32 was due to a transcriptional effect and it correlated with the severity of disease in BMD patients.

Knock-out models have shown that TRIM32 is involved in ubiquitination of muscle cytoskeletal proteins as well as of protein inhibitor of activated STAT protein gamma (Piasγ) and N-myc downstream-regulated gene (NDRG), two inhibitors of satellite cell proliferation and differentiation.

Accordingly, we showed that in DMD/BMD muscle tissue TRIM32 induction was mostly pronounced in regenerating myofibers rather than in necrotic muscle cells, thus pointing out an effect of this protein in the regulation of human myoblast cell fate. This finding evidences TRIM32 as a possible target to favour skeletal muscle regeneration in patients.

Study design of an assessment protocol for central and peripheral fatigue in myotonic dystrophy type 1 (DM1)

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Myotonic dystrophy type 1 (DM1) is an autosomal-dominant disorder characterized by myotonia, muscle weakness, and multisystemic involvement; a majority of patients complain of fatigue and daytime sleepiness with unclear relationship with clinical-pathophysiological variables.

Central and peripheral determinants of fatigue have been evaluated in a sample of 26 DM1 patients (17 males, 9 females, mean age 41.6 years, SD ± 12.7), that underwent Fatigue Severity Scale (FSS), Epworth Sleepiness Scale (ESS) and an intermittent incremental effort of the forearm muscle using a myometer. Muscle fatigue blood biomarkers were collected during aerobic exercise test. Statistical analysis revealed no significant differences in blood concentration of oxidative stress biomarkers (advanced oxidation protein products-AOPP, ferric reducing ability of plasma-FRAP, thiols; _mol/l) before and after the effort. The presence of central fatigue was detected; FSS score was significantly correlated to maximal voluntary contraction (MVC baseline and 60%, r-baseline = -0.583, $p < 0.01$, r-60% = -0.534, $p < 0.05$), and to motor disability measured by MRC ($r = -0.496$, $p < 0.05$); moreover we found a strong tendency towards significance in the association with lactate baseline ($r = 0.378$, $p = 0.057$). Multiple regression analysis has then been performed to see whether and how experienced and physiological fatigue measures relate to the other variables.

Results are to indicate as such exercise protocol, easily deployable and well-tolerated, may be suitable for proper management of DM1 patients Based on that, proper assessment of fatigue should therefore be considered to be included in algorithms for clinical-data collection in DM1 patient-registries.

Validation of a cell-based assay for large up-regulation in alpha-dystroglycanopathies

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Alpha-dystroglycanopathies are caused by loss of alpha-Dystroglycan (a-DG) functional glycosylation. Although exogenous overexpression of the glycosyltransferase LARGE has been extensively shown to rescue a-DG glycosylation and laminin-binding in distinct models of dystroglycanopathies, pharmacologically-active compounds able to increase endogenous LARGE are unknown.

Our study aimed to develop and validate a High Throughput Screening target-based platform for the identification of drugs/chemical compounds that may induce LARGE in myoblasts by acting at transcriptional level. We had previously generated clones of C2C12 cells stably expressing a 1.4 kb fragment of the LARGE promoter fused with a luciferase reporter (pPr-1.4).

To identify positive controls for the screening of FDA-

approved drugs, we tested three stable clones with a library, consisting of 95 substances that regulate gene expression by carrying out epigenetic modification of lysines. We considered "active" a substance when luciferase activity was greater than 3SD above the mean of DMSO-negative controls and the vitality greater than 80%. We identified three positive hits, PFI-1 (#27, Inhibitor of Bromodomains), Bromosporine (#79, non-selective Bromodomain Inhibitor), Trichostatin-A (#87, reversible Inhibitor of HDAC). The effects of these molecules on pPr-1.4 were further verified with a full-dose-response-curve and the resulting optimal concentration was tested in a time-course experiment. Accordingly, in wild-type C2C12 cells, treatment with compounds 27, 79, 87 led to an increase of endogenous LARGE transcripts of respectively 2.6,1.8 and 2.7folds over DMSO-treated cells.

In conclusion, we have identified and characterized three molecules, which consistently enhanced LARGE transcription in myoblasts and which may be adopted as positive controls for screening of FDA-approved drugs or larger chemical collections.

Lipomatosis incidence and characteristics in an Italian cohort of mitochondrial patients

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Lipomas have often been associated with mtDNA mutations and were mainly observed in patients with mutation in mitochondrial tRNA lysine which is also the most frequent mutation associated with MERRF. Up to date, no systematic studies have been developed in order to assess the incidence of lipomas in large cohorts of mitochondrial patients.

The aim of this study is to analyze the incidence and characteristics of lipomas among an Italian cohort of patients with mitochondrial diseases.

Retrospective, database-based study (Nation-wide Italian Collaborative Network of Mitochondrial Diseases) of patients with lipomas.

A total of 17 patients with lipomas have been identified among the 1086 mitochondrial patients, enrolled in the Italian database. About 20% showed a classical MERRF syndrome whereas the others disclosed myopathy (40%), one CPEO and othertwoonly an isolated lipomatosis. Lactate was elevated in almost all the examined patients. Muscle biopsy was available in 10/17 patients: in all of them mitochondrial abnormalities were present. 90% had mutations in mtDNA coding for tRNA lysine. Interestingly,two patients had multiple mitochondrial DNA deletions in muscle. Lipomas were multiple in 11 out of 17 patients and in about 50% were symmetric. In all patients, lipomas were localized along the cervical-cranial-thoracic region.

Our data confirm the strong association between multiple lipomas and lysine tRNA mutations, although the presence of two patients with muscle mtDNA multiple deletions confirm that mutation in other genes may lead to a similar phenotype.

Mobility shift of beta-dystroglycan as a marker of genetic defects in the *GMPPB* gene

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Dystroglycan (DG) consists of two subunits (alpha and beta), which are translated from a single mRNA as a propeptide. This is proteolytically cleaved into two proteins that undergo posttranslational modifications and remain associated at the membrane. Defects in glycosylation of alpha-DG cause autosomal recessive disorders with a wide spectrum of phenotypes ranging from severe congenital muscular dystrophies with brain and eye abnormalities, to milder adult onset limb girdle muscular dystrophies. An ever-growing number of genes involved in the pathogenesis of dystroglycanopathies have been reported, with 18 genes identified so far. Despite the genetic heterogeneity of this group of disorders, muscle biopsies from all affected individuals show variable reduction of immunoreactivity to monoclonal antibodies specific for glyco-epitopes of alpha-DG. Therefore, in the absence of specific markers to focus molecular investigations, the diagnosis must be achieved through the analysis of a large panel of genes. Substantial changes in beta-DG have not been described to date. However, Western blot analysis performed as routine diagnostic test showed a consistent shift in the mobility of beta-DG in samples from twelve patients with mutations in GDP-mannose Pyrophosphorylase B (*GMPPB*). This was not observed in any other sample analyzed, including cases with mutations in various dystroglycanopathy genes (*FKRP*, *POMT1*, *POMGnT1*, *ISPD*) and it is accompanied by reduction of laminin alpha2. *The significance of this alteration is currently under investigation.* Our data demonstrate that a change in beta-DG electrophoretic mobility in patients with dystroglycanopathy is a clear and specific indicator of a molecular defect in *GMPPB.*

DMD genotypes and loss of ambulation in the CINRG Duchenne Natural History Study

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We aimed to correlate time to loss of ambulation (LoA) and different truncating *DMD* gene mutations in a large prospective natural history study of Duchenne muscular dystrophy (DMD), with particular attention to mutations amenable to emerging molecular treatments. To this end, we analyzed data from the Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study (DNHS), selecting participants with frameshifting *DMD* single- or multi-exon deletions or duplications with defined exon boundaries (n = 186), or small mutations identified by sequencing (n = 26, including 10 frameshifting small mutations and 16 nonsense point mutations). We performed a time-to-event analysis of LoA, a strong indicator of overall disease severity, adjusting for glucocorticoid treatment and genetic modifiers. Participants with deletions amenable to skipping of exon 44 had later LoA (median 14.8 years, HR 0.31, 95% CI 0.14 - 0.69, p = 0.004). Age at LoA did not differ significantly in participants with deletions amenable to exon 45, 51, and 53 skipping, duplications, and small rearrangements. Nonsense mutation DMD also showed a "typical" median age at LoA (11.1 years), with a few outliers (ambulatory around or after 16 years of age) carrying stop codons within in-frame exons, more often situated in the rod domain. As exon 44 skipping amenable DMD have a later LoA, mutation-specific randomization and selection of placebo groups are essential for the success of clinical trials. We also provide relevant natural history data about the LoA endpoint in specific DMD subpopulations, amenable to novel molecular treatments.

Body composition and resting energy expenditure in late- onset Pompe disease

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Pompe disease (LOPED) is characterized by great variability of muscle weakness and respiratory insufficiency in relation to residual enzyme activity, onset age and disease progression rate. To date, few data are available on body composition (BC) and resting energy expenditure (REE). To this purpose, 7 patients (3M/4F, 56 ± 11years) and 7 healthy control subjects well matched for sex, age and body mass index were enrolled. BC by anthroplicometry, bioelectrical impedance and dual x-ray energy absorptiometry, REE by indirect calorimetry and routinary blood parameters were assessed. Physical activity level and food intake were investigated by International Physical Activity Questionnaire and 7-days food dietary records.

No significant differences occurred in BC and REE compared to controls. Fat mass by plicometry seemed to be underestimated when compared to DEXA (34,2 ± 7,4 *vs* 43,4 ± 7,4%; p = 0,03).

Creatinine levels were lower in LOPED (0.47 ± 0.08 *vs* 0.86 ± 0.34 mg/dl; p = 0.01), while GOT and GPT levels were higher in LOPED than controls (34.3 ± 8.1 *vs* 20.8 ± 5.9 UI/l, p = 0.002; 36.5 ± 10.5 *vs* 24.0 ± 11.0 UI/l, p = 0.04, respectively). Mean fasting glucose, HOMA IR and glycated hemoglobin were 101 ± 9.62 mg/dl, 2.13 ± 1.29 and 5.40 ± 0.37%, respectively. Impaired fasting glucose was found in 3 LOPED.

LOPED physical activity level was lower than controls (p = 0.03). Dietary intake of protein (1,2 g/body weight) and simple sugars (17%) were higher in LOPED than recommended values (*LARN, 2012*).

LOPED did not seem to impair BC and REE. Increased liver enzymes and reduced creatinine are in agreement with the neuromuscular disease. More detailed studies are needed to better understand nutritional management of LOPED.

Penetrance differences among subjects carrying leber's hereditary optic neuropathy (LHON) primary mutations

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Leber's hereditary optic neuropathy (LHON) is a maternally inherited disease characterized by a rapid bilateral central vision loss, typically associated to three primary mitochondrial DNA (mtDNA) mutations, including the m.3460G > A and m.11778G > A. However, the presence of primary mutations is necessary but not sufficient to cause the optic neuropathy. Incomplete penetrance, variable clinical expressivity and male prevalence, are LHON features that remain to be explained but that suggest that environmental and/or other genetic factors must influence the phenotype. We assessed genetic diagnosis in an Apulian cohort of patients with LHON and evaluated the contribution to the penetrance of three different factors: the proportion of mutated *versus* wild-type genotype; the presence of ancillary mutations; the mtDNA copy number. Our findings showed that a less severe LHON phenotype is mostly associated to both m.3460 and m.11778 when heteroplasmic; in heteroplasmic unaffected subjects an increased mitochondrial biogenesis is protective, such as in homoplasmic, thus discriminating affected and carrier subjects. This result was confirmed in an independent cohort of homoplasmic/heteroplasmic LHON Spanish subjects. Moreover, we identified the co-occurrence of three mutations in 12S rRNA in a LHON pedigree characterized by the presence of mental retardation and epilepsy.

Exome sequencing as strategy to identify the gene determining limb girdle muscular dystrophy type 1H

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More than 20 genes with autosomal recessive (LGMD2A-Q) and autosomal dominant (LGMD1A-H) inheritance have been mapped. We aim to identify the genetic features of a four-generation family from Southern Italy affected by LGMD1H, presenting onset during the fifth decade of life, of a slowly progressive proximal muscle weakness affecting both upper and lower limbs and a relatively benign course. We had previously mapped the LGMD1H *locus* on chromosome 3p23-25.1. To identify the gene variant responsible for the phenotype we performed Whole Exome Sequencing (WES) in 8 family members belonging to three generations, including 6 affected and 2 healthy/unaffected relatives. We identified a total of ~18,000 exonic and splicing variants in each sample, of which ~4,000 variants were common to all affected individuals. Considering the dominant mode of inheritance of LGMD1H, we focused on the heterozygous calls (~400 variants) and filtered out all variants with a frequency > 2% in the 1000 genomes, NHLBI Exome Sequencing Project and/or EXAC Broswer (~100 variants). From this analysis we selected ~50 variants including either those absent in dbSNP146, or those occurring in muscular-expressed genes and in all the known myopathy and dystrophy-related genes. To distil the causative mutation we performed in *silico* analyses for their damaging role. We also excluded gross genomic rearrangements through Array Genomic Hybridization (AGH) in 2 affected subjects. To date, we have restricted the number of potential causative variants to a very small number of candidate genes.

Skin alterations in myotonic dystrophy type 1: an observational, cross-sectional study

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Myotonic dystrophy type 1 (MD1 or Steinert's disease) is an autosomal dominant multisystem disorder caused by expansion of a CTG trinucleotide repeat in the non-coding region of dystrophia myotonica protein kinase (DMPK) gene, located on chromosome 19. Progressive muscular weakness, atrophy and myotonia are the most prominent features even though heart, central nervous system, endocrine system, smooth muscles are commonly involved. To date, few studies have been performed to evaluate skin features in MD1 patients compared to control subjects, showing controversial features: a significantly higher numbers of nevi, dysplastic nevi, melanomas and pilomatrixoma have been reported, whereas others authors do not rule out an increased prevalence of pre-neoplastic and neoplastic skin lesions. Herein, we present the largest group (103 MD1 patients) systematically screened for skin lesions by a complete clinical examination of skin and mucosae and an intra-vital digital videodermoscopy through the Fotofinder Dermoscope by a trained dermatologist to check for the presence of skin lesions. Aim of this study is to evaluate the prevalence of neoplastic, proliferative, functional and inflammatory skin lesions in MD1 patients, compared to controls and to compare our results with previous data. Our MD1 patients showed a variable increase of inflammatory, preneoplastic and neoplastic skin lesions, except from hidradenitis suppurativa, acne and fibrohistocytoma. This study adds new details to characterize the phenotype of MD1 patients and support the presence of cutaneous specific hallmarks in MD, ruling out a higher prevalence of preneoplastic and neoplastic cutaneous lesions.

Mutations in *CPT2* gene in patients with carnitine palmitoyltransferase ii deficiency

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Carnitine palmitoyltransferase II (CPT II) deficiency is one of the most common defects of oxidative lipid metabolism in humans with recessive autosomal inheritance. CPT II deficiency has three distinct phenotypes: a common adult onset myopathy, a severe late infantile hepatocardiomuscular form and a rare lethal neonatal form. The myopathic form occurs most frequently, with more than 300 cases and is characterized by episodes of muscle pain, cramps, elevated serum creatine kinase levels and myoglobinuria triggered by prolonged exercise or fasting. The myopathic form of CPT II deficiency may be under-recognized because symptoms can be mild and physical impairment may not occur.

The diagnosis is usually achieved by detection of reduced CPT enzyme activity but the molecular genetic testing of *CPT2,* the only gene known to be associated with CPT II deficiency, provides a definitive diagnosis.

To date, 60 mutations have been identified in the *CPT2* gene responsible for this disorder and 41 of them are predicted to produce amino acid substitutions or deletions. One mutation in the exon 3 namely c.338C > T (p.Ser113Leu), has been found in about 60% of adult cases.

The analysis provides the full screening of the five *CPT2* exons and their immediate flanking regions by Sanger sequencing. In a cohort of 8 Italian patients we found 3 mutated subjects: one compound heterozygote (p.Ser113Leu and p.Arg151Gln) and two homozygotes (p.Ser113Leu). Based on these findings, a targeted mutation analysis starting from the exon 3 of the *CPT2* gene can be an effective approach for molecular diagnosis of CPT II deficiency.

Muscle MRI in *POMT2* limb-girdle muscular dystrophies

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Among the heteorogeneus group of alpha-dystroglycanopathies, *POMT2* mutations are known to cause a spectrum of CMD disorders including the Walker–Warburg Syndrome with severe brain and ocular malformations and the limb girdle muscular dystrophies (LGMD) with and without mental retardation.

In contrast to many reports on brain MRI, there are very few descriptions of skeletal muscle imaging findings in patients with alpha-dystroglycanopathies LGMDs. To the best of our knowledge a distinct pattern of muscle involvement has been described only in *FKRP*-related alpha-dystroglycanopathy (LGMD2I).

We performed muscular magnetic resonance with axial

T1-weighted sequences of lower girdle and lower limbs in two patients affected by LGMD with mutation in POMT2 gene, identifying a common and recognizible pattern of selective muscular involvement with signal intensity variations depending from age.

Patients, a male and a female, of 22 and 12 years old respectively, presented a typical girdle weakness with waddling gait and hyperlordosis and normal brain MRI.

At pelvis level, marked fatty degeneration of glutei medium and minimum was evident, followed by gluteus maximus. At thigh level, most prominent changes were found in adductor magnus and to a lesser extent, in hamstrings muscles. Gracilis and sartorius seemed to be spared and hypertrophic. In the lower leg, striking fatty infiltration and atrophy of gastrocnemius medialis was detected, with mild involvement of soleus muscle and relative preservation of gastrocnemius lateralis and anterior compartment muscles.

These findings appear to be different from what reported in *POMT2* congenital muscular dystrophies patients, suggesting that muscle involvement could be "gene-unrelated".

3,4-diaminopyridine in Lambert-Eaton Syndrome: a five year experience

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The Lambert-Eaton Syndrome (LEMS) is characterized by proximal muscle weakness, autonomic symptoms, and depressed tendon reflexes with post-tetanic potentiation. The disorder can be either paraneoplastic, P-LEMS, (small cell lung cancer in about 60%) or associated to autoimmune disorders. The weakness results from a reduction in the quantal release of acetylcholine from motor nerve terminals, caused by autoantibodies to P/Q-type voltage-gated calcium channels (VGCCs). 3,4-Diaminopyridine (3,4-DAP) enhances the release of acetylcholine at the neuromuscular junction and it is widely used to treat LEMS.

We follow 8 patients (5 male and 2 female) affected by LEMS. All these patients are treated with 3,4-DAP. We evaluated the effects of 3,4-DAP trough LEMS Registry Worksheet which consist of a clinical and neurophysiological evaluation of the patients at the beginning of the therapy and at follow-up. Five out of seven patients had a clinical improvement which lasted till the follow-up; two out of these five patients get the improvement with the 3,4-DAP therapy alone, the others are treated also with corticosteroids and azathioprine. No one of the patient had clinical adverse effects.

Unlike other aminopyridines, 3,4-diaminopyridine (DAP) has limited penetration into the brain and thus produces few CNS side effects at doses sufficient to improve neuromuscular transmission. To the date 3,4-DAP is the first-line treatment for Lambert-Eaton syndrome, as suggested by the Task Force of the European Federation of Neurological Diseases.

Autosomal dominant central core disease caused by a novel heterozygous mutation in the ACTA1 gene

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Congenital myopathies (CMs) are a heterogeneous group of inherited muscle disorders, characterized by specific histopathologic findings in muscle biopsy. To date, more than 20 genes have been associated to different forms of CMs, but approximately one-third of patients remain genetically unresolved.

The ACTA1 gene encodes skeletal muscle alpha-actin, the principal actin isoform in adult skeletal muscle, which forms the core of the thin filament of the sarcomere where it interacts with a variety of proteins to produce the force for muscle contraction. ACTA1 is implicated in several CMs=including nemaline, cores, rod-core or actin aggregate myopathies and fiber-type disproportion.

We report four subjects from an Italian family presenting rhinolalia and diffuse mild muscle weakness (facial, axial, proximal and distal). Muscle MRI showed fatty replacement in gluteus minimus, quadriceps, biceps, abductors and gastrocnemius. Muscle biopsy showed the presence of myopathic changes associated with several cores in muscle fiber type I. The molecular analysis revealed a novel mutation in exon 3 of the ACTA1 gene in heterozygous state. Our findings enlarge the genetic spectrum of ACTA1 related core myopathies.

Inclusion body myositis and progressive muscular atrophy: muscle and nerve, who does mimic what?

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Progressive flaccid tetraparesis, muscle atrophy, cramps and hyperCKemia may be both muscular or nerve damage clinical features. Sporadic inclusion body myositis is an acquired inflammatory myopathy whose usual presentation is a progressive weakness and atrophy of the four limbs muscles, especially of the quadriceps femoris; fasciculations are always absent. Progressive muscular atrophy is a rare, sporadic, adult-onset, clinically isolated lower motor neuron syndrome, clinically characterized by progressive flaccid weakness, muscle atrophy, fasciculations, and reduced or absent tendon reflexes. Here we report the case of a 63 yrs-old man, came to our attention for lower limbs proximal hypostenia, myalgias and hyperCKemia (1600 U/L). He had no family history of neuromuscular disorders and no major comorbidities. Neurological examination revealed lower limbs mild hypostenia and quadriceps femoris hypotrophy, without upper motor neuron or bulbar signs; diffuse limbs fasciculation are also present, but not tongue fasciculations. The electromyography was consistent for a neurogenic

damage with diffuse denervation and fasciculations. Muscle biopsy revealed a mixed myopathic and neurogenic changes, an inflammatory lympho-monocyte endomysial infiltrate, rare vacuoles and no SMI-positive inclusions. The electronic microscopy confirmed the mixed picture and showed no inclusion bodies. *VCP* gene mutations were negative, as well as *SOD1*, *FUS*, *TARDBP* genes. The patient underwent i.v. immunoglobuline treatment with only initial partial benefit. The clinical features is rapidly progressed to severe tetraparesis in three years, needing for non-invasive ventilatory support. Actually, upper motor neuron or bulbar signs are not yet present. Eventually a diagnosis of second motor neuron disease was formulated and riluzole was recommended.

