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PROCEEDINGS OF THE XV CONGRESS OF THE ITALIAN SOCIETY OF MYOLOGY

Naples, Italy

May 20-23, 2015


Associazione Italiana di Miologia
15° Congresso Nazionale AIM
Naples, 20 - 23 May 2015
Hotel Royal Continental 4****
Azienda Ospedaliera Universitaria
UILDM
Seconda Università degli Studi di Napoli
SUN
Chairpersons:
Prof. Giovanni Nigro, President of the Congress
Prof. Luisa Politano
Prof. Vincenzo Nigro

XV CONGRESS OF THE ITALIAN SOCIETY OF MYOLOGY

Naples, Italy - May 20-23, 2015 - Program (Summary)

WEDNESDAY MAY 20, 2015

h. 17.00-20.00 Meeting with Patient's Associations: the role of families in caregiving and cure of patients with Neuromuscular Disorders

- C. Solimene: **Introduction**
- L. Magliano: Family burden in families of patients with Muscular Dystrophies
- C. Giacobini: Social and economic burden associated with a family member's disability condition
- G. Griffo: The CRPD approach for the caregiver and family activities to support persons with disabilities
- C. Bruno: Vaccines and Muscle Disorders
- M. Moggio: AIM-ASNP-Telethon Alliance
- *Pre-ordered interventions*
- **General discussion**
- G. Griffo - M. Moggio: **Conclusions**

THURSDAY MAY 21, 2015

h. 08.00-18.00 Registration of participants

- **Setting up of posters**

h. 08.30-09.00 Opening Ceremony

h. 9.00-10.30 Workshop 1. Spinal Muscular Atrophies

- A. Berardinelli: Clinical aspects of Spinal Muscular Atrophies
- F.D. Tiziano: Genetic aspects of Spinal Muscular Atrophies
- S. Corti: New therapeutic approach for Spinal Muscular Atrophies

h. 10.30-11.00 Coffee break

h. 11.00 -11.40 Lecture

- E. Tizzano: New insights in the pathogenesis and treatment of Spinal Muscular Atrophies

h. 11.40-13.00 Oral Communications

h. 13.00-14.30 Lunch

h. 14.30-15.30 Poster viewing and presentation

h. 15.30-16.45 Joint Workshop AIM-SIRN-SIMFER. Rehabilitative aspects in Muscular Dystrophies

- T. Mongini: Clinical Rehabilitative aspects in NeuroMuscular Disorders
- I. Riccio: Rehabilitative aspects in the early stages of Muscular Dystrophies
- G. Fiorentino: Ventilatory treatment strategies in Muscular Dystrophies: the Naples experience

h. 16.45-17.15 Coffee-break

h. 17.15- 18.30 Oral Communications

h. 18.30-20.30 AIM Members General Assembly - Election of the New Board - AIM Board Meeting

FRIDAY MAY 22, 2015

h. 08.30-09.45 Workshop 3. Laminopathies: Clinical and molecular update

- A. D'Amico: Early onset Laminopathies
- N. Carboni: Laminopathies with atypical phenotypes
- P. Bernasconi: Role of cytokines in the pathogenesis of laminopathies

h. 09.45 -10.15 Lecture

- E. Pegoraro: New emerging phenotypes in LGMDs

h. 10.15-10.45 Coffee break

h.10.45-12.00 Workshop 4. Autosomal Dominant and Recessive LGMD New phenotypes

- C. Angelini: Insights and phenotype of LGMD1F
- M. Mora: TAMs: Clinical, histological and genetic features
- L. Politano: Fatal early onset dilated cardiomyopathy caused by mutations in FKTN gene

h. 12.00-13.00 Oral Communications

h. 13.00-14.15 Lunch

h. 14.15-15.15 Poster viewing and presentation

h. 15.15-16.15 Future Projects and Programs

- Italian Network of Laminopathies-French Network of Laminopathies
- LGMD Euro-Net
- Italian Study Group on Pompe Disease

h. 16.15-16.45 Coffee-break

h. 16.45- 18.00 Workshop 5. Advances in the treatment of lysosomal disorders

- G. Parenti: Pharmacological chaperone therapy for lysosomal storage diseases
- M. Moggio: Effects of ERT on muscle tissue of patients with Pompe Disease
- A. Toscano: ERT in adult onset Glycogenosis

h. 18.00-19.15 Oral Communication

h. 20.30- 23.00 Social Dinner

SATURDAY MAY 23, 2015

h. 08.30-09.30 Muscle Club

h. 09.30 -10.45 Workshop 6. Advances in the treatment of Muscular Dystrophies

- E. Bertini: Ataluren in DMD. Results and perspectives
- G.P. Comi: Antisense therapy for DMD: the lesson from exon skipping approach

h. 10.45-11.15 Coffee break

h. 11.15-12.30 Workshop 7. Neuromuscular Junction Diseases: an update

- C. Rodolico: Infantile myasthenic syndromes. Clinical and molecular characterization
- A. Evoli: Lambert-Eaton Myasthenic Syndrome (LEMS) management
- R. Mantegazza: European database for myasthenia gravis: a model for an international disease registry.

h. 12.30-13.30 Oral Communications

h. 13.30-14.00 Administration of the ECM questionnaire

h. 14.00 Congress Closure

ABSTRACTS OF INVITED LECTURES

Meeting with Patient's Associations: the role of families in caregiving and cure of patients with Neuromuscular Disorders

Burden, social network and professional support in the families of patients with muscular dystrophies: results from the GUP10002 study

L. Politano[1], M. Scutifero[1], A. Zaccaro[1], U