Risk of myopathy in statin treated patients: identification of CLC-1 chloride channel as a pivotal biological marker

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Statin therapy can produce skeletal muscle dysfunction that range from myalgia to severe rhabdomyolysis. Our preclinical studies in rat model showed that statin-induced myopathy involve the reduction of ClC-1 chloride channel expression and of resting chloride conductance (gCl), sustained by this channel. This reduction is detrimental for muscle function. The reduction of gCl is also due to statin-induced activation of protein kinase C (PKC), which negatively regulate the ClC-1 channel activity. Our goal is to translate these observations into clinical studies at the aim to identify biological markers useful to predict and prevent statin-induced muscle damage especially during aging. For this we examined ClC-1 mRNA and protein expression in muscle biopsies of 10 patients of different age under statin-therapy and affected by myopathy, and compared the results with those of untreated patients. We found a marked reduction of ClC1 protein by 40% in statin-treated patients, independently from age. This effect is associated with an alteration of electromyography. Also, PKC expression and activity is increased, contributing to ClC-1 channel inactivation. However compensatory mechanism are elicited, as we found a significant increase of the ClC-1 channel mRNA, which suggest enhanced transcription. Although MuRF1 is increased as a result of muscle atrophy, here we found that Notch-1 was highly expressed, suggesting active regeneration. An increase of PGC-1-alpha and of isocitrate dehydrogenase suggest mitochondrial biogenesis in accord with increased citrate synthetase activity. This study demonstrate the pivotal role of ClC-1 in the increased risk of myopathy due to statin therapy in humans.

Contractile efficiency of dystrophic MDX mouse muscle: in vivo and ex vivo assessment of adaptation of functional endpoints to exercise

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Progressive weakness is a typical feature of Duchenne muscular dystrophy. In vivo weakness is exacerbated in the mdx mouse model by protocols of chronic treadmill-exercise and its correlation with impaired muscle function is unclear. We focused on the occurrence of functional adaptation/maladaptation of dystrophic muscles in relation to duration of treadmill-exercise protocols. In vivo weakness was confirmed by grip strength after 4, 8 and 12 weeks of exercise in mdx. Torque measurements in anesthetized mdx mice led to a weakness correlated with the duration of exercise protocol, while wt mice were stronger. Contractile parameters of diaphragm and extensor digitorum longus (EDL) muscle were impaired in mdx mice; a worsening by exercise, significant after a single exercise bout for both muscles or after long exercise protocol in EDL muscle, was also observed. Mdx EDL muscle was highly susceptible to eccentric-contraction and didn't show the exercise-induced adaptation observed in wt mice. qRT-PCR analysis in mdx EDL muscle confirmed the impairment of exercise-sensitive genes. The increased levels of plasma biomarker metalloproteinase-9 and the worsening of histopathology in mdx mice supported the exercise-induced damage. Then the dystrophic muscles showed a certain degree of functional adaptation to chronic exercise which is however not sufficient to overcome the weakness typical of the pathology, likely related to the mechanical-metabolic uncoupling accounting for general exercise-induced worsening. These results support the importance to better understand the mechanisms underlying in vivo and ex vivo weakness for paving the way to more effective treatments. (Supported by Prin-MIUR n°20108YB5W3_004).

A possible involvement of endosomal toll-like receptors in the pathogenesis of laminopathies

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Laminopathies are a group of rare genetic disorders caused by mutations in the *LMNA* gene, encoding proteins of the nuclear lamina, affecting skeletal and cardiac muscle and many other tissues. Although the genetic cause of laminopathies is known, the specific factors that initiate and perpetuate disease progression are not well understood. Recent studies revealed the important contribution of innate immune system and, particularly, of endosomal Toll-like receptors (TLRs) in the degeneration/regeneration processes undergoing Duchenne muscular dystrophy, and the relationship between nuclear lamina defects and activation of NFκB, a transcription factor of TLR pathway; therefore, we proposed to evaluate the role of TLRs in skeletal muscle laminopathies (SML) and other muscle diseases. By immunohistochemistry/immunofluorescence, we observed that in SML muscle biopsies TLR4 and TLR9 were highly expressed on capillaries and inside muscle fibers, characterized by a small size or/and a high nuclei number, whereas TLR7 was mainly localized on the sarcolemma and, occasionally, in the sarcoplasm of some myofibers. A similar expression pattern was observed with facioscapulohumeral muscular dystrophy. In Pompe disease and in sporadic inclusion body

myositis (sIBM), a TLR4 and TLR9 positivity was observed in the sarcoplasm of degenerating/regenerating muscle fibers, especially in association with vacuolar formations, whereas TLR7 mainly localized at the level of immune infiltrates. A significant increase in TLR4 and TLR7 transcriptional levels was observed in SML and sIBM muscles.

Therefore, a key role in the pathogenic mechanisms of skeletal muscle laminopathies might be hypothesized for specific endosomal TLRs.

Mutations in *HSPB8* causing autosomal dominant distal hereditary motor neuropathy and myofibrillar myopathy: report of a novel family

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Mutations in the small heat shock protein 22 gene (*HSPB8*) are associated with Charcot-Marie-Tooth type 2L (CMT2L) and distal hereditary motor neuronopathy type IIa (dHMN2A). More recently, *HSPB8* mutations (p.K141E and p.P173SfsX43) have been reported in two unrelated families with distal myopathy and motor neuropathy. Here we report a third family with autosomal dominantdHMN and myofibrillar myopathy.

The proband, a 30-year-old woman, presented at age 20 with progressive difficulties in walking and muscle cramps. Similar complaints were reported by her trizygotic. On examination there was moderate lower limb proximal and distal weakness, as well as axial weakness involving neck flexors. She could not raise from supine to sitting position, nor from sitting to standing position. Reflexes were present throughout. Head and upper limb examination was uneventful and sensation was intact at four limbs. Previous nerve conduction study showed chronic neurogenic changes at distal lower limbs. CK was 227 U/L. Lower limb MRI showed a selective pattern of fatty-replacement predominantly affecting peroneal and biceps muscles consistent with a primary myopathic process. Quadriceps muscle biopsy showed mixed dystrophic and neurogenic features. Myofibrillar aggregates reactive for myotilin and aB-crystallin were also observed. Exome sequencing identified a heterozygous p.K141E change in *HSPB8* in the proband, which segregated with the disease in the family.

Our report further confirms the association of the p.K141E mutation in *HSPB8*with motor neuropathy and myofibrillar myopathy.

Consensus conference on orthotopic liver transplantation *versus* allogenic hematopoietic stem cell transplantation in MNGIE

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Mitochondrial neuro-gastro-intestinal encephalomyopathy (MNGIE) is a rare autosomal recessive mitochondrial disease caused by mutations of TYMP gene (with a markedly reduced TP activity) which is known to result in nucleoside accumulation and related mtDNA damage. The phenotype is characterized by gastrointestinal and neurological manifestations with an unavoidable fatal outcome. The ideal treatment is based on TP replacement, which has been first achieved with allogenic hematopoietic stem cell transplantation (AHSCT), which is associated to a very high mortality. Thus, other tissue sources of TP have been investigated. We have recently characterized TP in human liver and demonstrated that TYMP transcript is endogenously synthesized and expressed in comparable amounts to that detectable in the bone marrow. This in turns pointed to orthotopic liver transplantation (OLT) as an alternative to AHSCT. So far 2 MNGIE patients have received worldwide, one in the US and one in Italy, with encouraging results both in terms of biochemical correction and outcome. In view of the availability of such a novel approach to treatment of MNGIE, an international consensus conference was convened in Innsbruck on February 2016 to discuss the clinical and laboratory criteria which should be met in order to select patients for either procedure. Other useful options and future genetic therapy have been included in the discussion.

Genetic and proteic study of CLC-1 channel expression in rat fast and slow skeletal muscle, from birth to aging

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ClC-1 chloride channels play a critical role in controlling sarcolemma excitability. Loss of function mutations of ClC-1 gene cause myotonia congenita that is characterized by delayed muscle relaxation after voluntary contraction. Previous studies on extensor digitorum longus (EDL) rat muscle have shown that 7 days after birth, the resting chloride conductance (gCl), sustained by the ClC-1 channel, and ClC-1 mRNA are very low and increase rapidly with age. During aging a decrease of gCl and ClC-1 mRNA expression was also found with respect to adulthood. On this basis, our aim is to perform a systematic study of ClC1 protein and mRNA, in slow-twitch (SOL) and fast-twitch (EDL) rat muscles from birth to old age by western blot and qPCR analysis. Our preliminary data show that ClC-1 protein increases 10-fold from birth (1-day) to 8-months and decreases by 50% in aged muscles (27-months) both in SOL and EDL, while in SOL muscle ClC-1 protein is undetectable at birth. Since ClC-1 can be inactivated by protein kinase C (PKC), we evaluated PKC activity by ELISA analysis showing that it decreases from birth until 8-months and slightly increases in aged muscles. We are currently investigating ClC-1 cellular distribution by immunofluorescence analysis. Interestingly, we found sarcolemma and cytoplasmic co-localization of ClC-1 from birth to 24-days and a peculiar membrane localization in adult rats. Our findings provide the direct evidence that ClC-1 expression varies during muscle development offering a point of comparison for all the situations in which ClC-1 protein is altered.

Late onset nemaline myopathy in a patient with autoimmune polyendocrine sindrome type I

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Nemaline myopathy encompasses a group of disorders characterized by muscle weakness and the presence of rods in affected muscle fibres. Clinical presentations vary from neonatal to late onset forms. This latter form typically affects adult patients with onset in the third to sixth decade and may not have a genetic basis. Late onset form has heterogeneous clinical presentation with progressive weakness and often with immunological abnormalities or associated with inflammatory changes on muscle biopsy.

We describe a 48-year-old man with autoimmune polyendocrine syndrome (APS) type I, a rare systemic autoimmune disorder with autosomal-recessive inheritance, that developed muscular disease. At 37 years of age, he started complaining progressive muscle weakness that involved proximal and limb girdle muscle and that lead to loss of walking ability. Since one year muscle weakness progressed till involving diaphragm.

A first muscle biopsy revealed features of inflammatory myopathy. Histological examination showed degenerative changes and the presence of inflammatory cell infiltrate with increased expression of major histocompatibility complex class I.

Examination of a repeated muscle biopsy, 8 years later, revealed marked variation in fiber size and modified Gomori trichrome method showed an increased staining suggestive of nemaline rods. Transmission electron microscopy revealed the presence of electron dense structures with rod-like shape, mainly localized at the periphery of the fibers but also dispersed between myofibrils.

Muscle biopsies suggest the appearance of a late onset nemaline myopathy in a patient affected by APS type I, probably as secondary pathophysiological response of skeletal muscle to primary autoimmune disorder.

De novo dominant mosaic mutations in Collagen 6 genes: uncommon cause of Bethlem and Ullrich myopathies that may be missed by Sanger sequencing

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Collagen type VI-related dystrophies (COL6-RD) are a spectrum of conditions ranging from severe Ullrich congenital muscular dystrophy (UCMD) to intermediate phenotype and the milder Bethlem myopathy (BM).

COL6-RD are caused by mutations in the three different genes, COL6A1, COL6A2 and COL6A3.

COL6-RD are characterized by inter and intra-familial phenotypic variability. Somatic mosaicisms have been recently reported partially explaining the phenotypic heterogeneity in 4 families. We report on clinical, immunohistochemical, and genetic data about 3 unrelated patents affected by a COL6-RD who carried a de novo mosaic mutations in col VI genes. All patients had clinical, histochemical and myoimaging findings consistent with a diagnosis of COL6RD, athough mutations in none of three patient were detected by Sanger Sequencing. Whole Exome Sequencing allowed the identification of a de novo mosaic COL6A3 mutation in one patient who presented an intermediate phenotype and two de novo mosaic COL6A2 mutations in the additional 2 patients who manifested a Bethlem phenotype. This study highlights the importance of a complete diagnostic workup when clinical and histological finding are consistent with a COL6-RD.

The respiratory symptom check-list for patients with myotonic dystrophies: preliminary results

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Chronic Respiratory Insufficiency is frequent in patients with Myotonic Dystrophy type 1. Symptoms are however often overlooked. A respiratory symptom checklist was discussed for patients with DM1 and DM2 at the 207th ENMC workshop and was revised at the OMMYD-3 Respiratory Special Interest Group in 2015 with the purpose of capturing symptoms of respiratory involvement at onset. The general aim of our project is to determine whether the respiratory symptom check-list is able to detect symptoms of respiratory involvement even in apparently asymptomatic patients.

51 patients with DM1 and DM2 were given the Respicheck questionnaire for reliability analysis and 30 initial patients were then subjected to: (i) Neuromuscular Assessments; (ii) Respiratory Assessments; (iii) Quality of Life assessments for Content and concurrent validity. Preliminary results in 51 patients showed good test-retest reliability (Spearman correlation coefficient $p < .0001$ and $r = 0.87$; ICC = 0.86). Each item in the Respicheck questionnaire was significantly correlated with al least one motor or respiratory parameter. The strongest correlation was found between maximal Borg dyspnea at Six Minute Walking Test and Respicheck, both total and single items scores. The Respicheck questionnaire appears to be a reliable instrument. The preliminary results described will be verified in a larger cohort of patients to confirm whether the questionnaire allows to identify symptoms of respiratory involvement early in the disease process and to confirm the validity trend showed in our small sample. Future analysis will explore whether the Respicheck questionnaire will be able to capture changes over time or after treatment.

Respiratory and skeletal muscle strength: correlation analysis and functional impact in myotonic dystrophy type 1 (DM1)

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Reduced mobility and fatigue as well as chronic respiratory insufficiency are well described in DM1. How respiratory and muscle weakness interact and contribute to functional limitations needs to be further explored. To study the correlation between respiratory and motor function tests in DM1.

28 consecutive patients with adult-onset classical DM1 (42,51 ± 10,08 years), without respiratory failure were subjected to: (i) the 6-Minute Walk Test (6MWT); (ii) the Ten Meter Walk Test (10mWT); (iii) respiratory function (Forced Vital Capacity-FVC-, and Peak cough Expiratory Flow-PcEF-) and muscle strength tests (Maximal Inspiratory and Expiratory Pressure- MIP and MEP-); (iv) muscle strength and function tests. Correlation analysis was performed as appropriate. FVC (both sitting and supine) significantly correlated with 6MWT distance and 10mWT time and MIRS. MIP and PcEF significantly correlated with 6MWT distance (r = 0,46826 and r = 0,49643 respectively). In our DM1 patients, respiratory function significantly correlates with walking tests variables. Which respiratory parameters are best predictors of reduced mobility needs to be further explored and will be potentially useful in the design and construction of future clinical therapeutic trials.

Teriparatide (rhPTH 1-34) in Duchenne muscular dystrophy related osteoporosis: a case report

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Patients with Duchenne muscular dystrophy (DMD) experience secondary osteoporosis with high fracture rate due to immobilization and glucocorticoid (GC) use. Vertebral and symptomatic fractures may occur leading to severe pain and worsening of restrictive respiratory impairment. Recombinant human PTH (rhPTH 1-34, teriparatide) acts by stimulating bone formation, leading to gain of bone mineral density (BMD) and reducing vertebral fracture rate in men, postmenopausal women and glucocorticoid-induced osteoporosis. No guideline exists concerning bone loss management in DMD and no published data are available about teriparatide in these population.

We describe the case of a 20-year old GC treated DMD subject, suffering from multiple painful vertebral fractures, treated with teriparatide. BMD was measured by dual-energy X-ray-absorption (DXA) at lumbar spine (L1-L4). DXA and spine X-ray were performed at baseline and after 18 months. Bone resorption (CTX) and bone formation (BGP) markers were assessed in addition to other laboratory and clinical data every 6 months for 18 months.

At baseline, Z-score value was -6.7 SD. Calcifediol supplementation, previously given to maintain adequate 25 (OH) D levels, was administered in addition to teriparatide. After 18 months, Z-score increased significantly (+18%) and no further vertebral fractures were detected. Bone turn-over markers changed as expected (within 6 months) and BGP peaked at 6 months, remaining higher than baseline value at the end of our observation. Moreover, teriparatide significantly reduced back pain intensity after 3 months, enhancing quality of life.

These encouraging new data suggest to test the efficacy of teriparatide on osteoporosis, back pain and quality of life in a larger cohort of DMD patients.

Long term follow-up of two patients with cerebellar ataxia and coenzymeq10 deficiency due to mutations in *ADCK3* gene

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Ataxia is the most common phenotype associated with CoQ_{10} deficiency. Mutations in *ADCK3*, a protein-kinase involved inCoQ biosynthesis,have been identified in about 30 patients with autosomal recessive cerebellar ataxia. Severity and progression of ataxia varied greatly. Aim of this study was to provide a long-term clinical follow-up of two unrelated patients with cerebellar ataxia due to mutations in *ADCK3*.

Clinical data were collected longitudinally until 2015. Biochemical andhistochemical studies were performed on available tissues. Sanger or next-generation sequencing techniques were applied to identify the molecular defect. Case1: a 16 year-old young womandeveloped exercise intolerance and ataxia in early childhood, and epileptic seizures at 4.5 years. CoQ10 concentration in muscle was markedly decreased. Brain MRI showed cerebellar atrophy. Next generation sequencing revealed two novel mutations in *ADCK3* gene: p.K276R and p.P603Tfs*25. Supplementation with high dose of CoQ10 partiallystabilized her clinical features. Case 2: a 24 year-old young man developed ataxia and corticospinal tract dysfunction at 7 years. MRI showed cerebellar atrophy. CoQ10 levels were reduced in muscle and fibroblasts. Genetic analysis showed two heterozygous *ADCK3*mutations: p.Y514C and T584del. He was started on high doses of CoQ10. His symptoms have been stable for over 10 years.

Our data confirm the clinical spectrum of *ADCK3*-related ataxia, characterized by slowly progressive or stable ataxia associated with other signs of central nervous system and muscle involvement. The correct diagnosis is crucial since this condition often responds to supplementation with CoQ10.

Analysis of AMH in Myotonic Dystrophy type 1 patients

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Myotonic Dystrophy type 1 is a multisystemic genetic dis-

order affecting muscle, heart, eye, respiratory and endocrine apparatus. Hypogonadism, defined as low serum testosterone levels, is an almost constant feature. Anti-Mullerian hormone (AMH) – produced by foetal Sertoli cells at the time of sexual differentiation and responsible for the regression of Mullerrian ducts in the male foetus – has been proposed as a useful marker of the prepubertal Sertoli cell function and paediatric male hypogonadism. Recent studies have also shown a possible role of AMH in the assessment of the adult gonadal function.

The present study aimed to evaluate the levels of serum AMH in DM1 patients, beside testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol. Therefore, 50 DM1 patients aged 28-60 years were selected, after informed consent obtained during the routine evaluation. Total testosterone, 17 beta-estradiol, LH and FSH levels were assessed by CLIA DiaSorin assay. Serum AMH concentration was measured by a 2nd generation ELISA kit (Beckman Coulter, Brea, CA,USA).

The preliminary results showed that serum testosterone levels are low or at the lower limits in almost all patients, irrespective of their age, while values of FSH and LH are in the normal range. AMH levels were within the normal limits in 30% of patients, at the lower limits in 20% and undetectable in 50% of patients. The latter group presented the highest levels of FSH and LH.

These preliminary results suggest that AMH might be a reliable marker of biological fitness in patients with DM1.

Isolation and characterization of human urinary stem cells from healthy donors and DMD patients as in vitro cell model for functional studies and drug testing

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Duchenne Muscular Dystrophy (DMD) is a rare hereditary disease due to mutations in the dystrophin gene and it is characterized by muscle weakness, cardiomyopathy, with a severe disease course. The novel therapeutic opportunities would benefit of an enhanced efficiency in terms of dystrophin rescue amount. Therefore there is a need to identify novel molecules and biomarkers to be used as surrogate endpoint of the trial efficacy. Patient-derived human induced pluripotent stem cells are a promising and ideal cell source for drug discovery, and urinary stem cells (USCs) can be accessed via an easy and non-invasive approach.

USCs provided by healthy and DMD donors were isolated and the expression of cell surface markers and dystrophin transcript were analyzed, before and after the myogenic differentiation. Myogenic USCs from DMD patients were treated with the antisense oligoribonucletide for exon 44 skipping.

USCs displayed a mesenchymal stem cell morphology and expressed the mesenchymal stem cell markers. USCs from both healthy and DMD donors express the full length dystrophin transcript and after myogenic differentiation the dystrophin levels increases. Both USCs and USCs transformed in myogenic cells show the mutation on the dystrophin transcript in DMD patients. The antisense treatment of DMD myogenic USCs allow to skip the exon 44.

These results may provide the scientific and technological know-how for a personalized strategy for the utilization of urine stem cells, leading to the development and banking of therapeutic bioproducts suitable for clinical application as drug efficiency tests, pre-screening studies and biomarkers identification.

Familial late-onset proximal myopathy with polyglucosan body and novel GYG1 gene mutation

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Polyglucosan Body Myopathy type 2 (PGBM2) has been recognized to be due to mutations in the *GYG1* gene encoding glycogenin-1, the same defective enzyme that causes glycogenosis type XV, where cardiac involvement is also present.

We report an Italian family including 3 affected relatives (2 siblings, 1 cousin) who presented a late-onset PGBM without cardiac involvement of unknown genetic origin. By targeted re-analysis of whole exome sequencing, and Motorplex data, we identified a mutation at the donor splice site in intron 2 of the *GYG1* gene (c.143+3G > C, p.Asp3Glufs*4), either in homozygous (the 2 siblings) or in compound heterozygote state (the cousin). In the cousin the second mutant allele was identified by cDNA analysis, which showed the inclusion of a cryptic exon, resulting from a deep intronic base change. Genomic sequencing revealed a novel variant (g.148.717.967 C > G) that enhances a cryptic 5' splice site in intron 4.

The c.143+3G > C homozygous mutation has been identified in other PGBM2 patients, in whom it was associated with some residual amount of glycogenin-1 protein, thus explaining the late-onset myopathic phenotype observed in such patients (later than 50 years of age in our series).

In this family, a role of ubiquitin-proteasomal and autophagic degradation pathways was suggested: accumulated polyglucosan is likely to be insufficiently degraded by the ubiquitin-proteasomal system, and may cause and involvement of the autophagic flux, as documented by increased LC3-II and p62/SQSTM1 accumulation.

The deficiency of glycogenin-1 should be considered and investigated as a possible genetic cause of PGBM.

Translational approach to address therapy in non-dystrophic myotonia due to Nav1.4 sodium channel mutations

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Gain-of-function mutations of hNav1.4 sodium channels cause paramyotonia congenita or sodium channel myotonia. By blocking hNav1.4 channels, the orphan drug mexiletine reduces

sarcolemma excitability and counteracts myotonia. Yet, reduced efficacy or tolerability of mexiletine have been reported by some patients. We have shown that the G1306E hNav1.4 mutant causing severe myotonia permanens is less sensitive to mexiletine in vitro compared to wild-type channel, and that patients carrying G1306E can obtain great benefits by shifting treatment to flecainide, another hNav1.4 blocker (Desaphy et al. Neurology 2001; J. Physiol. 2004; Eur. J. Clin. Pharmacol. 2013). Here we studied the function and pharmacology of myotonic hNav1.4 mutations located in channel fast inactivation machinery, as G1306E.

Whole-cell hNav1.4 currents were recorded in transfected tsA201 cells with patch-clamp technique. Similarly to G1306E, many mutations induce a marked slowing of channel inactivation and a shift of fast inactivation voltage dependence toward positive voltages. Such effects likely account for the sarcolemma hyperexcitability and muscle stiffness in carriers. Those mutant channels showed a reduced sensitivity to mexiletine, while flecainide effects were preserved. Thus flecainide appears as a valuable antimyotonic drug, especially in patients carrying mutations inducing a positive shift of fast inactivation voltage dependence. Accordingly, therapy was successfully shifted to flecainide in a patient carrying one of the new mutations (Desaphy et al. Neurology, in press). This study paves the way toward a bench-to-patient pharmacogenetics approach in myotonia.

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Beyond SMA-LED: cerebellar hypoplasia associated with novel mutation in *BICD2*

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Bicaudal-D2 (BicD2) belongs to the family of dynein adaptor motor proteins with key role in cargo trafficking and microtubule transport.

A defect of the human *BICD2* gene has been recently associated with a form of spinal muscular atrophy with lower extremity dominance (SMA-LED) and nearly 50 patients have been described so far.

Unlike the human phenotype, genetic ablation in mice (*Bicd2*$^{-/-}$) is responsible of a defective laminar organization of cerebellar cortex due to impairment of granule cells migration.

We have identified the novel c.2048T > G/p.Leu683Arg in *BICD2* in a 7-year-old Italian boy who presented typical SMA-LED phenotype associated with a developmental disorder of the cerebellum, characterized by posterior fossa enlargement and vermis hypoplasia. The child was diagnosed with congenital hip dysplasia and motor development delay. Last neurological examination showed severe trunk hypotonia with lumbar hyperlordosis and selective atrophy of lower limbs with fixed contractures of ankles and knees. There was neither cognitive nor cerebellar impairment. EMG showed denervation pattern and muscle biopsy was consistent with neurogenic damage. Muscle MRI displayed the typical pattern of previous SMA-LED patients. Brain MRI revealed mild cerebellar hypoplasia with mega cisterna magna which remained stable at follow-up neuroimaging.

To our knowledge there are no previous report of cerebellar developmental disorder due to *BICD2* mutation, despite this can be expected in view of the animal model. We believe these findings expand the phenotype of *BICD2*-related diseases and link a defect of microtubule trafficking to the pathogenesis of cerebellum hypoplasia in human. To confirm this hypothesis we suggest to investigate this aspect in SMA-LED patients.

Analysis from the Italian National Registry: patient-reported impact on employment status

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The value of employment has been worldwide pointed out to be an important factor for preservation of _mental and physical health. This statement is even more relevant in people presenting chronic disabilities, above all in the ones affected by a multisystemic disease, such as Myotonic Dystrophies (DM). Therefore, it is important to recognize that DM can be used as a valuable model to assess the relative impact of the disease on the employment status to further enhance the understanding of this important topic. Data from 328 adult DM patients (> 18y), the ones who correctly filled in self-report sheets consecutively enrolled in the Italian National Registry from February 2013, were analyzed. 307 (93,60%) patients were affected by DM1 and 21 (6,40%) patients were affected by DM2. 121 (36,89%) DM patients were actually gainfully employed and 66 (20,12%) unemployed, 15 (22.72%) of which lost their job because of the disease. The remaining patients were students, homemakers or pensioned. Employment status has been correlated with gender, educational level, having affected relatives and severity of the disease. Between-group analyses revealed significant factors that proved their being related to employment. These results identified previously unrecognized factors influencing the disease and its personal and social implications; therefore, they should be taken into account in the design of future clinical trials.

Influence of *ACTN3* and *ACE* genotypes and mitochondrial genome in elite soccer players

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We assessed the genotype distribution of the *ACTN3* and *ACE* genes, both genes involved in different biological pathways crucial in skeletal muscle structure and functioning, as markers of athlete performance. *ACTN3* gene encodes for alpha-actinin-3 protein and *ACE* gene encodes for angiotensin converting enzyme. Alpha-actinin-3 is almost exclusively expressed in type IIA fibers, being responsible for generation of rapid contractions during activities such as sprinting. Intriguingly, a premature stop codon truncates the ACTN3 protein but does not result in muscle disease. ACE has an important role in the regulation of blood pressure that is necessary to sustain the increased metabolic and physiological demand of skeletal muscle during exercise. An *ACE* polymorphism is associated with lower level of circulating enzyme, lower sprinting ability and muscle strength. Our study aims to evaluate the frequency of *ACTN3* and *ACE* polymorphisms in elite soccer players *versus* healthy non-athlete control group. Moreover, since exercise training induces metabolic changes in response to the energetic requests of skeletal fibers, we evaluated the effect of training on mitochondria content in soccer players as skeletal muscle bioenergetics biomarker.

Mechanical in-exsufflation improves the breathing pattern in patients with Duchenne Muscular Dystrophy

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In Duchenne muscular dystrophy (DMD) with the progression of the disease, muscular weakness contributes to diminish the effectiveness of the cough. Mechanical insufflation-exsufflation is a therapy in which the lung is alternatively inflated and deflated using positive and negative pressures respectively, simulating a natural cough. The aim of this study is to evaluate, using OptoElectronicPlethysmography (OEP), the short-term effects after a single treatment with mechanical assist devices to cough in patients with DMD, in terms of change of cough peak flow (PCF), breathing pattern and ventilatory strategy.

20 DMD patients received a single in-exsufflation treatment. Quiet breathing, PCF and slow vital capacity (SVC), before and after treatment, were analyzed and total and compartmental chest wall (CW) volumes were measured by OEP. Changes in breathing pattern parameters emerged considering quiet breathing before and after a single treatment with in-exsufflation device; total time of the respiratory cycle significantly increased ($p = 0,007$) between pre-treatment and post-treatment. Breathing rate significantly decreased ($p = 0.0009$) after treatment. Data show a significant increment ($p = 0.041$) of abdominal contribution to total tidal volume between pre-treatment and post-treatment. The rapid shallow breathing index (RSBI) after a single treatment device significantly decreased ($p = 0.0011$). The changes in the parameters of the quiet breathing before and after a single treatment with mechanical in-exsufflation suggest that a more efficient ventilatory strategy is adopted, diminishing the effort of the respiratory muscles, improving lung volume recruitment and optimizing gas exchanges within the alveoli.

Tubular aggregate myopathy with miosis caused by a novel missense mutation in *ORAI1*

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We present clinical, histological, muscle imaging and molecular data from 3 patients (proband, his brother and father) of an Italian family affected by Tubular Aggregate Myopathy (TAM). The proband was a 53 years-old woman with cramps and CK elevation. The muscle biopsy of his father, performed when he was 62 years-old to investigate an asymptomatic hyperCKemia, revealed a tubular aggregate myopathy at histochemical and ultrastructural levels. The same findings were observed in the muscle biopsy of the brother at the age of 55. He had CK elevation and proximal lower limb weakness. All three patients had bilateral miosis. Muscle MRI revealed a very mild muscle involvement in the proband and his father, and a severe fatty replacement in the brother. All the patients carried a novel pathogenic mutation in *ORAI1* gene, confirmed by a functional essay on patient myoblasts and HeLa cells harboring the missense mutation that proved a dysregulation of calcium homeostasis in the cells related to a gain-of-function effect of CRAC channel. Our findings expand the clinical and genetic spectrum of tubular aggregate myopathies.

Frequencies of autosomal recessive limb-girdle muscular dystrophies: a systematic literature analysis

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Autosomal recessive limb girdle muscular dystrophies (LGMD2) represent a class of hereditary muscle diseases with heterogeneous genetics and severity. Their frequency is variable among different populations, due to different carrier frequencies. To ascertain the prevalence of each form of LGMD, all the studies in PubMed and Google Scholar, published between 1995and 2016 were reviewed. The literature research has been performed using the terms "LGMD2A/LGMD2W prevalence", "LGMD2A/LGMD2W incidence" as well as sarcoglycanopathies, calpainopathy, dysferlinopathy and so on.

The estimated prevalence of all LGMD2 forms is reported between 0,2and 2,500 X 10^{-6}.

LGMD2A has a higher frequency in Italy but also in Spain, England, Turkey, Russia, China, Brazil and Australia. Sarcoglycanopathies are more prevalent in India. LGMD2I and LGMD2L are more frequent in Denmark, Norwegian, and Northern Europe, reflecting the presence of mutations with a founder effect.

This study is an overview of the prevalence estimates for LGMD2 reported so far. It also reflects the presence of clinical and technical bias (extensive scanning of specific genes and other uninvestigated genes, expression analysis not routinely performed for all the LGMD proteins).

The upcoming large use of next generation sequencing as a first tier test in the diagnostic approach to LGMD2s and to the other neuromuscular disorders will probably provide more accurate data.

Two cases of neck extensor myopathy responding to IGIV

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Describe two patients with neck extensor myopathy and secondary proximal arms weakness, responding to immunomodulating treatments. The patients present a history of progressive subacute weakness of neck extensor muscles and subsequent onset of proximal arms weakness. Both subjects underwent: routine laboratory studies (all in normal range) electrodiagnostic testing (ENG and repetitive nerve stimulation negative), anti-AChR and anti-MUSK antibody test (negative) and a total body TC (negative), in order to exclude the most frequent causes of dropped head. Patient 1: a 67 years old woman whose exams showed a moderate hyperCKemia (403 U/L), positive ANA (> 1:320) and ANCA P, altered EMG (presence of positive potential and fibrillation potential in left splenius and bilateral cervical paraspinal muscles) and muscle MRI (atrophy of the paraspinal muscles). The biopsy (deltoid muscle) was characterized by active inflammatory signs with chronic degeneration). Patient 2: a 68 years old woman underwent the dosage of CPK (418 U/L), ANA (> 1:320), ANCA P (Positive), PR3 ANCA (3,15 RU/ml), cervical MRI (diffuse spondylarthrosis), EMG (signs of myogenic damage), muscle MRI (hyperintensity of the trapezius and atrophy of paraspinal dorsal muscles) and biopsy of brachial biceps (modest signs of muscle impairment). We observed strength improvement in both patients after the first cycle of intravenous immunoglobulin. The patients were able to keep the head upright, with an increase of, at least, one point on MRC scale. The first patient requires a maintenance dose of 0,2 g/kg every month, while the second patient performs monthly infusions at the full dose of 0,4 g/kg. CONCLUSION: we confirm the myositic origin of specific cases of dropped head syndrome and highlight the efficacy of IGIV in those patients.

NGS *vs* Sanger in the diagnosis of neuromuscular disorders

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Because of the overlapping of clinical features in a great number of neuromuscular disorders the molecular diagnosis of these diseases is often challenging. Next generation sequencing (NGS) is a powerful tool that allows simultaneous wide screening of genes. The use of multigene NGS panels that can detect most of the mutations causing neuromuscular disorders is replacing targeted gene testing. We have used next generation sequencing with a panel of known neuromuscular genes to analyze a heterogeneous cohort of patients with neuromuscular disorders presenting at birth or in adulthood.

We have designed two different panels selecting genes based on onset of the disorder. The first panel contained 41 genes linked to childhood onset including congenital muscular dystrophies and myopathies, while the second contained 40 genes related to adult onset diseases including limb girdle muscular dystrophies and myofibrillar myopathies. We selected for the first round of analysis a cohort of patients that failed to receive a diagnosis by targeted gene Sanger sequencing. In the first panel 20 patients were analysed with 4 solved cases (20%) and 11 (55%) still waiting for further clinical follow-up. In the second panel, 9 cases were solved out of the 42 screened (21.4%), with 13 (30.9%) in which segregation in the family is still to be confirmed.

In addition to being faster and cheaper, NGS can be more reliable than Sanger sequencing, preventing technical failures such as allele selectivity in PCR primers annealing or mistakes in reading electropherogram data.

CGH array (motor chip) screening in diagnosis-resistant myopathic patients

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MotorPlex, a targeted NGS platform that covers over 90 genes, allowed us to identify putative causative mutations in 218 out of 504 cases (43%) of undiagnosed myopathic patients. After this screening 386 (57%) cases remained undiagnosed. These diagnosis-resistant cases may be due to three different causes: 1) holes in the NGS coverage; 2) novel genes involved; 3) elusive genetic changes, such as deletions or duplications.

To verify the third hypothesis, we recruited hundred-ten undiagnosed patients out of 386 cases: in seventy-five patients (69%) NGS has previously revealed a single mutation in a recessive disease gene, while in thirty-five patients (31%) NGS has been totally negative.

All patients were analyzed by Motor Chip, a custom CGH-array for the identification of deletions and duplications in neuromuscular disorders. Motor Chip allowed the identification of 13 different copy number variants (CNVs). In particular, this analysis solved the genetic diagnosis for 4 patients (3.6%) in which causative deletions, clearly involved in the observed phenotype were found. In four cases deletions included genes different from those of clinical suspicion and five out of thirteen

CNVs of unknown significance were detected.

These CGH array data were also useful to re-evaluate previous NGS data using five different algorithms to test sensitivity and specificity for CNV detection.

Our results indicate that CNVs do not contribute for a large proportion of elusive mutations in undiagnosed myopathic patients.

Application of electrotherapy to improve motor function in subjects with type II/III spinal muscular atrophy: an exploratory study

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Electrical stimulation (ES) is widely used in physical therapy to improve motor function. For Spinal Muscular Atrophy (SMA), ES effectiveness has not been adequately explored. We aimed at determining whether ES is feasible for subjects with SMA type II/III.

Two phases were conducted:

– Phase 1 (assessment): we assessed the presence of visible muscle contractions elicited by ES in 10 subjects (6 females; age: 2-31; SMA II/III) and evaluated the tolerance to ES.
– Phase 2 (treatment): we evaluated ES effectiveness in 2 male subjects with SMA III (EB: 14 y.o.; MB: 26 y.o.) who responded to ES in phase 1. Subjects underwent a home-based ES treatment for quadriceps strengthening (30 min/day; 5 days/week; 12-18 weeks). EB performed also cycling assisted by functional electrical stimulation (10 sessions; 25 min/week).

Patients showed good compliance (9/10) and responsiveness (8/10) to ES.

Treated patients showed excellent adherence, perceived meaningful changes after the intervention and reported no side effects.

Outcomes:

– EB: quadriceps strength from 1.7 to 2.9 kg (right), from 0.8 to 2.3 Kg (left); Tinetti Scale from 15/28 to 23/28; Hammersmith Scale (HFMSE) from 35/66 to 42/66.
– MB: quadriceps strength from 8.2 to 9 kg (right), from 7 to 9 Kg (left); Tinetti Scale from 24/28 to 25/28; HFMSE from 58/66 to 64/66.

This preliminary experience suggests the feasibility of using ES as a therapeutic intervention for SMA and, for the first time, indicates great potential for electrotherapy to strengthen skeletal muscles and improve motor abilities of subjects with SMA III.

Standards of care adherence and multidisciplinary management of DMD patients at a neuromuscular dedicated center

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Although currently incurable, Neuromuscular Disorders (NMDs) are not untreatable. The comprehensive management of the varied clinical problems associated is a complex task and a multidisciplinary approach has been emphasized by government agencies, clinicians and scientists. Advocacy organizations often report variable and inconsistent health care for individuals with NMD

NEMO Milan (Neuromuscular Omnicenter) is a neuromuscular dedicated clinical and research center having a multidisciplinary patient-centered approach. Aim was to analyze the type and timeline of assessments, interventions and laboratory tests performed by patients affected by Duchenne Muscular Dystrophy (DMD) followed at NEMO Milan center comparing them with Italian and International guidelines on multidisciplinary care

We retrospectively analyzed outpatient and inpatient charts of DMD patients consecutively admitted at NEMO Milan center between 1 January and 31 December 2014. Medications, rehabilitation and orthopedics, pulmonary, cardiac, GI and psychosocial management data were recorded. Preliminary results show a high level of adherence (90%) to assessments and interventions with respect to international guidelines in all items considered. This is in agreement with the high scores obtained on the customer satisfaction questionnaires outcome analysis. The multidisciplinary approach for the care of patients with NMD and the participation by committed providers with disease-specific expertise are key ingredients to the provision of optimal care for these patients which can contribute to the improvement of health, function, participation, and quality of life.

Family burden and impact of DM: a pre-test survey

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Cost of illness studies in neuromuscular diseases have been performed previously, but only on medical costs in limited populations, mostly outside Italy, not accounting for other significant costs to families and to society as a whole. Aim was to analyze preliminary data from a cohort of 24 patients with DM1 using a self-reported questionnaire on the social and economic burden of the disease.

We performed a pre-test qualitative and quantitave analysis using a 120-item questionnaire (single and multiple choice responses, open and closed questions) grouped in 4 domains (demographic; family impact including effects on job, relationships, life-style; state-related economic and social support; self-perceived quality of care) in 24 patients with DM1 (mean age 44; 58% women; 21 adult-onset, E2 CTG range; none with PEG tube; x on nocturnal NIV; 1 CDM with tracheostomy). 67% benefit from the national health-related 3-day permission, 58% have reduced motor ability recognition, 75% reported variable degrees of invalidity benefits (88% > 46%), 5% have access to health-related chauffeuring services. None fulfill requirements for regional economic benefits. 9% report a limitation in independence perception and 21% require 24/7 support. 25% of workers reported problems at work regarding health-related assistance and the majority describe economical problems and only a small amount is dedicated to health-related expenses. 13% of questions were unanswered. The pre-test analysis has

allowed to identify specific domains and indicators to conduct an in-depth survey on the impact of the disease. This will potentially help to allocate more resources to alleviate the costs absorbed by individuals and families living with DM.

Novel variants of collagen VI subunits genes and proof of the consequent assembly alteration and network disorganization

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Collagen VI is a heterotrimeric protein expressed in several organs including skeletal muscle and skin. This complex localizes at the cell surface and links the cytoskeleton to the extracellular matrix, therefore it is involved in cell anchoring, integrity and signal transduction. The heterotrimer consists of three main chains, alfa1, alfa2 and alfa3, organized to create a microfilamentous network. These proteins are encoded by *COL6A1*, *COL6A2* and *COL6A3*. Mutations in these genes cause two severe collagen related myopathies (Ullrich congenital muscular dystrophy and Bethelem myopathy), and other milder phenotypes, myosclerosis myopathy and limb-girdle muscular dystrophy.

We present 3 patients with Collagen type VI-related disorders and carrying 4 variants in the three main genes. The detection of these variants was achieved analysing 53 Italian undiagnosed probands affected by muscular dystrophies. We applied a massive parallel sequencing strategy to analyze 89 genes involved in neuromuscular disorders. We found in patient 1 a missense variant in COL6A2 and another in COL6A3, in patient 2 a dominant COL6A1 splicing mutation in the conserved TH domain and in patient 3 a missense variant in the C terminal lysine-proline rich domain of COL6A3. In order to validate these data we investigated the protein expression by Immonohistochemistry and Western Blot studies performed on muscle biopsies and skin fibroblasts cultures. Finally we evaluated the side effect on the expression of Collagen VI alpha 6 and we observed a protein reduction and disruption of the microfilamentous network, confirming the pathogenicity of the identified mutations.

Italian validation of the Myotonic Dystrophy Health Index (MDHI)

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The Myotonic Dystrophy Health Index (MDHI) is patient-reported measure of disability and impact on the patients lives (Heatwole 2014). The way this instrument has been constructed is based on the idea that it highlights those symptoms which are most relevant and significant for patients with DM1. The aim of this study is the translation and validation of the MDHI in Italian.

The questionnaire is made up of 17 questions exploring several domains of the disease (mobility, difficulties in the hands and arms, limitations in everyday activities, fatigue, pain, gastrointestinal problems, eye problems, difficulties with speech and communication, sleep, emotional aspects, difficulties with attention and concentration, swallowing problems, hearing limitations). There are 114 questions usually completed in 20 minutes. The validation process will include: a) translation and back translation of the questionnaire; b) administration of the MDHI to a sample of 8 patients; c) adaptation and changes to the wording and phrasing according to the patients' comments; d) administration of the final MDHI version to a sample of 50 patients with Myotonic Dystrophy type 1; e) content validity; f) reliability by test-retest. We expect to provide the Myotonic Dystrophy community including patients, families and health operators with a measure of symptoms and impact in this disorder that will potentially be an outcome measure in the near future international clinical trials. If future international clinical trials will include MDHI, the availability of the questionnaire in Italian will represent an advantage for Italian patients and health operators.

First report of a family with a DMD out of frame exon 2 deletion associated with asymptomatic phenotypes

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We present the first case of dystrophic patient carrying an "out-of-frame" exon 2 deletion of the dystrophin gene with a asymptomatic phenotype.

Patient's neuromuscular, cardiac and pulmonary examinations were normal up to 20 years of age. Muscle biopsy showed only a mild fiber size variability and immunofluorescent analysis using a C-terminal antibody showed the presence of dystrophin at the membrane that western blot revealed to be of a smaller molecular weight (~410 kDa) and mutational analysis revealed a deletion of exon 2. Peptide sequencing using tandem mass spectrometry confirmed the absence of any residues encoded by exons 1 through 5, consistent with translation initiation within exon 6.

In particular it has been demonstrated the presence of a IRES (internal ribosome entry site) within exon 5, activated from disruption of the reading frame caused by deletion of exon 2, which allows an alternate translation initiation beginning in *DMD* exon 6 that leads to expression of a highly functional N-truncated dystrophin. We also studied patient's family with neuromuscular examination that was normal in all relatives and with molecular analysis that showed the carrier status of patient's mother and sister and the same mutation of proband in his eighty year old grandfather. Interestingly, we recently performed a muscle MRI in the patient and his grandfather which were within normal limits with a mild fatty infiltration of the lower limb muscles in the grandfather compatible with age. This report shows for the fist time that the expression of N-truncated but functional isoform of dystrophin can lead to a phenotype which remains asymptomatic *throughout the* advanced age.

Muscular laminopathies: state of the art on molecular and cellular pathways

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Muscular laminopathies include Emery-Dreifuss muscular dystrophy, Limb-girdle muscular Dystrophy 1B, *LMNA*-linked congenital muscular dystrophy and cardiomyopathy with conduction system disease. The molecular defects associated with these disorders are mutations in *LMNA* or related genes, from EMD to SYNE 1/2, from SUN1/2 to FHL1. Cellular pathways involved in muscular laminopathies include those regulating myoblast differentiation, not only at transcriptional level, but also at structural level, as well as those regulating cellular signaling, protein degradation and autophagy. How these pathways interconnect to each other and elicit deleterious effects leading to muscle deterioration is an intriguing yet unanswered question. However, recent advances from diverse research centers have implicated new cell types such as tenocytes and inflammatory cells in the pathogenesis of muscular disorders linked to lamin and nuclear envelope protein mutations. Further, new interpretations suggest that the pathogenetic mechanisms so far proposed can be recapitulated in a network of interactions starting from altered signaling pathways triggered by mutated proteins up to defective transcriptional response leading to structural defects of muscle fibers. This model, unexpectedly, fits both skeletal and cardiac muscle disorders found in laminopathies.

New genes and pathomechanisms in mitochondrial disorders unraveled by NGS technologies

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Next Generation Sequencing (NGS) technologies are revolutionizing the diagnostic and research screening for rare diseases, particularly for those genetically and clinically heterogeneous like primary mitochondrial disorders. NGS approaches are particularly suitable for investigating the causative mutations in small families and even in single individuals, for which the traditional linkage analysis is limited. These technologies, in fact, are contributing to deepen the knowledge on the heterogenic genetic causes of mitochondrial diseases and to significantly reduce the percentage of cases lacking a molecular diagnosis, with several new disease genes discovered. In this study we analyzed a cohort of 125 patients, that failed to show mutations in mtDNA and in specific nuclear genes after traditional Sanger's sequencing, performing a combined, two-step strategy, based on targeted genes panel as a first NGS screening, followed by whole exome sequencing (WES) in still unsolved cases. This approach has allowed us to reach a molecular diagnosis in the 20% of these difficult cases, but it has also revealed unexpected and conceptually new findings. These include the possibility of marked variable penetrance of recessive mutations, the identification of large-scale DNA rearrangements, which explain spuriously heterozygous cases, and the association of mutations in known genes with clinical phenotypes never described before. Importantly, WES on selected cases allowed us to discover pathogenic mutations in genes encoding non-mitochondrial proteins, an observation that widens the complex genetic heterogeneity of mitochondrial disease and suggests a new area of investigation in mitochondrial medicine.

Myasthenia-like phenotype in aromatic L-amino acid decarboxylase (AADC) deficiency

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We report the case of a 3-year-old boy who presented with fluctuating palpebral ptosis since birth. Hypotonia, fatigability and fluctuation of alertness became, in addition, evident from his early age, in association with sleep and eating disorders and *nasal* stuffiness. Psychomotor and language developments were delayed. Routine electromyography (EMG) showed non-specific myopatic abnormalities; single-fiber electromyography (SFEMG) was not performed, however. Under the hypothesis of an atypical congenital myasthenic syndrome, we screened DOK7 for pathogenic mutations and proposed salbutamol therapy in this child, with limited clinical response. Oculogyric crises and dystonic movements of the upper limbs were observed at the age of 2.6 years and suggested a neurotransmitter disorder. The decrease of both homovanillic acid and 5-hydroxyindoleacetic acid combined with the increase of 3-O-methyldopa and 5-hydroxytryptophan in CSF examination suggested aromatic L-amino acid decarboxylase (AADC) deficiency, a rare disorder of biogenic amine synthesis. Sequencing of *DDC* detected two predictably pathogenic mutations (c.272C > T/p.Ala91Val and c.1228T > G/p.Cys410Gly), the second being novel. Dopamine and transdermal rotigotine treatments resulted in an initial improvement of the clinical picture (the child experienced reduced fatigability and less fluctuating palpebral ptosis and alertness).

The present case suggests that a myasthenia-like picture could mimic an AADC deficiency; oculogyric crises are valuable "red flag" of AADC neurotransmitter disorder.

A peculiar concomitance of two different monogenic syndromes

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Down syndrome (DS), caused by the inheritance of an additional copy of chromosome 21, is the most common chromosome disorder in live born infants, affecting several body systems, but usually sparing skeletal muscles. We present here the case of a child with coexistence of DS and dystrophinopathy. Only one similar case has been reported so far. A 8-year old boy with DS had a history of incidental finding of increased serum CK levels up to 1775 U/l (normal 38-174 U/l). Medical history was negative for other inherited disorders, he presented no delay in motor development nor did he show progressive muscular wasting; at the neurological examination no muscle weakness or fatigability were detected in two different evaluations performed over a six-month period. Skeletal muscle biopsy specimen revealed marked dystrophic changes with patchy immunostaining for dystrophin. Multiplex PCR analysis of the genomic DNA extracted from peripheral blood lymphocytes confirmed a diagnosis of Becker muscular dystrophy (BMD). This peculiar "double trouble" case exemplifies the value of careful clinical evaluation and adequate clinical experience to identify the concomitance of two different monogenic syndromes in the same patient. Moreover, it underlines how a multidisciplinary approach is essential in managing and treating these kind of patients.

Growth hormone secretagogues prevent dysregulation of skeletal muscle calcium homeostasis in an animal model of cisplatin-induced cachexia

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Cachexia is a wasting condition associated with cancer types and, at the same time, is a serious and dose-limiting effect of cancer chemotherapy. Skeletal muscle loss is the main characteristic of cachexia and the primary cause of function impairment, fatigue and respiratory complications. Calcium-dependent signaling pathways are believed to play an important role in skeletal muscle decline observed in cachexia, but whether intracellular calcium homeostasis is affected in this situation remains uncertain. Growth hormone secretagogues (GHS), ghrelin mimetics, represent a therapeutic option for cancer cachexia. However, the exact mechanism by which GHS interfere with skeletal muscle is not fully understood. By a multidisciplinary approach, here we characterized the calcium homeostasis in fast-twitch EDL muscle of adult rats with cisplatin-induced cachexia and established the potential beneficial effects of two GHS (hexarelin, JMV2894). Cachetic fibers are characterized by a reduction of the muscle weight and fiber diameter with an alteration of *atrogin1*/*Murf-1* and *Pgc1-a* genes, and a 2-fold increase in resting intracellular calcium, $[Ca^{2+}]_i$, compared to control rats. Caffeine or depolarizing solution responsiveness and the store-operated calcium entry was reduced in cisplatin-treated rats. The changes of some calcium-dependent functional outcomes support the impact of calcium homeostasis alteration on muscle functionality in cachetic animals. GHS efficaciously prevents cisplatin-induced muscle weight loss and $[Ca^{2+}]_i$ increase and improve the functional parameters. Our findings provide the first direct evidence of a calcium homeostasis dysregulation in cachexia as well as contribute to the elucidation of the mechanism of action through which GHS could potentially ameliorate chemotherapy-associated cachexia.

MR findings in a child with spinal muscular atrophy type II: a clinical case study

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of alpha motor neurons in the spinal cord, due to a mutations in the survival motor neuron (SMN) gene in chromosome 5q13. Four phenotypes are known, which differ in severity and age of onset. We present a girl with SMA 2, who showed neuroradiologic features. She's the first child of a non-consanguineous parents. She was born at term after an uneventful pregnancy, without post-natal complications and she moved her extremities spontaneously at birth, but gradually over seven months, she displayed signs of progressive hypotonia. At the age of 12 months, the patient was admitted to our department for further evaluation. Neurological examination revealed symmetrical hypotonia, with lower limbs weaker than upper limbs, lying in a frog-like position, poor trunk control and fine tremors of upper extremities. Deep tendon reflexes were absent. Gene analysis confirmed homozygous deletion of exons 7 and 8 in the SMN1 gene. Brain MRI revelead delayed myelination and dysplasia of the corpus callosum with a lipoma, and the more affected part was the splenium. Patients with SMA associated with delayed myelination or cerebral atrophy have been reported. The pathological changes in the brain are attributed to repeated hypoxic episodes; however, there are patients with no demonstrable hypoxia or respiratory failure, that show evidence of neuropathological alterations in the brain, which may be part of this disease. Generally, MRI is not regarded as mandatory for making the diagnosis of SMA and the first level diagnostic test should be the genetic analysis. Nevertheless, neuroradiological and electrophysiological examinations might be helpful in the diagnosis and follow-up of this rare disease.

Kaposi's sarcoma in a patient with inflammatory myopathy: immunosuppressive therapy and its management

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A 77-years-old male patient was admitted to our clinics because a progressive history of upper limb proximal weakness, head-drop and severe dysphagia with significant weight loss. Elevation of muscular enzyme and electromyographic myopathic-neurogenic mixed pattern were found. Left deltoid muscle biopsy confirmed the suspected diagnosis of myositis, brachio-cervical variant. Because of the extremely severe dysphagia patient underwent percutaneous endoscopic gastrostomy. Prednisone 1 mg/kg ameliorated

the clinical conditions and was progressively tapered after 3 months when azathioprine was started. Patient presented a significant clinical response both on muscular weakness and on dysphagia. After about 21 months of treatment with prednisone and azathioprine 18 months patient presented numerous and confluent purple skin lesion on the arms and the legs. Biopsy of a similar lesion in the larynx confirmed diagnosis of Kaposi sarcoma and patient started therapy with systemic and local alpha-2 interferon with skin lesions stabilization. We immediately suspended treatment with azathioprine and kept a low dosage of prednisone. Kaposi's sarcoma is a vascular tumor with four clinical variants: classic, endemic, HIV-associated and iatrogenic. Rare cases of Kaposi sarcoma in patients treated for myositis are described. These cases presented wide variability regarding immunosuppressive therapy duration, which varies from months to several years; cases of sarcoma after treatment with prednisone alone are described. Kaposi's sarcoma complicates clinical management because myositis relapses need prompt pharmacological intervention that requires a strict risk/benefit ratio assessment. This case peculiarity lies in the visceral involvement of Kaposi that is usually limited to skin in iatrogenic forms.

Clinical and serological features in myasthenia gravis with musk antibodies

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Myasthenia Gravis (MG) with MuSK antibodies is a form of MG characterized by a predominant facial, bulbar and respiratory involvement. Unlike the AChR antibodies, which are mainly of the complement-fixing IgG1 and IgG3 subclass, the MuSK antibodies (MuSK-Abs) belong to IgG4 subclass, they don't fix complement, and their pathogenic role is still unknown.

Aim of this study is to analyze retrospectively the clinical and serological features of our population of anti-MuSK patients.

We describe 84 patients with MuSK antibodies (15 men, 69 women; mean age of onset of MG: 40 ± 7.7 years, mean level of MuSK-Abs: 0.15-2.62 nmoli/l). Clinically, 89% of patients had a bulbar form.

Thanks to steroid and immunomodulatory therapy, 44,5% of patients achieved a complete stable remission (CSR) or a pharmacological remission while the response to the anticholinesterase therapy and to thymectomy was very poor.

Serologically, each blood sample was analyzed according to the various subclasses of IgG; besides the prevalence of IgG4, a high presence of IgG1 and IgG2 was detected. Furthermore, the end point titres were plotted according to MGFA (Myasthenia Gravis Foundation of America) classification: the results suggested that higher end-point titres were associated with higher MGFA scores.

In conclusion, our data confirm the demographic and clinical characteristics of myasthenic patients with MuSK-Abs. It was interesting to note in a fair number of sera the presence of IgG1 and IgG2 and higher end-point titres in the most severe classes of MG.

Disruption of sleep wake continuum in myotonic dystrophy type I

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Excessive daytime sleepiness is a common complaint in Myotonic Dystrophy type I (DM), and although it may be related to comorbid sleep disorders such as sleep disordered breathing, a central sleep dysregulation may be prominent. Our aim was to evaluate sleep macrostructure in 8 DM (6M, 37,5 ± 13.3 yy) and 16 healthy controls (12M, 27,8 ± 5,7 yy) through in-lab polysomnographic sleep recording. DM patients showed increased slow wave sleep and REM sleep; two subjects had a sleep onset REM period and another one a first REM latency of 21 minutes. The peculiar macrostructural pattern suggests a narcoleptic-like phenotype in DM and a role for other events that go beyond the conventional sleep staging, particularly slow wave related sleep transients, related to both comorbid sleep disorders and a central dysregulation per se, in determining excessive daytime sleepiness in DM1.

Jagged1 rs6104632 polymorphism as modyfing factor in Duchenne and Becker muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an untreatable disorder caused by mutations in the dystrophin gene.

Several genetic modifiers, such as *LTBP4, SPP1* and *TGF-BR2* gene polymorphic variants, are known to affect the severity of the clinical symptoms in human.

Recently, the polymorphism rs6104632 in *JAGGED1* gene, leading to overexpression of Jagged1, a known regulator of the Notch signaling pathway, has been demonstrated to ameliorate the dystrophic phenotype in Golden Retriever muscular dystrophy (GRMD) dogs.

We studied a large sample of dystrophinopathic patients to estimate the prevalence of this polymorphism and to determine its role as modulator in human pathology.

JAGGED1 polymorphism was screened in 125 patients affected with Duchenne or Becker Muscular Dystrophy. For each patient data about molecular diagnosis, level of protein expression, age at loss of ambulation were defined. All kind of *DMD* mutations, including deletion, duplications and point mutations, were represented.

The *JAGGED1* rs 6104632 polymorphism was absent in our sample of patients as far as in a cohort of 50 healthy subjects. Conversely we found the following polymorphic variants: rs910118/rs910119 (26/125, 20.8% of the sample) and rs143085701 (1/125, 0.8%). These variants did not correlate with disease severity.

JAGGED1 rs6104632 polymorphisms do not influence the

severity of clinical presentation in subjects affected with dystrophinopathy. However the study of polymorphisms in other proteins involved in Notch signalling pathway could reveal new potential genetic modifiers.

Histological features of mitochondrial dysfunction in late onset Pompe disease (LOPD): a case report and review of muscle biopsies from a cohort of LOPD patients

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The recognized clinical and histological heterogeneity of late onset Pompe disease (LOPD) represents a challenge for a rapid diagnosis which is based on the demonstration of decreased/absent acid alpha-glucosidase (GAA) activity and of GAA gene mutations.

We report on a 46-year-old patient with a 3-year history of fatigue, myalgia and proximal lower limb muscle weakness who was referred to perform a muscle biopsy for suspected limb-girdle muscular dystrophy. His past medical history included dilated cardiomyopathy and early onset type 2 diabetes mellitus (DM2) which was present in other family members, obstructive respiratory insufficiency and thyroid nodules. Biopsy of vastus lateralis muscle showed cytochrome c oxidase negative fibres, respiratory chain enzyme activities were normal and mitochondrial DNA sequencing revealed the point mutation at position A15924G which has been reported as polymorphism. The patient was examined one year after the muscle biopsy and the detection of diaphragm weakness raised the suspicion of LOPD. Reduced GAA activity was demonstrated on muscle biopsy and Pompe disease was genetically confirmed.

We retrospectively analyzed the muscle biopsies from a cohort of LOPD patients diagnosed in our centre. Significant features of mitochondrial dysfunction were found in the muscle biopsy from a HIV-positive patient who complained of muscle weakness during antiretroviral therapy. Two other biopsies showed mild, probably age-related, signs of mitochondrial dysfunction.

This report confirms the histological heterogeneity of LOPD and suggests to consider Pompe disease despite atypical features on muscle biopsy to reduce the diagnostic delay.

Myopathy as the herald manifestation of Cushing's syndrome: a case report

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Cushing's syndrome (CS) is due to prolonged hypercortisolism and includes hypertension, weight gain with moon face and abdominal obesity, reddish striae, fatigue, and glucose intolerance. Muscle weakness is reported in nearly 60% of the patients and myopathy is a well-recognized complication. The most common endogenous cause of CS is Cushing's disease (CD), i.e. the excessive secretion of adrenocorticotropin hormone (ACTH), often by a pituitary tumor.

We report on a 46-year-old woman who was referred for muscle biopsy because of a 10-year history of myalgia and progressive muscle weakness and atrophy, affecting legs first and arms few years later. Previously she had practised sports and dance without any problem. Her past medical history included hypertension, since the age of 28, which was investigated with no conclusive aetiological diagnosis, subclinical hypothyroidism, and polycystic ovary syndrome. Her family history was unremarkable. On evaluation she showed moon face and abdominal obesity with thin arms and legs. Muscle biopsy revealed selective type 2 fibers atrophy.

Considering past medical history and suspected steroid-induced myopathy, endocrine tests were performed. Urinary free cortisol was at the upper normal values, ACTH was elevated, and dexamethasone high- and low-dose suppression tests were consistent with the diagnosis of CD. Brain MRI revealed a pituitary microadenoma. The patient underwent transsphenoidal surgery with normalization of blood pressure values and disapperance of the moon face.

In this patient myopathy preceded by years the appearance of typical biohumoral markers of CS. Full endocrine workup should be performed in patients with suspected endocrine myopathies.

Chronic fatigue syndrome and mitochondrial myopathy: a clinical case

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Chronic Fatigue Syndrome (CFS) or Myalgic Encephalomyelitis (ME) is a disease characterized by fatigue, cognitive dysfunction, muscle pain, disturbed sleep, postexertional malaise following activity or exercise and other symptoms regarding immune and gastrointestinal system. The pathophysiology of CSF is still unknown, but several studies show a dysfunction of cellular energy metabolism and ion transport, suggesting a mitochondrial involvement. We report the case of a patient diagnosed with CSF 16 years ago and revaluated 2 years ago. Histochemical study of muscle biopsy, spectrophotometric analysis of the complexes of the mitochondrial respiratory chain and genetic studies were performed.

The results revealed some cytochrome oxidase (COX) negative fibres, deficiency of complexes I and IV and multiple deletions of mtDNA. Our case shows that it is possible to misunderstand symptoms of mitochondrial disease in adults wth CFS.

Chronic inflammation and altered mechanotransduction in degenerative myopathies: validation of druggable targets via animal models and pharmacological studies

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Chronic inflammation is a hallmark of degenerative

myopathies, such as Duchenne muscular dystrophy (DMD) and polymyositis (PM). In the mdx mouse model of DMD, a standard protocol of chronic treadmill exercise leads to a disequilibrium between protective and damage-related pathways, supporting our hypothesis that, in DMD, chronic inflammation is caused by the absence of dystrophin via a failing mechanical-metabolic coupling. This was presently validated performing a head-to-head comparison of drugs able to activate PGC-1alpha signalling (resveratrol, 100mg/kg/day, i.p.) or reduce oxidative stress (taurine, 1g/kg/day per os; apocynin, 38mg/kg/day, per os), in parallel with gold standard alpha-methyl prednisolone (PDN, 1mg/kg/day, i.p.), upon 4-week treatment in exercised mdx mice. Resveratrol ≥ taurine > apocynin enhanced in vivo mouse force similarly to PDN. All drugs significantly reduced superoxide anion production and improved electrophysiological biomarkers of oxidative stress. Force of isolated muscles was little ameliorated. The compounds improved mdx muscle histopathology and taurine > apocynin > PDN significantly decreased activated NF-kB-positive myofibers. Resveratrol also reduced plasma CK and LDH. Our results validate the working hypothesis and support the interest in drugs counteracting inflammation and oxidative stress in DMD. In parallel, a novel double-transgenic model of myositis (the H^+T^+ mouse) was used to verify our hypothesis that, in PM, inflammation is the event that triggers metabolic dysfunction, oxidative stress and mechanical impairment. A preliminary characterization of the first female H^+T^+ mice born from our colony, confirmed the functional, biochemical and histological profile described in literature, further supporting the usefulness of this model for future mechanistic and pharmacological studies on PM (Supported by MIUR-PRIN-n°20108YB5W3_004).

DPM synthase depletion in zebrafish leads to dystrophic muscle with hypoglycosylated alpha-dystroglycan

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Defective dolichol phosphate mannose (DPM) synthase complex is a cause of congenital muscular dystrophy characterized by reduced glycosylation of alpha-dystroglycan (alpha-DG). We used the zebrafish (*Danio rerio*) to model muscle abnormalities due to defects in the subunits of DPM synthase. The three orthologue subunits in zebrafish (*dpm1*, *dpm2*, and *dpm3*) showed high similarity to the human proteins, and their expression displayed a localization in the midbrain/hindbrain area and somites. Using antisense oligonucleotide morpholinos targeting each subunit, morphants showed muscle disorganization, low Dpm synthase activity, early lethality and increased levels of apoptotic nuclei together with an almost absent expression of hypoglycosylated alpha-DG in muscle fibers, thus recapitulating the characteristics seen in patients with mutations in DPM synthase. Our results in zebrafish suggest that DPM synthase plays a role in stabilizing muscle structures and in apoptotic cell death.

High-troughtput nutraceutical screening in a zebrafish model of muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an inherited disorder that cause muscle weakness, loss of muscle mass and motor skills, and it is caused by mutations in *DMD*, encoding dystrophin. In the era of genomic medicine, there is no known cure for DMD, and physical therapy and steroid treatments can only slow the loss of muscle strength, and help to control symptoms. In clinical practice, there is increasing attention to a proper nutrition in chronic and neuromuscular diseases. This is often the result of empirical observations made by the patients or caregivers on how antioxidants and pharmacologically active substances derived from food alleviate their fatigue and improve muscle strength, and quality of life. To offer a more solid experimental setting to these observations, we have developed a research pipeline to investigate a vast compendium of nutraceuticals that might have "clinical" significance by improving the muscle and locomotor as well as metabolic phenotypes in the *sapje* fish strain, a validated zebrafish model of DMD.

The primary objective is to obtain experimental data that complement inflammatory and molecular parameters in mutant zebrafish model.

This study would offer an innovative approach of analytic investigation and might establish a preclinical tool to identify strategies to study bioelements and supplements useful in DMD.

Electromyographic findings in 11 patients with late-onset pompe disease

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Glycogen storage disease II (GSD II), also known as Pompe disease is an autosomal recessive disorder caused by mutations in the *GAA* gene encoding the enzyme acid alpha-glucosidase. Two different clinical forms have been described: infantile (IOPD) and late-onset (LOPD). LOPD is usually characterized by progressive limb-girdle and axial muscle weakness, and respiratory muscle impairment, but rarely distal muscles are involved. There are only few studies that have tried to describe electromyographic (EMG) features and their distribution in LOPD patients. The aim of our study was to evaluate these EMG findings in a cohort of LOPD patients.

We studied 11patients (5 M and 6 F), the mean age was 48 years (range, 20-62 years). 8/11 patients had a proximal and axial muscle weakness and 3/11 presented only iperCk-emia and myalgia, none of them had distal nor respiratory muscle weakness. All patients underwent a complete electroneurographic and electromyographic evaluation at four limbs, also including paraspinal muscles. EMG evidenced the presence of myotonic discharges (MD) in paraspinal muscles in 8/11 patients (73%) and in distal limb muscles in 3/11 patients (17%). Fibrillation potentials occurred in all muscles where MD were found. Short duration, polyphasic motor unit potentials (MUPs) were found in the paraspinal (6/11), proximal (5/11) and distal limb muscles (4/11). Nerve conduction studies were normal in all patients. Our results confirm that in LOPD patients, MD are quite common in paraspinal mus-

cles; a myopathic EMG pattern, usually observed in proximal muscles, could also be detected sometimes in distal muscles. In LOPD patients EMG study of the distal muscles study can reveal a subclinical involvement.

The contribution of clinical and paraclinical indicators to the characterization of the phenotype in a large group of molecularly defined hereditary spastic paraplegias

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Hereditary spastic paraplegias (HSP) are a genetically heterogeneous group of conditions expressed by the impairment of the central motor system. The definition of an investigation protocol capable to fully represent the extent of the motor system impairment, would help both the clinical handling of these conditions and contribute to our understanding of their pathogenesis.

We applied a clinical and paraclinical protocol which included tools exploring motor and non motor functioning, neurophysiology and MRI to a cohort of 70 molecularly defined HSP patients, to define for each indicator its significance in detailing the presence and the severity of the pathology.

MRI diffusion tensor imaging (DTI) highlighted a significant alteration of FA and MD. Combining the sampling of the various portion of the cortico-spinal tract (CST) DTI consistently discriminated patients from controls. Increased deep tendon reflexes and lower limb (LL) weakness are constant findings in all patients. Peripheral motor impairment, cognitive and cerebellar involvement are the most frequent additional clinical findings. The Spastic Paraplegia Rating Scale efficiently reflects the severity of functional problems and correlates with disease duration and DTI changes. Neurophysiology consistently documents the impairment of the central motor pathway to the LLs.

We propose a clinical and paraclinical protocol for HSP phenotype definition, indicating for each tool the discriminative and descriptive capacity. Our protocol applied to 9 different forms of HSP showed that the functional impairment often extends beyond the CST. The novel DTI approach may add significant elements in disease recognition, staging and mapping.

Use of the 6-minute walk (6MWD) in studies on Duchenne Muscular dystrophy (DMD)

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6MWD is the proven endpoint for DMD ambulatory studies, used in ataluren, drisapersen, tadalafil and eteplirsen trials. Decline in the 6MWD predicts progression of the disease, time to loss of ambulation and subsequent disease events. Reinforcement of the inclusion criteria of 6MWT has resulted in exclusion of patients with close to normal or severe ambulatory impairment. Recent studies were reviewed to determine the evolution of 6MWT as a significant clinical endpoint.

6MWT inclusion criteria for DMD studies have evolved from the original 75m without maximum criteria for the first two trials started in 2008. These baseline criteria are reduced to a minimum value of up to 300m for phase III open eteplirsen trial (Sarepta 4658-301 begun in 2015) and maximum values up to 400m in a phase III tadalafil trial (Eli Lilly H6D-MC-LVJJ begun in 2013). Ataluren Phase 3 Study (ACT DMD; begun in 2013) included patients with baseline ≥ 150m and < 80% predicted, with a pre-specified baseline 300-400m subgroup. In the ACT DMD study, the benefits of ataluren compared to the placebo observed in the general population (difference 48 weeks = 15m; p = 0.213) was improved in the pre-specified 300-400m subgroup (47m; p = 0,007). Sensitivity analyses confirmed an ataluren effect with 6MWD ≥ 250 - < 400m (29.5m; p = 0.035); ≥ 200 - < 400m (26.6m; p = 0.0501); and ≥ 300 - < 450m (24.4m; p = 0.0504). In evaluating drugs considered capable of slowing the progression of the disease, a more reduced range of 6MWD is used as inclusion criteria. For ataluren, a pre-specified range of 300-400m showed the greatest therapeutic effect. Significant effects were observed with 6MWD from 200-450m.

Study financed by: PTC Therapeutics Inc.

Revised North Star ambulatory assessment for young boys with Duchenne muscular dystrophy

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The recent development of therapeutic approaches for DMD has highlighted the need to identify clinical outcome measures for young boys with Duchenne muscular dystrophy.

The aim of this study was to developed a revised version of the NSAA suitable for boys between the age of 3 and 5 years by identifying age appropriate items and revising the scoring system accordingly.

Using the scale in 168 typically developing boys between the age of 2.9 and 4.8 years, we identified items that were appropriate at different age points. An item was defined as age appropriate if it was completed, achieving a full score, by at least 85% of the typically developing boys at that age. At 3 years (± 3months) there were only 8 items that were age appropriate, at 3 years and 6 months there were 13 items while by the age of 4 years all 17 items were appropriate. A revised version of the

scale the items was develop with items ordered according to the age when they can be reliably performed. The application of the revised version of the scale to data collected in young DMD boys showed that none of the DMD boys was able to complete with a full score all the age appropriate items. In conclusion, our study suggests that a revised version of the NSAA can be used in boys from the age of 3 years to obtain information on how young DMD boys acquire new abilities and how this correlates with their peers.

Can the Muscular Strengthening help maintain function in ALS patients?

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In ALS a combination of damage to various cell types (motoneurons, muscle) may act synergistically to exacerbate the disease. Skeletal muscle is a source of anabolic signals that influence neuron survival. Thus, skeletal muscle might be a target for therapeutic intervention. Previous studies have demonstrated that exercise induces a maximal recruitment drive motor and can be worthwhile also in denervated muscles. In the isometric muscle contraction, the motor unit recruitment is complete and the MU, rarely triggered, are recruited. Beside, this contraction might be proposed also to avoid the "weakness overload" muscular of the fast twitch muscular fibers. In the present study, has been designed an exercise program based on a standardized training of isometric contractions in early-stage ALS; resistance exercises with elastic bands have been well set, defining the type of contraction, isometric sub maximal, the muscle segments to be reinforced, and the number of repetitions of the sessions. The objective has been to strengthen the weak muscles (MRC 3. 4-, 4) and to prevent further atrophy that overlaps on the ALS primary symptoms. The purpose of this pilot study is to verify the clinical efficacy and safety through an objective assessment of muscle strength. Twenty-three patients with ALS (13 males-10 females, mean age 61,63 ± 10,9 years, mean value ALSFRS-r score 35.7 ± 4.6, mean ALS duration 30,7 months ± 11,8) participated. All patients have presented an increase of MMT dynamometry scores at two months. The pts able to walk has presented an improvement of 6MWT /10MWT.

Impaired rotational mechanics and strain revealing subclinical left ventricular dysfunction in children with Duchenne muscular dystrophy: a speckle tracking study

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Myocardial dysfunction is part of Duchenne muscular dystrophy (DMD) natural history, although timing of onset, progression and severity can vary. Standard echocardiography has known limitations in DMD patients since left ventricular (LV) abnormalities are often subclinical and detectable by standard echocardiography only in the advanced stage of the disease.

We evaluated if the two-dimensional (2D) strain with speckle tracking technique may detect early LV changes not detected by standard echo and the correlation with age and functional performances measured by 6 minute walk test (6MWT) and North Star Ambulatory Assessment (NSAA) in the ambulatory phase of the disease.

We enrolled 39 patients with DMD with normal standard echo (age range: 5-15 yrs, mean 10.2 yrs ± 3.6 SD), divided in Group 1 aged under 9 years and group 2 over this age, and 20 healthy controls. Global longitudinal strain (GLS) was significantly reduced in patients *vs* controls (Group 1 *vs* controls $p = 0.020$, Group 2 *vs* controls $p < 0.001$). Furthermore, GLS was lower in older *vs* younger patients (Group 2 *vs* Group 1 $p < 0.005$). Also global circumferential strain (GCS) was reduced in patients (both groups) *vs* controls (MV-Sax $p = 0.002$, PM-Sax $p = 0.003$, AP-Sax $p = 0.049$). In the ambulant group ($n = 24$, age range: 5-11 yrs, mean: 9.2 yrs ± 2.6 SD) we found a significant positive correlation with the 6MWT ($r = 0.38$, $p < 0.05$).

With the increasing evidence of the efficacy of cardioprotective drugs and the ongoing therapeutic trials targeting at younger ambulatory DMD patients, this technique can represent a valuable tool to monitor cardiac function in the earliest phase of cardiac involvement.

Differential diagnosis in hypokalemia paralysis: a pale grey zone between neurologist and endocrinologist

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Hypokaliemia is a pathological condition defined as serum kalemia < 3.5 mmol/l and is caused by many disorders. It may be symptomatic and present as myalgia, muscular weakness, rhabdomyolysis, muscular cramps, paresthesia, and cardiac arrhythmias.

The first case is 48 years-old woman, arrived to our attention for acute myalgia and proximal muscle weakness. Her personal history revealed uncontrolled hypertension. Her blood tests showed severe hypokalemia (1.9 mEq/L) and hyperCKemia (19.360 UI/L). Viral serology and reumatological analysis were normal. Electromyography showed myophatic pattern. Muscle biopsy revealed mild myophatic signs and genetic test for hypokalemic periodic paralysis was negative. The patient was treated with potassium and her symptoms markedly improved. Further analyses have diagnosed a primary aldosteronism caused by bilateral adrenal hyperplasia.

The second case is a 36 years-old man, complaining of muscular cramps and proximal weakness associated with severe hypokalaemia (2.19 mEq/L). The patient was treated with potassium and his symptoms markedly improved. The patient reported significant weight loss (25 Kg in the last two years). Physical examination revealed bilateral exophthalmos for which the patient has performed blood dosage of thyroid hormones with detection of thyrotoxicosis.

Hypokalemic periodic paralysis is a neurological disease caused by genetic defect (*CACNA1S* and *SCN4A* gene*).* Clinically is characterized by paralytic episodes associated with severe hypokalaemia. The episodes are triggered by carbohydrate-rich meals and rest after exercise. Our cases presented similar

clinical features. This focused attention to the importance of a correct differential diagnosis between hypokalemic periodic paralysis and some endocrinological disorders.

The efficacy of nutraceuticals in primary muscle diseases: scoping review

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Muscle diseases are characterized by weakness, muscle pain and fatigue, resulting in a considerable limitation in activities of daily living (ADL) and a negative impact on patients' quality of life. One of the most widely used approaches to patients with myopathy is the prescription of nutraceuticals. The goal of our scoping review is to define the state of art about the use of nutraceuticals in the integrated approach to primary muscle diseases. We selected from the "EU Register of nutrition and health claims made on foods" micronutrients which are supposed to have a beneficial effect for the skeletal muscle, planning a research on PubMed. We identified for each selected micronutrient the most relevant studies published in the last 10 years, categorized according to the EBM pyramid. From the 65 micronutrients listed on the Register, we identified 23 micronutrients having a role on muscular function, but for only 6 of these a strong scientific evidence was reported: a Cochrane review confirmed the efficacy of creatine in short term improvement of muscle strength; also idebenone, in a double-blind randomized placebo-controlled phase 3 trial, showed to be effective in improving respiratory muscle strength in patient with Duchenne muscular dystrophy. Vitamin C, E, Zinc gluconate and selenomethionine, in a double-blind RCT demonstrated to increase quadriceps strength in patients with facioscapulohumeral dystrophy. Some micronutrients have scientific evidences about their efficacy in primary muscle diseases, but further studies are necessary to justify their use in clinical practice.

Identification of ICF categories in patients with primary muscle diseases

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Primary muscle diseases are characterized by progressive loss of muscle strength, different ages of onset, phenotypic variability, clinical evolution and progression rate, with multisystemic involvement. In 2006, Wynia et al. proposed a selection of ICF items for patients with chronic neurological disorders, not including the muscle disease. The purpose of our study is to determine the most common problems in patients with primary muscle diseases, selected according to the ICF domains.

In a cohort of patients with primary muscle diseases referring to our rehabilitation service, we performed an evaluation protocol including: Manual Muscle Testing, Hand Grip Strength Test, Tinetti Performance Oriented Mobility Assessment, 6-Minute Walk Test, QuickDASH, Functional Independence Measure, Fatigue Severity Scale, 12-Item Short Form Survey, Brief Pain Inventory, and were identified the ICF categories involved and compared with that proposed by Wynia et al.

We recruited 31 patients with mean age of 33.65 years, 9 with myotonic dystrophy type I, 9 with facio-scapulo-humeral dystrophy, 8 with dystrophinopathy 4 with limb-girdle muscular dystrophy, one with inclusion body myositis. Fifty-one ICF categories were identified as relevant: 18 body functions, 7 more than Wynia et al. (+38.89%); 7 body structures, not previously identified (+100%); 20 activities and participation, including 3 categories not identified by Wynia et al. (+15%); 6 the environmental factors, of which 2 selected for the first time (+33%).

ICF categories identified in this preliminary study should expand the classification previously proposed and can be a great starting point to better define the ICF core sets for muscle diseases.

The 24-month PUL changes and steroids correlation

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The aim of this study was to evaluate the 24-month PUL changes and the possible effect of glucocorticoid treatment on upper limb function in a cohort of 100 non-ambulant DMD boys and adults of age between 10 and 20 years.

All 100 were assessed using the Performance of Upper Limb test at baseline, 12 month (T1) and 24 month (T2).

As in the previous study, even in the two years evaluation the most significant changes are highlighted in the middle domain (elbow), We divided the patients in three groups based on the score obtained in the elbow domain. The first group with score ≤ 8; the second group with a score between 9-20 and the third group with scores > 20, and we analyzed the average of changes and standard deviation in the three domains of the scale and in the total.

In the total score the first group shown the mean change -3.0 (5.8 SD); the second group shown the mean change -8.0 (7.2 SD); the third group shown the mean change -10.6 (6.7 SD).

The our data shown that the T2 changes affecting the middle domain influence the most points in total and the patients with steroids show a lower functional impairment.

Our results confirm that continuing glucocorticoids throughout teenage years and adulthood after loss of ambulation appears to have a beneficial effect on upper limb function.

Inclusion body myopathy mistaken for amyotrophic lateral sclerosis: report on a family

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Inclusion body myopathy associated with Paget disease and frontotemporal dementia (IBMPFD) is an autosomal dominant multisystem disorder, caused by missense mutations in Valosin containing protein (VCP) gene. In some families other associated features are hepatic fibrosis, cardiomyopathy, neuropathy and cataract. We report on a 60-year-old man who was referred for a second neurological evaluation. He had been discharged from another neurological unit with a diagnosis of probable amyotrophic lateral sclerosis. The patient complained of slowly progressive thigh muscle weakness and cramps from two years with recent involvement of the upper limbs. On examination muscle atrophy and fasciculations of the tights were evident. CPK was within the normal range and EMG showed myopathic signs with denervation activity, therefore, the patient underwent a muscle biopsy which was consistent with inclusion body myopathy. The patient's father, who died at the age of 76, had received a diagnosis of muscular dystrophy thirteen years before and developed cognitive impairment in the last years of his life. The 64-year-old patient's brother complained of myalgia and mild leg muscle weakness from two years. Both the patient and his brother performed a lower limb MRI which showed besides muscular changes also Paget disease of the bone. Molecular analysis showed the mutation c.277C > T in exon 3 of VCP gene in the patient, his father and his brother. Our report confirms the need to differentiate motor neuron diseases from other neurological conditions, suggests to perform a muscle biopsy in questionable cases and demonstrates the clinical heterogeneity of VCP-related disorders.

Late onset Pompe disease: is there any correlation between muscular mri and cardiopulmonary functional tests?

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Late-onset Pompe disease (LOPD) manifests, in most of the patients, with proximal lower limbs muscle weakness and respiratory insufficiency. Patients under enzyme replacement therapy are monitored with the 6MWT and FVC, however, these parameters do not account for the increasing difficulty in climbing stairs or raising from the floor. Four LOPD patients (three women and one man), followed in our centre with an almost stable 6MWT through the years, were submitted to lower limbs muscular MRI, spirometry and cardiopulmonary exercise test (CPET) to try to better estimate the muscular damage and dysfunction. Information about exercise performed at home was obtained through International Physical Activity Questionnaire (IPAQ). The muscular signal alteration observed by MRI was related with subcutaneous adipose tissue in different muscles at middle thigh level and mean value was calculated. We found an apparent relationship between the residual muscle tissue, the exercise performed at home and the maximal work load (measured in watt/Kg). Maximal oxygen consumption adjusted for weight (VO2/Kg), obtained at CPET, does not seem to be influenced by residual muscular bulk. Vital capacity in seated position does not seem to be related to the parameters measured during CPET. These observations should be confirmed in a larger patient population sample, and should be repeated after an individualized exercise protocol to verify whether a rehabilitation program may improve muscular performances in LOPD.

Revised upper limb module for spinal muscular atrophy: development of a new module

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There is a growing need for a robust clinical measure to assess upper limb motor function in Spinal Muscular Atrophy (SMA) as the available scales lack of sensitivity at the extremes. We used a stepwise approach, starting from the ULM, a module that was specifically devised for SMA focusing on activities observed in the younger and weaker SMA patients. The RULM fits the conceptual framework for SMA with an increased number of items capturing a wider spectrum of functional abilities across ambulant and non-ambulant individuals with SMA. An international expert panel with specific neuromuscular expertise convened to perform a thorough review of the current scales used to assess upper limb function in SMA. Subsequently, they applied clinical expertise and modern psychometric methods to revise and adapt items in the RULM to make it a suitably robust scale. Multiple revisions of the scale included statistical methods that focus on capturing clinically relevant changes that reflect requirements by regulators and advocacy groups. There was consensus among the clinical evaluators that the module is easy to perform and score. It was generally well accepted even by the younger children, allowing us to capture upper limb activities across a wide spectrum of both ambulant and non-am-

bulant individuals. The RULM can continue to identify change in motor function after an ambulant individual becomes non-ambulant. These results together with the statistical robustness suggest that the RULM may represent a valuable addition to the existing scales that have so far been used in clinical trials in SMA.

Proposal of a mobile app designed for patients with Pompe disease

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One of the most important device in our lives is a mobile phone. For now, it is a powerful computing platform equipped with various sensors that can be used in multiple domains, such as environmental monitoring, social networks, safety and also healthcare. Smart Phones are becoming increasingly successful in the area of health monitoring. We are developing an app mobile designed for patients with Pompe disease, with the following purposes: to help patients affected by Pompe disease to manage illness related issues and to reduce burden, being continuously aware of health and quality of life; to provide clinicians with continuous tracking of disease in each patient in real-time and ecological conditions of everyday life; to collect data about Pompe disease natural history. Our device will perform, by using friendly interface, a fast acquisition/visualization of clinical parameters, of disease progression and of all the activities of patients' daily living with the possibility to be constantly connected with referred medical staff. Moreover it will provide the possibility to register physical activity (with various proposition of exercises/workout/ training programs tailored for Pompe patients), with tutorial videos to workout both safely and independently.

Study financed by: Genzyme Corporation

Difficulty in defining role of genetic mutations: a case report

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The symptomatic heterozygotes for recessive LGMD were well documented in literature. The summation effect of different heterozygous mutations in recessive genes was in contrast not demonstrated.

We report a case presenting with muscular cramps, normal CK levels and persistent mild elevation of transaminases. Age at onset was acute at 43 years old. Electromyography founded evidence of neurogenic finding due to radiculopathy. Patients underwent needle muscle biopsy of quadriceps. Morphological examination by light microscopy showed inflammatory myopathic findings with minimal sarcolemmal HLA expression. Muscle biopsy performed after a year of immunosuppressive therapy showed the same histopathological pattern. Western blotting showed normal weight and size of all protein tested. Molecular analysis showed the following mutations: c.3893A > G (p.Ile1298Val) in DYSF gene, c.427C > A (p.Arg143Ser) in FKRP gene.

Histopathological findings contrast with previous literature report of reduced amount of protein in LGMD2B carriers. The mutation in another recessive gene described in literature as pejorative complicate the diagnostic challenge. Our data, lead us to think about dysferlinopathy in patient with late onset mimicking treatment resistant polymyositis o liver disease.

A peculiar case of LGMD with rimmed vacuoles

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The patient is a 26-year-old soldier who came to our attention complaining about progressive difficulty in climbing stairs and getting up from a squatting position. His family history was unremarkable. He presented with scapular winging, proximal weakness of the upper and lower limbs, and mild impairment of thigh flexion and adduction. Facial power was normal with a full range of eye movements and so was his respiratory and cardiac function. The serum CK activity was increased (> 1000 UI/L) and EMG results showed mild myopathic abnormalities. A muscle MRI of the lower limbs showed bilateral fatty replacement of a large part of the thigh muscles (especially adductor magnus, longus and brevis). A muscle biopsy revealed a myopathic pattern with several necrotic fibers and rimmed vacuoles. Extensive immuno-histochemical and WB analysis were normal. Dysferlin and GNE gene mutations were excluded by genetic testing. A DNA sample was analysed by whole exome sequencing and a homozygous variant in the *HNRNPDL* gene (c.1130A > G, p.Tyr377Cys) was detected. In the literature *HNRNPDL* gene mutations are linked to LGMD1G, which was described in two families (Brazilian-Caucasian & Uruguayan) bearing two different heterozygous missense mutations affecting the same codon in the HNRNPDL gene (D378N, D378H); the transmission pattern was consistent with autosomal dominant inheritance and incomplete penetrance. The significance of this new homozygous variant is under investigation in the proband's family.

Innovative quantitative testing of handgrip myotonia

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Dystrophic (DM) and Non-dystrophic Myotonias (NDM) are diseases characterized by the presence of myotonia with or without muscle weakness. A standardized assessment is important to quantify this aspect. Herein, we describe a new test to quantitatively evaluate handgrip myotonia. In particular, we screened patients affected by Myotonic Dystrophy type 1 (MD1) and Myotonia Congenita (MC). This test was performed using the sensor-engineered glove test (SEGT) on 10 patients with MC (5 on mexiletine and 5 drug-free) and 10 patients with MD1, compared with a cohort of healthy controls, evaluating touch duration and inter-tapping interval. The protocol consisted in: repeated finger tapping (FT) between thumb-index, making the fist, started after 5 minutes of rest and lasting for one minute, to assess time to fully release the hand; then, after

2 minutes of ice-application at the hand, the same exercise was performed again. Both groups (MD1 and MC) showed a significantly increased finger tapping interval compared to controls. Ice application and mexiletine influenced this value in some patients. The SEGT is a non-invasive and sensitive test to quantify handgrip myotonia. We suggest that it should be applied for monitoring treatment efficacy and for clinical trials.

Early macular dysfunction in mitochondrial diseases

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The aim of this study was to determine if the macula, the retinal locus with the highest energy demand, is functionally impaired in patients with genetically-defined mitochondrial disease (MD) without clinical evidence of retina-wide involvement. Twenty-one patients with various mitochondrial DNA mutations (13 PEO, 5 MERRF, 1 MELAS and 2 MNGIE) and no evidence of retina-wide pathology and 20 age-matched control subjects were enrolled in the study. All patients underwent complete ophthalmologic examination including best-corrected visual acuity (BCVA) measurement, fundus examination, spectral-domain optical coherence tomography (SDOCT), full field electroretinogram (ERG) and focal ERG (fERG) recordings.

Compared to controls, visual acuity and fERG amplitude of patients were reduced on average, by 13% ($p < 0.005$) and 62% ($p < 0.0001$) respectively. Acuity and fERG amplitude losses were found in 16 and 36 out of 42 eyes, respectively ($p < 0.01$). All eyes with reduced acuity and twenty eyes with normal acuity had fERG losses. Linear regression analysis showed a significant negative correlation between fERG fundamental harmonic amplitude and disease severity assessed with NMDAS score, while no relationship was found with patient age.

The results indicate that mitochondrial insufficiency in patients with MD is associated with macular dysfunction which can be detected by fERG and, to a lesser extent, by visual acuity measurement in absence of retina-wide involvement. Our results pave the way to the design of clinical trials based on antioxidant strategies and to the use of corresponding sensitive and specific outcome measures such as macular cone fERG.

Efficacy of lacosamide in patients with mitochondrial diseases

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The central nervous system is metabolically very active and is therefore vulnerable to defects of the mitochondrial respiratory chain. Furthermore, mitochondria have a central role in many process of seizure generation such as neurotransmitter synthesis, calcium homoeostasis, redox signaling, production and modulation of reactive oxygen species and neuronal death.

Consequently, epilepsy is associated with a long list of pathogenic mutations in the mitochondrial genome and in nuclear-encoded mitochondrial proteins. Three consecutive patients (one male and two female, respectively 35, 16 and 33 years of age) affected by typical Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-Like Episodes (MELAS) associated with the A3243G mutation of mitochondrial DNA and drug-resistance epilepsy (more than one seizure a week; in treatment with levetiracetam, clobazam and lamotrigine) came under our observation. Symptoms include partial epileptic fits, which sometimes become generalized later, and associated with stroke-like episodes (SLEs) in two patients. In all three patients lacosamide was started with dramatic reduction of seizure frequency and seizure freedom that persisted after 3 years of follow-up without sides effects. Lacosamide, one of the very last approved antiepileptic drugs, is believed to exert its anticonvulsant effects through stabilization of hyperexcitable neuronal membranes and inhibition of repetitive neuronal firing by selectively enhancing slow inactivation of voltage-gated sodium channels. Epilepsy strongly influences course and prognosis of mitochondrial disease, often triggering metabolic crisis or SLEs. This is the first report of efficacy and safety of long-term therapy of lacosamide in fragile patients such as subjects affected by mitochondrial diseases and drug-resistance epilepsy.

Validation of ultrasound technique for the study of rats skeletal muscle atrophy

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Muscle atrophy is defined as a decrease in the mass of the muscle occurring in many diseases, which reduce quality of life increasing morbidity and mortality. Using the non-invasive ultrasonography technique, we developed a new method to measure soleus and gastrocnemius lateralis muscle atrophy in the hindlimb-unloaded (HU) rat, a well accepted model of muscle disuse. Soleus and gastrocnemius volumes were calculated using the conventional truncated-cone method and the innovative sinusoidal method. The structural parameters physiological cross sectional area (PCSA), pen nation angle (PA) and fascicle length (Lf) have been evaluated. For Soleus muscle, the ultrasonographic volume determined *in vivo* with either method was linearly correlated to the volume determined *ex-vivo* from excised muscles as muscle weight-to-density ratio. For both soleus and gastrocnemius muscles, a strong linear correlation was obtained between the ultrasonographic volume and the muscle fiber cross-sectional area determined *ex-vivo* on muscle cryosections. According to the Soleus muscle atrophy, a reduction of the structural parameters has been observed. Thus ultrasonography allowed the longitudinal *in vivo* evaluation of muscle atrophy progression during hindlimb unloading. The measure of the structural parameters closely related to the functional parameters has allowed us to understand the biomechanical mechanism underlying the soleus muscle atrophy induced by HU. This study validates ultrasonography as a powerful method for the evaluation of rodent muscle atrophy *in vivo*, which would prove useful in disease models and therapeutic trials. Supported by the Italian Ministry of Education, Universities and Research (PONa3_00395"Biosciences & Health") and the Italian Ministry of Health (GR-2009-1580433).

Clinical characterization and study of methylation profiles in carriers of borderline D4Z4 Alleles from the Italian National Registry of FSHD

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In the last years, the level of D4Z4 DNA methylation and mutations in SMCHD1 gene have been proposed as new diagnostic genetic markers of FSHD, especially in cases carrying alleles with 9-10 D4Z4 repeats, considered borderline allele, and in contraction-independent patients (FSHD2). We present a large genotype-phenotype correlation study on 262 subjects carrying 36-41 kb DRA (146 index cases and 116 relatives), selected from the Italian National Registry for FSHD. We observed that 51.8% of probands showed a FSHD phenotype (category A), 21.4% presented incomplete features of disease (category B), 21.4% did not fulfill the clinical diagnosis of FSHD, showing atypical clinical features (category D). Among FSHD probands, we detected a wide variability of clinical expression, both in term of age at onset and motor disability, ranging from almost asymptomatic carries to severly affected subjects. Importantly, 68.1% of relatives, carrying the same DRA of the proband, did not display any muscle functional impairment (category C). Among affected relatives, only 20.8% showed FSHD phenotype (category A). To investigate the molecular basis of incomplete penetrance we investigated the level of D4Z4 methylation and sequenced *SMCHD1* gene analysis in a subgroup of patients and informative families. Our analysis failed to identify specific epigenetic settings exclusively associated with the presence of disease**.**

Autophagic vacuolar myopathies with ultrastructural features of myofibrillar myopathy: case reports

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Autophagic vacuolar myopathies are a spectrum of disorders unified by distinctive myopathologic features, characterized by the presence of autophagic vacuoles containing lysosomal, autophagosomal and sarcolemmal membrane components. While not being classified as autophagic vacuolar myopathies, also muscle disorders including myopathies with "rimmed vacuoles," inclusion body myopathies and myofibrillar myopathies (MFM) have autophagic pathology.

Here, we present the diagnostic algorithm which led us to the diagnosis of MFM in four unrelated patients with autophagic vacuolar myopathies. Patient 1 is a 39 years old male, that came to our attention at age of 35 for hyperCKemia (5x normal value) and a mild exercise intolerance; his clinical conditions have worsened in the last years of follow up, with the appearance of a progressive limb girdle weakness resembling a LGMD-like phenotype. Patient 2 is a 74 years old woman with a 7-years history of a progressive axial and pelvic girdle weakness, in association with a moderate increase of blood CK (4x normal value). Patient 3 and 4 are respectively a 19 years old and a 37 years old males, that present an asymptomatic hyperckemia (5x normal value), without muscle weakness. None of them is affected by cardiomyopathy. Their family history is inconsistent for neuromuscular diseases. The ultrastructural studies of the muscle biospies have showed myofibrillar myopathy features, including progressive myofibrillar degeneration commencing at the Z-disk, accumulation of degraded filamentous material and entrapment of dislocated membranous organelles in autophagic vacuoles.

An exercise test protocol to study muscle fatigue in facioscapulohumeral muscular dystrophy

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Muscle fatigue, weakness and atrophy are basilar clinical features that accompany facioscapulohumeral dystrophy (FSHD). Although a wide range of clinical severity is observed in FSHD patients, abnormal muscle fatigue appears to be a primary component of disease, often appearing before evident functional impairment. Despite its relevance in the clinical picture, of FSHD and other muscle dystrophies, very little is known about the pathophysiology of muscle fatigue in muscle disorders. The aim of the study has been to define an exercise protocol for FSHD patients in order to detect and quantify the impairment of motor performance. We enrolled 8 FSHD subjects, aged between 20 to 45 yrs, affected by a mild-moderate form of disease and without a severe motor impairment at lower limbs, and 5 unrelated healthy controls, matching for sex-, age- and basal level of physical activity. The study design included: -clinical evaluation and phenotypic classification; - evaluation of subjective experience of daily life fatigue by Individualized Neuromuscular Quality of Life (INQoL) and Fatigue Severity Scale (FSS); incremental exercise test on cycle ergometer with serum biochemical evaluation of markers of anaerobic metabolism and oxidative stress. Results are discussed to define whether such exercise protocol may be easily deployable and suitable for a proper clinical assessment of fatigue in FSHD patients.

Facial weakness, bulbar symptoms and epilepsy: which possible links?

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We describe a 10-year-old boy who developed dysarthria and nasal voice, dysphagia and easy fatigability. Respiratory function test was seriously impaired. The boy came to our attention after 5 years of carbamazepine therapy for epilepsy with secondarily generalized tonic-clonic seizure.

Needle EMG was normal in deltoid muscle, while it showed reduced area of action potential of motor unit in facial muscles (orbicularis oculi and orbicularis oris muscles). Routine blood examinations with CK analysis were normal, brain CT scan was normal.

Early neurodevelopment was referred to be normal and family history was negative for neuromuscular disorders and consanguinity.

Clinical picture suggested a possible myasthenia gravis (MG), confirmed by Repetitive nerve stimulation and Anti Ach-R antibodies were positive.

It is well known that Carbamazepine (CBZ) is associated with a significant incidence of hypersensitivity reactions and has been already linked with drug-induced systemic lupus erythematosus-like syndromes and have already been reported 4 girls affected by MG being treated with Carbamazepine.

Our child showed a good response to pyridostigmine treatment and he is now free of MG symptoms, but he still has positive Ach-R antibodies.

We wondered if signs and symptoms of myasthenia gravis in our case could be hypothetically related to CBZ therapy.

Even if this association could be coincidental, this case report suggests that drug-induced myasthenia gravis is an important side-effect to be suspected even after long-term treatment with CBZ and during childhood.

Dysautomic symptoms due to peripheral nerve involvement in myotonic distrophy type 2 (DM2)

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A peripheral neuropathy has been only seldom described in DM2 patients. Here we report two related DM2 patients manifesting peripheral neuropathy with dysautonomic symptoms among their cardinal disease features.

The proband, a 50 year-old male, from age 36 referred erectile dysfunction. At age 37 he was evaluated by cardiologists for episodes of cold sweating, palpitations and syncopes, and refused prophylactic ICD for severe ventricular tachyarrythmias.

Recently, he had been complaining of muscle stiffness and pain. Neurological examination documented frontal balding, no muscle weakness or atrophy, reduced lower limbs deep tendon reflexes. Blood tests showed a two-three fold increase of CK levels and hypogammaglobulinemia, while glycemia, thyroid and sexual hormones were normal. EMG studies showed rare pseudomyotonic discharges and a sensory-motor polyneuropathy. Nerve biopsy showed a chronic aspecific axonal neuropathy. Muscle, brain MRI and neuropsychological tests were normal. The patient again refused prophylactic ICD pacing despite persistence of severe ventricular tachyarrythmias.

Also his 27 year-old son recently manifested similar muscle and dysautonomic symptoms. Neurological examination revealed mild frontal balding, handgrip myotonia and diffuse hyporeflexia. CK levels were increased (490 UI/l, n.v. 30-170), while other blood tests were normal. He also showed pseudomyotonic discharges and a mixed polyneuropathy at EMG studies. Muscle MRI and biopsy, extensive cardiac, cognitive and endocrine diagnostic assessments were all normal.

In both cases, molecular testing for FAP, CMT1B and CMTX1 resulted negative, whereas DM2 testing was positive. This report emphasizes that a sensory-motor neuropathy may be part of the clinical manifestations of multisystem involvement in DM2.

Novel mutations in *OPA1* with leigh-like neuroimaging features

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Behr syndrome is characterized by the association of early onset optic atrophy, cerebellar ataxia, pyramidal signs, peripheral neuropathy and mental retardation. Recently, some cases were reported to be caused by bi-allelic OPA1 mutations. We describe a 11-year-old girl (pt1) and a 7-year-old boy (pt2) presenting during early infancy cognitive delay, ataxic gait and clinical signs suggestive of a peripheral neuropathy. In pt1 ocular fundus examination showed optic disk pallor whereas pt2 presented severe optic atrophy. In both children neuroimaging showed a progressive cerebellar involvement accompanied by basal ganglia hyperintensities with pathological peak levels of lactate. Muscle biopsy showed a diffuse reduction of cytochrome *c* oxidase stain, the presence of some atrophic fibers and type II fiber grouping in both cases. Using a targeted resequencing panel in next generation sequencing (NGS), we identified the homozygous c.1180G > A/p.Ala394Thr in pt1 and the c.2779-2A > C in compound heterozygosity with the c.2809C > T/p.Arg937Cys in pt2. The mutations were novel and segregated in healthy parents. Expression of OPA1 protein was significantly reduced in muscle tissues of both patients by Western blotting analysis. We also observed in patients' fibroblasts a higher proportion of fragmented and intermediated mitochondria upon galactose treatment compared to controls, as already seen in other patients harboring mutations in *OPA1*. The presence of Leigh-like MRI features is a novel finding in Behr syndrome and further complicates genotype-phenotype correlations in *OPA1*-associated disorders.

Cerebellum in Duchenne Muscular Dystrophy

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Delay in cognitive development, particularly in language, is more frequent in patients with Duchenne Muscular Dystrophy (DMD) than in general population. The occurrence of psychopathology in DMD patients, including attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD) and obsessive compulsive disorder (OCD), was documented in several studies. In DMD patients, reported prevalence of ADHD varies from 11.7 to 33% and of ASD is higher than expected in general population. In ADHD and ASD were described cerebellar abnormalities such as hypotrophy of the worm and reduction of the volumes of both grey and white matter. Diffusion tensor imaging (DTI) in Asperger syndrome showed a reduction of specific axonal density of thin efferent fibers from the right cerebellar peduncle. Dystrophin isoforms present in nervous system are mostly in cerebellum, hippocampus and cerebral cortex. In DMD patients reduced cerebral and grey matter volume and regional reduction of glucose metabolism, especially in sensorimotor areas, temporal neocortex and cerebellum were reported.

In our study, cerebral Magnetic Resonance Imaging of 13 patients, compared to 13 healthy controls, showed: aspecific alterations, such as mild enlargement of ventricles, cortical and subcortical punctiform hyperintensities; statistically significant volumetric reduction in bilateral caudate, putamen, thalamus and hippocampus; reduction of cerebral volume; at DTI analysis, fiber axis reduction in cerebellar white matter, medium and superior cerebellar peduncles, mesencephalon, temporal deep white matter, optic radiations, capsules, cingulum and corticospinal tracts. Furthermore, was found a thickening in left temporal and paracentral, right parietal and fronto-mesial cortex. These preliminary data suggest a dystrophin role in neurological development and cognitive processes.

Psychological profile in McArdle's patients

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Much is known about the physiopathology at the level of muscle energy production and response to exercise of McArdle's disease. The condition is associated with substantial disability and requires continuous adaptation of life habits. This may result in psychological stress.

We established a multiprofessional team for the assessment and management of McArdle's patients. The team included the psychologist, who evaluated the emotional impact of the disease and the individual coping strategies. The assessment included a structured interview and admistration of personality test.

13 patients have been evaluated. Three psychological phases could be identified: before diagnosis, trying to get a diagnosis having identified a problem and after diagnosis. While the first two phases are very similar in all patients, the last one largely depends on the individual coping strategies. Most McArdle patients were not diagnosed until adulthood and often they were not believed when they complained about exercise induced pain. This was reported as depressing and frustrating. When they were finally diagnosed, they felt relieved because they could now justify their complaints. After diagnosis they react in two ways: they either avoid or suppress emotions or they externalize. This will determine their coping style. All patients but one appreciated the attention given to their emotional world. Conversely the team could better taylor the treatment indications taking into consideration the psychological evaluation.

The psychological profiling in non-fatal but chronic neuromuscular diseases, may reveal hidden emotional difficulties which may be exhacerbated by the "invisible" nature of the condition.

Clinical and genetic heterogeneity in four patients with Dysferlinopathies

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Dysferlin is a sarcolemmal protein that plays an important role in patching defects in skeletal membrane by regulating vesicle fusion with the sarcolemma. Mutations in the dysferlin gene can lead to a variety of clinical phenotypes. Affected individuals usually present with early involvement of the posterior calf muscles (Miyoshi myopathy), but can present with proximal greater than distal weakness similar to other limb-girdle muscular dystrophies (LGMD2B), with anterior tibial weakness, an axial myopathy (e.g. rigid spine syndrome or hyperkyphosis resembling bent spine syndrome), or any combination of the above. To describe clinical, histological and molecular findings of four patients carrying *DYSF* mutations. Each patient underwent a complete neurological examination, muscle CT scan and muscle biopsy with histological, and immunohistochemical studies. Molecular genetic studies on DYSF gene were performed; mutation analyses included the coding exons of *DYSF* gene and their flanking intronic sequences. Muscles biopsies showed dystrophic changes. The patients had variable phenotypes. Pathogenic variants in *DYSF* were detected in four patients. In two patients we found the already reported c.6124C > T (p.Arg2042Cys) and c.5509G > A (p.Asp1837Asn) variants in homozygous state. Instead we could detect only the heterozygous variants c.6139A > G (p.Ile2047Val) and c.6017G > A (p.Arg2006Gln) in the last two patients. The p.Arg2006G. ln mutation (rs539697079) was not associated with a clinical significance therefore we performed *in silico* analysis and all tools predicted the missense mutations as pathogenic. Overally, our study expands the spectrum of DYSF mutations. Evidence for pathogenicity of the p.Arg2006Gln mutation is highly suggestive for the following reasons: 1) It affected conserved amino acids 2) Rare variant (allele frequency = 0.0002001) 3) *in silico* tools predicted the missense mutations like pathogenic.

A new mutation of ACAD9 causative of a late-onset cardiomyopathy syndrome

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Acil-CoA dehydrogenase-9 (ACAD9) is critical in the assembly of mitochondrial complex I of the respiratory chain, which acts mainly in heart, brain, skeletal muscle, liver and kidney. Either homozygous and heterozygous mutations are

capable to cause early-onset hypertrophic cardiomyopathy, encephalopathy and myopathy with lactic acidosis. A 43 year old man, who suffered from non-obstructive hypertrophic cardiomyopathy (NHCM) since his twenties, came to our observation complaining with exercise intolerance, breath shortness and iperCKemia. Married to a first cousin, he had a 12 and a 9 year old sons, suffering for muscle weakness with iperCKemia. Cardiac evaluation, electrophysiology, muscle biopsy were performed in the proband and his sons while genetic studies involved the mother also. Cardiac study being normal in both the children at admission revealed NHCM at two years follow-up in the oldest son. Electromyograpy showed primitive myopathy. Muscle biopsy disclosed the pattern of mitochondrial myopathy with several ragged red fibers and fibers with marked sub-sarcolemmal SDH/NADH storage. Genetic analysis showed the c.1240C > T mutation in exon 12, leading to p.Arg414Cys aminoacid substitution in homozygous in the proband and his children and in heterozygous in the mother. The proband decesead for sudden cardiac death at age 44. Cardioverter defibrillator was implanted in the eldest son when he was 16; the younger complains with exercise intolerance and fatiguability without cardiac involvement. Both the children receive 300 mg riboflavin a day with subjective amelioration. The c.1240C > T ACAD9 mutation being firstly reported in our family seems to be associated to a later-onset of cardiomyopaty.

In vitro and in vivo preclinical evaluation of therapeutic potential of SRC-TK inhibitors in Duchenne Muscular Dystrophy

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Src Tyrosine Kinase (TK), a redox-sensitive protein, is overexpressed in dystrophin-deficient muscles and can be overactive due to excessive production of reactive oxygen species. Thus, the pharmacological inhibition of Src-TK seems a feasible strategy to ameliorate the pathology.

We focused on two Src-TK inhibitors, PP2 and Dasatinib. First, we conducted *in vitro* studies on a murine muscle satellite cell line (C_2C_{12}) to evaluate the drugs' effects on cell viability and their potential protection against oxidative stress-induced cytotoxicity. We tested increasing concentrations of PP2 (0.1-300 μM) and Dasatinib (0.1-150 μM). Starting from PP2 3 μM, a significant cytotoxic effect was shown, while no protection was observed at lower concentrations. By contrast, Dasatinib showed a concentration-dependent decrease of cell viability from the concentration of 5 μM onward. We used two cytotoxic concentrations of H_2O_2 (300 μM and 1 mM) to evaluate potential cytoprotective effects of Dasatinib. Cytotoxic effect of H_2O_2 300 μM was significantly counteracted by Dasatinib 0.1 μM, while higher concentrations (0.5 μM and 1 mM) showed cytoprotection against H_2O_2 1 mM.

Secondly, we evaluated the effects of PP2 (5mg/kg,three times a week;s.c.) by means of five-week proof-of-concept preclinical study in treadmill-exercised mdx mice. PP2 improved *in vivo* forelimb-strength and slightly exercise resistance, while no effect was observed on torque. No protection was observed on contraction parameters of EDL muscle. PP2 did not counteract the high plasma levels of CK and LDH. Histopathology of treated mdx muscles was improved. Our results support the interest of further studies of Dasatinib and PP2 as potential treatments for DMD (Supported by Duchenne Parent Project NL_DPP).

Muscle MRI in late-onset Pompe disease (LOPD): pattern of muscle involvement, follow-up and atypical features

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We collected muscle MRI data from 15 LOPD patients with different degrees of clinical severity. In 7 out of 15 patients we also performed follow up MRIs which allowed to correlate clinical and radiological evolution.

Consistently with previous reports, most patients presented a typical MRI pattern of fatty substitution of axial (paraspinal, abdominal belt), limb girdle and posterior thigh muscles with some exceptions. In two patients there was a marked tongue involvement which correlated with dysphonia and respiratory insufficiency. Muscular damage in T1-weigthed images showed substantial stabilization or mild evolution in follow-up exams. Radiological involvement did not strictly correlate with disease duration but with clinical severity and in some cases it anticipated functional motor impairment.

The most interesting feature was the presence of significant STIR hyperintensities (inflammatory edema) in 6 patients, especially localized in posterior thin muscles. All patients were in early stages of the disease and inflammatory alterations were localized both in normal and in fatty infiltrated muscles. Follow-up MRIs showed that STIR hyperintensities ameliorated, but not resolved in most cases. In one patient with subacute onset steroids therapy determined marked clinical improvement. Inflammation can thou be considered as an early marker of pathology, but also as a chronic process not always evolving to fatty substitution of muscle.

Muscle inflammatory involvement can represent a "double trouble" condition in Pompe disease: if misunderstood it can result in diagnostic delay. On the other hand, inflammation is a potentially treatable condition that can significantly influence clinical and functional progression in LOPD patients.

Can MRI imaging of a muscle set follow FSHD natural history?

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Facioscapulohumeral dystrophy (FSHD) is characterized by extremely variable degree of facial, upper and lower limb muscle involvement and by a remarkable asymmetry. This unique combination of variables makes difficult to establish a valid biomarker to follow the natural history of the disease. Muscle MRI has been successfully used for diagnosis and clini-

cal characterization of FSHD patients, and given its non-invasive nature might constitute a suitable candidate tool to evaluate disease progression. We have already reported a cohort of 30 FSHD patients and 23 patients affected by other myopathies (NFSHD) examined by whole-body muscle MRI. We showed that FSHD patients had a specific pattern of muscle MRI involvement, primarily at the scapular girdle limbs. Here we extend our MRI study including other 40 FSHD (M:F, 27:13). We evaluated the presence of edema, the frequency and severity of fat infiltration and atrophy, and the asymmetric involvement of upper and lower limbs muscles. We confirmed the prevalent involvement of trapezius, teres major, serratus anterior, gluteus minimus and maximus, semimembranosus and obliquus muscles, as well as that the degree of involvement correlates with the severity of the disease. Finally, we are characterizing single set of muscles that could be used as a biomarker of the natural history of the disease, including asymptomatic patients and advanced stage of the disease.

POPDC1S201F causes muscular dystrophy and arrhythmia by affecting protein trafficking

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The Popeye domain-containing 1 (POPDC1) gene encodes a plasma membrane-localized cAMP-binding protein that is abundantly expressed in striated muscle. In animal models, POPDC1 is an essential regulator of structure and function of cardiac and skeletal muscle; however, POPDC1 mutations have not been associated with human cardiac and muscular diseases. Here, we have described a homozygous missense variant (c.602C > T, p.S201F) in POPDC1, identified by whole-exome sequencing, in a family of 4 with cardiac arrhythmia and limb-girdle muscular dystrophy (LGMD). This allele was absent in known databases and segregated with the pathological phenotype in this family. We did not find the allele in a further screen of 104 patients with a similar phenotype, suggesting this mutation to be family specific. Compared with WT protein, POPDC1S201F displayed a 50% reduction in cAMP affinity, and in skeletal muscle from patients, both POPDC1S201F and WT POPDC2 displayed impaired membrane trafficking. Forced expression of POPDC1S201F in a murine cardiac muscle cell line (HL-1) increased hyperpolarization and upstroke velocity of the action potential. In zebrafish, expression of the homologous mutation (popdc1S191F) caused heart and skeletal muscle phenotypes that resembled those observed in patients. Our study therefore identifies POPDC1 as a disease gene causing a very rare autosomal recessive cardiac arrhythmia and LGMD, expanding the genetic causes of this heterogeneous group of inherited rare diseases.

LMNA mutation in a case of axonal peripheral neuropathy: what has the muscle to say?

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Complex pictures of genetic disorders are linked to the *LMNA* mutations.

A 42 years old man, with_diagnosis of axonal sensitive-motor neuropathy since the age of 30 years old, came to our attention for the worsening of the foot drop gait and muscle weakness at the lower limbs. In the medical history, diagnosis of myeloid chronic leukemia (treated with Imatinib, discontinued for increased CPK levels) and positive family history for the axonal neuropathy (father, dead suddenly during the sleep at the age of 71 years old, and son). An extensive diagnostic work-up searching for CMT or *TTR*-related amiloyd neuropathies; mild increased levels of CPK and reduced lactate response to ischemic exercise test were detected. A muscle MRI showed diffuse hypotrophy, fatty infiltration and oedema at the lower limbs. Based on familiar anamnesis, CPK level, negativity for main genetic neuropathies, a molecular search for *LMNA* mutations was performed, revealing a missense variant, in heterozygous, c.1004 G > A in exon 6 (p.Arg335Gln aminoacidic replacement). Up to date, a *LMNA* mutation (892 C > T) was described in three Algerian families with AR-CMT2, but our mutation is reported only in one patient with dilatative cardiomyopathy. *LMNA* mutation search is ongoing in the patient family.

Distinct functional domains in LMNA need for the homeostasis of different cell lineages. Muscle wasting, common in CMT disease, is usually considered secondary to nerve damage; mutations in single genes may lead tooverlapping phenotypes suggesting that the muscular involvement may be primarily associated with nerve fibers loss or degeneration and should not be only a delayed consequence of nerve degeneration.

Transcriptomics analysis in collagen VI myopathy: role of circadian genes using novel fluidic card tools

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Collagen VI myopathies are genetic disorders due to mutations in collagen VI A1, A2, and A3 genes, ranging from the severe Ullrich congenital muscular dystrophy (UCMD) to the milder Bethlem Myopathy (BM), which is recapitulated by collagen VI null (*Col6a1*$^{-/-}$) mice.

UCMD and BM can be inherited accordingly to both dominant and recessive models, and generally, neither the type of mutation (missense, nonsense, splicing insertion or deletion), nor the effect of the mutation allows the discrimination between two phenotypes. This suggests that the genetic background of patients may influence the mutated genes expression.

To unravel the expression profiling perturbation in muscles with collagen VI myopathies we performed a deep RNA profiling in both *Col6a1*$^{-/-}$ mice and collagen VI patients. An interactome map that highlights the connection between the altered pathways, including circadian rhythms, and collagen VI pathology was built up. In order to profile both circadian and COL6 genes, we designed two novel TaqMan® low-density arrays, one covering 10 circadian genes (CLOCK, ATF5, ARNTL, EGR1, DBP, CCRN4L, FKBP5, PER1, PER2, PER3) and the other covering the full-exon composition of the three collagen VI genes. All the transcriptomic analyses revealed a profound deregulation of the circadian genes with a CLOCK upregulation in the more severe (UCMD) phenotype.

We propose that CLOCK gene might be a severity biomarker for collagen6 myopathy. In addition our TaqMan assays may be used as diagnostic tools for collagen-VI related myopathy lacking molecular characterization.

New genotype-phenotype aspects of neurodegeneration with brain iron accumulation (NBIA)

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Objectives. Neurodegeneration with brain iron accumulation (NBIA) comprises a group of brain iron deposition syndromes that lead to progressive neurological impairment with mixed pyramidal-extrapyramidal features and progressive dementia. To date ten genes have been identified as associated with different NBIA subtypes. The two core syndromes are the pantothenate kinase-associated neurodegeneration (PKAN) caused by mutation in the PANK2 gene and PLA2G6-associated neurodegeneration (PLAN) caused by mutation in the PLA2G6 gene, both with autosomal recessive transmission and a variable phenotype. To better understood the genotype-phenotype correlations of such disorders we performed clinical and genetic studies in 7 patients with NBIA.

Materials and methods. Our patients were subjected to extensive clinical investigations. Three of the 7 patients had a clinical phenotype compatible with PKAN, 1 patient presented with INAD and 3 with an atypical phenotype. Mutation analysis of the PLA2G6 and PANK2 genes was performed using direct DNA sequencing in all patients.

Results. Genetic analysis showed the homozygous mutation c.1169A > T (N390I) in PANK2 gene in two patients with PKAN, two novel compound heterozygote mutations (G206D and IVS3-1G to A) of the same gene in the third patient PKAN, the novel c.1786C > T (L596F) homozygous mutation of PLA2G6 in the patient with INAD and the novel c.17G > A (R6H) heterozygous mutation in one patient with atypical phenotype.

Conclusions. In this work we performed clinical and genetic studies in 7 patients with NBIA, 3 of whom presented mutations in PANK2 gene and two in PLA2G6 gene. We describe new genotype-phenotype correlations in our patients.

EMG diagnosis of McArdle disease with long exercise test

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Standard electromyography (EMG) is routinely performed during the diagnostic process of myopathies, but its role is limited in metabolic myopathies with exercise intolerance, such as McArdle disease (glycogenosis type V, GSDV).

The aim was to evaluate a provocative test (the Long Exercise Test, LET) in the EMG diagnosis of GSDV.

Twenty-five patients (17 males, 41 ± 17 years) with confirmed diagnosis of GSDV underwent an EMG study (including nerve conduction studies, repetitive nerve stimulation, needle EMG) completed with LET. Briefly, the compound muscle action potential (CMAP) responses were recorded from right *abductor digiti minimi* (ADM) muscle before and after 5 min of maximal ADM isometric contraction.

Needle EMG showed myopathic pattern only in 5/25 patients. LET disclosed a post-exercise decrease in CMAP amplitude in 22/25 patients (88%). The decrement appeared immediately after exercise (average -20%) and reached its maximum (-30%) at 30 min, after a transient plateau phase (lasting 5 to 15 min). This pattern was not observed in controls (healthy subjects, other metabolic myopathies, periodic paralysis). Interestingly, GSDV patients with normal LET (n = 3) presented milder symptoms and minimal myophosphorylase activity on muscle biopsy.

EMG combined with LET detects a peculiar pattern in

McArdle disease. This test is sensitive, safe and non-invasive. It may orient molecular analysis toward myophophorylase gene in patients referred for exercise intolerance with hyperCKemia at rest. The normal response of LET in patients with milder symptoms indicates a correlation with clinical severity, thus suggesting its use as a possible outcome measure in clinical trials.

Can heterozygous carnitine palmitoyltransferase II (CPT II) deficiency be symptomatic?

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Carnitine palmitoyltransferase II, is an enzyme encoded by CPT2 *gene*, participant in fatty acid oxidation. The deficit of this enzyme is the most common inherited disorder of long-chain fatty acid oxidation affecting skeletal muscle. Clinically, the myopathic picture is caracterized by recurrent episodes of muscle pain, muscle weakness, and rhabdomyolysis triggered by prolonged exercise, fasting and fever.

Here we describe two unrelated sympthomatic patients heterozygous for CPT II mutation.

The first case is a 29 years old man arrived to our attention for hyperCKemia (13.000 UI/L) and myalgia exercise-related. His electromyography showed myopathic pattern and muscle biopsy showed mild myopathic signs. Genetic test revealed the common c.338C > T heterozygous mutation (p.S113L) in CPT II gene.

The second case is a 45 years old man complaining of recurrent episodes of muscle pain and myoglobinuria exercised related, associated with hyperckemia. His family history revealed similar sympthoms in father and brother and his brother was homozygous for c.338C > T mutation (p.S113L) in CPT II *gene*. The genetic test of our patient proved the common c.338C > T heterozygous mutation (p.S113L) in CPT II *gene*.

In literature, few cases of heterozygous mutation in CPT II *gene* clinically manifested are reported. Although CPT II deficiency is considered as an autosomal recessive disorder, both cases indicate that heterozygotes with only one mutant allele might also show the typical phenotype. We can hypotize that other factors such as genetic background, additional epigenetic, environmental factors or other mutations not detectable by sequencing (like intronic regions or large detection) may play a role.

Biomarkers in ALS: combining microRNA expression and phenotype at onset

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The identification of circulating biomarkers is needed to facilitate amyotrophic lateral sclerosis (ALS) diagnosis and prognosis, and to offer indicators of therapeutic response in clinical trials. We aimed to investigate the levels of muscle-specific microRNA in serum of ALS patients subdivided according to bulbar or spinal onset.

In 14 ALS patients (10 spinal, 4 bulbar) we measured the serum levels of muscle-specific miR-206, miR-1, miR-133a/b, miR-27a, and the expression of myostatin and follistatin, which are negative regulators of muscle growth. Morphometric analysis of muscle fiber size was used to correlate muscle atrophy with biochemical-molecular parameters.

In ALS patients the expression of miR-206 and miR-133 were significantly increased and that of miR-27a was significantly reduced as compared to controls, and also between spinal versus bulbar ALS. Myostatin/follistatin ratio was significantly higher in ALS than in controls and in bulbar versus spinal ALS. Bulbar ALS patients present higher degree of muscle atrophy than spinal ALS, as documented by our muscle fiber morphometric analysis.

Muscle mass regulators are particularly down-expressed in bulbar ALS, suggesting a more rapid and diffuse atrophic process. These biomarkers may be considered as useful biochemical and molecular indicators involved both in neuromuscular junction maintenance and reinnervation process.

Identification of elusive mutations in the DMD gene by mRNA analyses and MotorPlex

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The genetic testing for dystrophinopathies may be time-consuming and often inconclusive, when typical mutations are excluded. Mutations that remain invisible after DNA analyses are conceivable. Other negative cases, mostly BMD, may require a differential diagnosis with LGMD forms. On the other side, patients classified as LGMD may carry DMD gene mutations.

We re-evaluated a group of undiagnosed dystrophin-deficient patients in which no deletions/duplications or point mutations have been found. In six cases, for which a muscle biopsy was available, the mRNA study was able to direct the analysis to the correct solution: 1) r.10798-9_10798-1 ins due to an insertion in intron 75; 2) r.1332_1359 del due to a missense variation in exon 12; 3) r.2949+889_2949+1147ins, due to a deep intronic (+964) insertion in intron 22; 4) r.1812+480_1812+600ins, not yet explained; 5) r.9283_9305 del, due to a cryptic splice site activation; 6) r.1331_1332ins1332-8_1332-1, 1332_1482del, where two transcripts are present: one with an insertion of 9 bases of intron 11 and the other with deletion of exon 12, due to a cryptic splice site at -9 in intron 11.

On the other side, we applied the targeted next generation approach (MotorPlex) to study over 700 undiagnosed patients with LGMD or other myopathies and identified seven cases with a stop codon in the DMD gene.

The detection of these elusive mutations indicates that mRNA analyses should be performed in addition to a wide NGS approach for the genetic diagnosis of dystrophinopathies and LGMDs.

A novel SYNE1 gene mutation in a family with dominant muscular dystrophy

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SYNE1 gene mutations may cause Emery Dreifuss Muscular Dystrophy (EDMD) type 4 and Spino-Cerebellar-Ataxia type-8. We report a family with three subjects affected by dominant muscular dystrophy with joint contractures and no cardiac abnormalities. The mother was suffering from progressive muscle weakness since 6-7 years of age, with associated foot and elbow joint contractures. Still ambulant, she died at age 44 because of melanoma. Her elder son, a 28-year-old man, showed mainly proximal weakness since early childhood; when 7, he presented foot elbow and knee progressive contractures. His cardiac investigation was negative. The younger son, a 26-year-old man, had a similar clinical phenotype. Their muscle biopsies showed dystrophic changes with normal nuclear morphology. Targeted Next Generation Sequencing identified a novel heterozygous mutation in *SYNE1* gene (c.323C > T, p.N108S). A causative genetic role for the identified mutation was suggested by a series of concordant clues, as its predicted deleterious effect, its crucially functional localization and its absence in a large series of controls. This mutation apparently determines an "EDMD-like" variant clinical phenotype, characterized by progressive muscular dystrophy, joint contractures but no heart involvement.

Tele-assistance in pediatric neuromuscular disorders requiring home mechanical ventilation; preliminary results of a multicentric study

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We present the preliminary results of a two-years tele-assistance (TA) longitudinal observational multicentric study in ventilated pediatric patients with different neuromuscular disorders (NMD) in term of feasibility, patient/caregiver's compliance and satisfaction, associated costs, hospitalization rate.

TA programme includes once a week scheduled nurse/physiotherapist phone call, telemetric monitoring of oxygen saturation, heart rate and breathing patterns and, in symptomatic cases, non-scheduled calls by the physician. At baseline, an *ad hoc* clinical score has been administered to each patient (Vitacca et al. Eur Respir J 2009). Score variations > 3 points from baseline were considered exacerbations. Disease severity has been defined according to daily hours of ventilation in low (< 12 h), moderate (12-20 h), high (> 20 h). Hospitalizations and emergency room admission rate were compared with those of an age-disease-severity-matched control population. Patient's satisfaction has been assessed monthly by a three-items questionnaire.

Fourty-one patients were enrolled (mean age 16.2 ± 8.5 years). Thirty-six were on mask ventilation while 5 had tracheostomy. Compliance and satisfaction to TA was excellent in all caregivers and patients, except for two children complaining respiratory discomfort due to respiratory flows detector, Total number of exacerbations were 23 (7 in low-, 10 in moderate-, 6 in high-severity group), while ward admission were 4 but none in emergency room. In control group total exacerbations were 27, hospitalizations 14, four of which in emergency room.

Our TA programme for NMD pediatric patients requiring HMV shows promising results in terms of home management and reduction of hospital admissions. However, further data are being collected throughout the second year.

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Efficacy of air stacking techniques in stabilizing vital capacity in neuromuscular disorders

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Impairment of vital capacity (VC) in neuromuscular disorders (NMD) leads to poor cough peak flow (PCF) and to impaired airways clearance (Bach JR et al). Lung expansion to optimize lung recoil pressure and increase PCF can be achieved by air stacking (AS) techniques.

This two-years longitudinal study describes PCF and forced vital capacity (FVC) variations in 30 non-ambulant NMD (20 DMD, 10 SMA II) subjects and role of AS in halting FVC and PCF drop. The cohort was divided in a group receiving AS (n = 11) and a control group (n = 19). AS was achieved by delivering for three times five consecutive air volumes with a manual resuscitator, 3 times/day. PCF was detected by a peak flow-meter and FVC as percentage of predicted value. Measures were collected at baseline and after 2 years (t0-t2). Non-parametric analysis was used to compare t0-t2 values and the two sub-groups.

Median age was 14.5 years (11.7-17.3). The two sub-groups were homogeneous at baseline for age (15.7 vs 14.4, p = 0.98), for FVC (p = 0.85) and for PCF (p = 0.74). In the whole cohort after 2 years PCF did not significantly differ (p = 0.14), while FVC significantly decreased (p = 0.003). However, in AS group FVC was less reduced (47 to 35, p = 0.34) than in the control group (50 to 34, p = 0.019).

Our work demonstrates that regular AS respiratory physiotherapy is effective in preserving lung vital capacity expressed as FVC. PCF seems less sensitive in measuring progression of respiratory involvement; further studies are needed in a larger series of patients.

Deflazacort treatment and *SPP1* rs8357094 genotype impact OPN protein level in primary muscle cells of Duchenne muscular dystrophy patients

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Osteopontin (OPN), the protein product of the *SPP1* gene, plays a role in Duchenne muscular dystrophy (DMD) muscle pathology as a modulator of muscle inflammation and regeneration. A polymorphism in *SPP1* promoter region (rs28357094)

has been recognized as a genetic modifier of disease severity and progression. The effect of the glucocorticoid drug deflazacort (DFZ) on OPN mRNA and protein expression was investigated in DMD proliferating primary human myoblasts and differentiated myotubes with defined rs28357094 genotype (TT versus TG).

OPN protein was detected as two 55/50 kDa bands confirmed by gene silencing. Our results show that both healthy and DMD myoblasts and myotubes express high levelof OPN which is not up-regulated in DMD cells as it happens in DMD muscles. A shift towards the 50 kDa protein band was observed in the transition from myoblast to myotube and to mature muscle. Increase in OPN protein was observed in DMD myotubes carrying the TT compared to the TG genotype at rs28357094. No significant difference in OPN mRNA or protein was detected in DFZ-treated DMD myoblasts or myotubes compared to control cells, however when DMD cells were stratified according to rs28357094 genotype, DFZ treatment resulted in a significant increase in OPN protein in both myoblasts and myotubes carrying the TG genotype. These results show a strong effect of the rs28357094 polymorphism on DFZ-mediated OPN protein expression underlining the relevance of the complex organization of the *SPP1* promoter region in predicting response to steroid treatment in DMD.

ABSTRACTS OF STUDY GROUPS

(in alphabetical order of the first Author)

Preclinical evaluation of pharmacogenetics and new therapeutic options in nondystrophic myotonias toward personalized medicine

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The study aims at identifying new therapeutic options for patients suffering from nondystrophic myotonias (NDM). More than 500 patients have been diagnosed with NDM in Italy. NDM are due to loss-of-function mutations in the ClC-1 chloride channel or gain-of-function mutations in the Nav1.4 sodium channel, leading to sarcolemma over-excitability. NDM can be chronically debilitating due to pain and muscle stiffness. Pharmacotherapy stands on the orphan drug mexiletine, which blocks sodium channels. Yet some patients do not benefit from mexiletine, due to contraindications, side effects, or suboptimal response. Alternative therapies are thus required to improve patients QoL.

We previously showed, from bench to bedside, that a specific Nav1.4 mutation (p.G1306E) impairs mexiletine block but responds well to flecainide, another sodium channel blocker; and that G1306E carriers obtain great improvement by shifting from mexiletine to flecainide (Desaphy et al, Neurology 2001; J. Physiol. 2004; Eur. J. Clin. Pharmacol. 2013). This encouraging result opens the way to a personalized medicine.

We are now characterizing, functionally and pharmacologically, a number of myotonic Nav1.4 channel mutants using patch-clamp technique. The examined sodium channel mutations and drug candidates, including mexiletine and flecainide, are chosen in close collaboration with Italian neurologists and the Italian Parents Association (MiA), on the basis of clinical experience, genotype/phenotype relationship, and biophysical defect of the mutation.

The anticipated output include the definition of a pharmacogenetics strategy and the discovery of new antimyotonic drugs to better address therapy in NDM patients. Our recent results allowed the successful shift to flecainide therapy in a severely-affected patient carrying a novel Nav1.4 mutation (Desaphy et al. Neurology 2016). The solid preclinical data obtained in this study should allow future clinical application, including pilot studies and possibly randomized clinical trials.

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Role of IgG antibodies to rh-GAA in modulating ERT therapeutic response in patients with LOPD: the IGERT study

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Factors contributing to a variable ERT (enzyme replacement therapy) response in patients affected with late-onset Pompe Disease (LOPD) are numerous and not entirely clarified: timeliness of diagnosis and, consequently, of starting therapy, severity of muscle damage, availability of mannose-6-phosphate receptors, presence and activity of neutralizing antibodies to recombinant enzyme.

The majority of patients treated with alglucosidase alfa develops anti rh-GAA IgG usually within the first three months of drug exposure, especially in childhood.

In patients with LOPD, the antibody titer varies greatly, usually being below 10,000, and its role in modulating ERT therapeutic response is still not defined.

The LOTS study found no clear correlation between antibody titers and clinical outcomes. The average time to conversion in the cohort of 59 patients which were evaluated post-ERT was approximately of 4 weeks. Six of sixty LOPD patients studied by Patel et al. developed a high antibody titer (≥1: 51,200) in two or more occasions after 6 months of treatment. In three of these patients, the development of antibodies correlated with a clinical decline as showed by respiratory and motor function testing.

These results suggest the need for systematic studies in order to accurately define the potential impact of anti rh-GAA antibodies in ERT therapeutic response in LOPD patients.

We propose a multicenter prospective study in a cohort of LOPD italian patients in order to evaluate the significance of the antibody conversion and the changes in antibody levels by systematically comparing antibody titers and clinical functional scale scores (primary outcome).

Secondary outcomes are 1) to assess whether the antibody titers/functional scale scores relation can be related to specific clinical and demographic variables (i.e. time from first dose of the drug, disease duration, age of patients, mutations, MRC, FVC, MIP, other medications, etc) and 2) to define an estimated model of the coefficients in order to obtain an equation predicting the functional scale scores based on the antibody titers and on the clinical-demographic characteristics taken into consideration.

Application of electrotherapy to improve motor function in subjects with type II/III spinal muscular atrophy

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Background. Electrical stimulation (ES) is widely used in physical therapy to preserve/increase muscle mass and improve

motor function in several neurologic conditions (e.g. stroke, spinal cord injury, myotonic dystrophy type I). Still, for Spinal Muscular Atrophy (SMA), ES effectiveness has not been adequately explored. To date, only one study was conducted by applying ES to SMA patients (Fehlings et al. 2002). In the study, ES was administered to 9 children (age > 5 years) at low intensity, i.e. able to reach the sensory threshold without inducing muscle contractions. The authors concluded that there was no evidence for ES to improve strength in patients with SMA.

Nevertheless, the critical limitation of the aforementioned study is represented by the low intensity of stimulation. Indeed, the only way of improving muscle strength through ES is to apply a stimulation intensity capable of evoking visible muscle contractions and therefore generating a proper mechanical stress which represents the essential prerequisite to induce new myofibril synthesis (Gobbo et al. 2011).

In this view, we conducted an exploratory study aimed at determining whether ES was feasible for subjects with SMA type II/III. The results are promising. This preliminary experience suggested the feasibility of using ES as a home-based therapeutic intervention for SMA indicating, for the first time, great potential for electrotherapy to strengthen skeletal muscles and improve motor abilities of subjects with SMA III.

Aim of the collaborative study is to test ES effectiveness on a wider sample of subjects with SMA type II/III.

Methods. The program will be conducted in two phases:

– Phase 1 (eligibility assessment): the operators will first assess the possibility to elicit visible muscle contractions through ES, define the target muscles according to each subject's condition, and evaluate the tolerance to the recommended stimulation parameters.
– Phase 2 (treatment): eligible subjects will undergo a home-based ES treatment for muscle strengthening (30 min/day; 5 days/week; 12-18 weeks). The stimulation protocol will be personalized and the stimulator programmed accordingly. Tele-assistance will be constantly provided. Participants or care givers will be instructed for proper electrode placement and stimulation amplitude settings (following recommendations by Gobbo et al. 2014).

If possible, also functional electrical stimulation (FES) will be applied in order to provide a systemic conditioning and enhance the fitness status.

Expected outcomes. We expect ES to strengthen the stimulated skeletal muscles and to improve physical performance and independence.

Since the most relevant problems in SMA patients are represented by muscle weakness and immobilization, which in turn lead to bone demineralization and restricted physical fitness, ES may represent a useful tool to hinder these conditions. Evoked muscle contractions can provide mechanical external loads on the bones in order to compensate for immobilization and reduce fracture risk. Also, by preserving muscle trophism, ES may delay motor function decline and support physical activities.

Myofibrillar myopathies: clinical, histological and molecular data in the Italian population

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Myofibrillar myopathies (MFM) are rare neuromuscular diseases with similar morphological features, but clinically and genetically heterogeneous. So far we have about 30 patients characterized at molecular level, with mutations in the genes *DES*, *MYOT*, *FLNC*, *CRYAB*, *DNAJB6, TTN*.

We propose to collect MFM patients from the Italian centres willing to participate, in order to characterize the natural history of the disease(s), the histological features, the neuroimaging data, and to correlate them to the genetic defects. For molecular characterization of the genetic defect we propose a preliminary screening by NGS using a panel of genes, including some genes that cause distal, autophagic, or protein aggregate myopathies, followed by whole exome/genome sequencing in unsolved cases.

Clinical, histopathological and molecular characterization of MFM is important in order to provide a diagnostic guide for this expanding and often under-recognized group of myopathies.

The Italian LGMD Registry: epidemiology, differential diagnosis and natural history

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Limb girdle muscular dystrophies (LGMDs) are genetic disorders characterized by progressive muscular impairment, predominantly involving proximal and limb girdle muscles, and by histological signs of degeneration and regeneration in muscles. Their molecular definition is often difficult due to their high molecular heterogeneity, their clinical overlap and the paucity of specific biomarkers. Furthermore a detailed description of their natural history and of outcome measure are still lacking.

However all these aspects are fundamental both for prognostic and therapeutic purposes.

The collaboration between 12 Italian Centers specialized in the diagnosis and treatment of neuromuscular diseases enabled the creation of an Italian LGMD registry. The registry included 370 molecularly defined patients and contained detailed molecular, histological and clinical data. Age of onset, cardiac and respiratory involvement as far as description of peculiar features were registered for each patient.

The data collected allowed to define the epidemiology of the different LGMDs, LGMD2A and 2B being the most frequent forms in Italy.

The age at disease onset, clinical progression, and cardiac and respiratory involvement can vary greatly between each LGMD subtype. The definition of these specific clinical features can guide the differential diagnosis.

Natural history is still lacking especially as far as motor function is concerned. The prospective collection of data about functional evaluations, such as six-minute-walking-test, Motor Function Measure and other scales, will be useful in order to better delineate natural history and efficacy of potential therapeutic trials.

The experience of the Nation-wide Italian Collaborative Network of Mitochondrial Diseases

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Mitochondrial disorders are among the most common genetic disorders. In contrast to the extraordinary progress in our understanding of the molecular bases, we are still extremely limited in our ability to treat these conditions. Small patient populations represent the major impediment to progress. The development of a web-based register of patients with mitochondrial disease is needed to better understand the phenotypes and the natural history of these diseases.

Eleven centers with expertise on mitochondrial medicine have been involved in this project. To date, we have collected 1300 patients, with both adulthood and childhood onset. The network has reached the following goals: 1. establishment of an Italian network with specific expertise; 2. creation of a validated web-based database (supported by Telethon GUP09004), harmonized with other European databases and networks; 3. characterization of a big cohort of cases.

Our database allows many phenotype-based and genotype-based studies. Two examples are given:

– Phenotype-based approach: exercise intolerance. More than 20% of patients complained of exercise intolerance. This symptom was more strongly associated with specific mutations (i.e. 3243A>G). CK levels were increased in ≈34% of the patients with exercise intolerance, not confirming the notion that CK are normal in mitochondrial patients. Moreover, all the other myopathic signs included in our database were associated with exercise intolerance. Ragged red fibers and, especially, COX-negative fibers were more frequent in the subjects with exercise intolerance, whereas lactate levels could not predict the presence of exercise intolerance.
– Genotype-base approach: the 8344A>G mutation in our database. Myoclonic epilepsy with ragged-red fibers (MERRF) is a rare mitochondrial syndrome, mostly caused by the 8344A>G mitochondrial DNA mutation. 42 patients carrying the 8344A>G mutation were identified. Myoclonus was present in one out of five patients, whereas myopathic signs and symptoms, generalized seizures, hearing loss, eyelid ptosis and multiple lipomatosis represented the most common clinical features. Our results showed higher clinical heterogeneity of the 8344A>G mutation than commonly thought. Moreover, MERRF could be better defined as a myoclonic ataxia rather than a myoclonic epilepsy.

Large, multicenter studies are strongly needed to better characterize the clinical picture and natural history of these diseases, in order to identify some countermeasures capable of benefit the patients suffering with these chronic, still incurable disorders.

The natural history of Becker Muscular Dystrophy. A National Collaborative Study.

Politano L. and the AIM Clinical Centers Representatives

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Becker muscular dystrophy (BMD) is part of a spectrum of muscular disorders caused by pathogenetic variants in DMD gene encoding for dystrophin protein; this ranges in severity from asymptomatic increased levels of CK, cramps and myoglobinuria to progressive muscle diseases classified as BMD, when skeletal muscle is primarily affected and as dystrophin associated dilated cardiomyopathy (DCM) when the heart is primarily affected.

The most common mutational events are represented by intragenic deletions, found in 60-80% of patients, while the frequency of duplications may range from 5 to 15%. The remaining cases are caused by a combination of small mutations, pure intronic deletions or exonic insertion of repetitive sequences. Patients with deletions in the rod-domain display a high clinical variability ranging from a classical BMD phenotype to asymptomatic patients with high concentration of creatine kinase as the only sign. Despite the milder skeletal muscle involvement, intractable heart failure from dilated cardiomyopathy is a common cause of morbidity and the most common cause of death; it may be the main clinical feature in patients affected by subclinical and mild BMD.

We intend to carry a national study on the natural history of BMD trying to correlate the phenotype to the genotype. The results will be useful for both BMD patients to select those to be treated early and more aggressively, and DMD patients in order to establish which type of mutation better respond to gene therapy (exon skipping).

Ongoing projects of the Italian Network for Laminopathies

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The mission of the Italian Network for Laminopathies is to share knowledge on lamin-linked diseases and to spread this knowledge among clinicians, patients and researchers. In seven years of continuous activity, partners from sixteen Italian regions joined the Network and three patient associations and a company have been also involved. This allowed us to design and complete three major research and clinical projects. The definition of the cytokine profile of laminopathic patients, a project coordinated by Giovanna Lattanzi and Pia Bernasconi, and the analysis and description of phenotypes associated with muscular laminopathies (Emery-Dreifuss muscular dystrophy and Limb-Girdle muscular dystrophy type 1B), a project coordinated by Lorenzo Maggi, are the major achievements of the Italian Network for Laminopathies. Ongoing projects include the analysis and characterization of the cardiac pathology linked to mutations in lamins, coordinated by Sara Benedetti and the setting up of a registry of laminopathic patients to be shared with the French Network for Laminopathies and Nuclear envelope diseases. Finally, dissemination and networking activity, in the past years accomplished through the organization of national and international meetings on laminopathies, will include the organization of an International Meeting on Laminopathies in Bologna on April 6-8 2017. The interdisciplinary approach, which is exploited through collaboration and exchange of knowledge among researchers and clinicians of far different expertise represents a unique feature of the Italian Network for Laminopathies.

Building a Nation-wide Italian collaborative network for muscle glycogenoses (MGSD): registry and natural history

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Messina, Torino, Venezia, Firenze, Genova, Milano, Pisa, Verona, Conegliano, Brescia, Roma, Udine, Napoli, Catania, Padova, Pavia, Chieti

Muscle glycogenoses' or " muscle glycogen storage diseases (MGDS) indicate a group of diseases due to defects of glycogen metabolism with impaired energy production and dysfunction of different tissues, mainly skeletal muscle and heart. In the past 15 years there has been an increasing interest in MGSD. In contrast to the progress of our understanding about biochemical and molecular bases of muscle glycogenoses, we still feel an extreme limitation in our ability to manage and treat those conditions. For rare diseases, small patient populations represent the major obstacle to progress in research and care. This limit could be overcome by a specific patient register. Our principal goal is to develop a web–based register of patients with MGSD to better understand their phenotypes and natural history of diseases. This goal could be more specifically reached by:

1. establishing an Italian network of clinical Centers where neurologists and neuropaediatricians with a wide expertise in MG are present;
2. by setting up a web–based database, which will be harmonized with other registries for rare diseases and by interfacing them with European Database (i.e. Treat–NMD);
3. by planning longitudinal studies.

The partners of the project will actively work on both recruitment and phenotyping of the MGSD patients, with special focusing on the natural history of these pathologies.

In agreement to the Telethon policy, this research project is aimed to create a clinical and laboratory database for MGSD, building up a nation–wide network of clinicians and researchers with recognized expertise in MGSD of both children and adults. We believe that these fundamental steps are necessary to finally contribute to improve the management and treatment of these disorders. This will allow the direct translation of clinical research results in patient care.

Italian Clinical Network for FSHD. Phenotypic and molecular characterization of FSHD families: a systematic approach towards trial readiness

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Based on the 7-year experience of the Italian Clinical Network for FSHD, we defined the new Comprehensive Clinical Evaluation Form (CCEF), a standardized clinical protocol for patients and relatives recruited in the Registry with validated inter-rater reliability that allows the classification of patients in homogeneous groups according to their clinical features. The classification of phenotypes is mandatory to demarcate similar clinical conditions, offer proper counseling and prevention, correctly design experimental trials, select homogenous clinical group of patients and define outcome measures. Results of clinical trials could be affected by a biased selection of patients.

The need to collect patient data in a harmonized manner, also across multiple countries, has become increasingly evident in neuromuscular research, where locating patients suitable for a particular trial or therapy poses a particular challenge. Moreover, the extensive use over the last years of next generation DNA sequencing analysis has revealed that the spectrum of clinical features associated with a specific gene as well as the number of gene variants associated with similar phenotypes has grown. The overlaps with other disease-gene phenotypes challenges the definition of a precise molecular signature and entagling true associations. It is now critical to harmonize clinical approaches in inherited muscle diseases to create a solid phenomic framework supporting the interpretation of molecular data, to facilitate their translation into clinical practice.

Sport activity in muscle diseases

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Individuals with a severe genetic neuromuscular disorder are commonly believed not to be able to perform sports. Physicians very rarely suggest patients to perform sport activity and, when they do, they recommend to avoid supramaximal exercise. Although such a prejudice, endurance training resulted to be a safe method to increase exercise performance and daily function in Becker muscular dystrophy. Moreover, the feasibility and tolerance of progressive resistive exercise, without any evident decline in muscle strength or motor function, have been reported in spinal muscular atrophy (SMA), even with limited improvements.

We report here a wheelchair-bound woman with Charcot-Marie-Tooth (CMT) type 4A who became a Paralympic champion swimmer. When we compared evaluations before initiating sport activity with those after five years of competitive swimming activity, we found a slight improvement of physical performance but a greater improvement of mental functions chacterized by reduced trait anxiety and depression, enhanced self-esteem and increase of her overall quality of life. In addition, two patients with SMA type II and III are wheelchair hockey players, active in the Italian National Championships, and with improved quality of life.

We propose to perform a survey study in order to investigate, through ad hoc self-administered questionnaires, the patient and family/caregiver perspective on sport and perceived benefit from it. Results from these questionnaires will be analysed in order to acquire information to support provision of evidence-based advice to patients and families. Furthermore, longitudinal studies on effect of chronic training on metabolic demand, heart rate variability and biomarkers will be planned.

AUTHOR INDEX

FORTHCOMING MEETINGS

2016

May 26-28

8th European Conference on Rare Diseases & Orphan Products. Edinburgh, UK. Information: www.eurordis.com

June 8-11

16th Congress of the Italian Association of Myology. Lecce, Italy. Information: website: www.aim2016.it

July 5-9

14th International Congress on Neuromuscular Diseases. Toronto, Canada. Information: website: http://icnmd2016.org/

August 1-3

9th World Cardiology Congress. Manchester, UK. Information: http://worldcardiology.conferenceseries.com

September 2-6

45th European Muscle Conference. Montpellier, France. Information: website: www.emc2016-montpellier.com

September 4-9

International Congress of Human Genetics 2016. Yokohama, Japan. Information: website: www.esgh.org

September 12-13

2nd International Conference & Exhibition on Tissue preservation and Bio-banking. Philadelphia, USA. Information: website: http://biobanking.conferenceseries.com/

September 12-14

6th International Conference on Genomics & Pharmacogenomics. Berlin, Germany. Information: website: http://genomics.conferenceseries.com/

September 26-28

2nd International Conference and Exhibition on Molecular Medicine and Diagnostics. Orlando, USA Information: website: http://molecularmedicine.conferenceseries.com/

October 4-8

21st Congress of World Muscle Society. Granada, Spain. Information: website: www.worldmusclesociety.org

October 20-24

ASHG Annual Meeting. Vancouver,Canada. Information: website: www.ashg.org

October 17-21

ASHG Annual Meeting. Orlando, Florida, USA. Information: website: www.ashg.org

October 31- November 2

World Congress on Human Genetics. Valencia, Spain. Information: website: http://humangenetics.conferenceseries.com/

2017

April 6-8

International Meeting on Laminopathies - 2° French-Italian Meeting on Laminopathies. Bologna Italy Information: E-mail: elisaschena83@gmail.com

May 9-12

ISBER 2017 ANNUAL MEETING & EXHIBITS. Toronto, Canada. Information: website: http://www.isber.org/

May 27-30

European Society of Human Genetics: ESHG Conferences. Copenhagen, Denmark. Information: website: https://www.eshg.org/94.0.html

To be announced

22nd Congress of World Muscle Society. St. Malo, France. Information: website: www.worldmusclesociety.org

2018

October 16-20

ASHG Annual Meeting. San Diego, CA,USA Information: website: www.ashg.org

To be announced

23rd Congress of World Muscle Society. Mendoza, Argentina. Information: website: www.worldmusclesociety.org

2019

October 22-26

ASHG Annual Meeting. Toronto, Canada. Information: website: www.ashg.org

To be announced

24th Congress of World Muscle Society. Copenhagen, Denmark. Information: website: www.worldmusclesociety.org

2020

October 27-31

ASHG Annual Meeting. San Diego, CA,USA .*Information*: website: www.ashg.org

To be announced

24th Congress of World Muscle Society. Toronto, Canada. Information: website: www.worldmusclesociety.org

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Books and other monographs: Dubowitz V. Muscle disorders in childhood. London: WB Saunders Company Ltd; 1978.
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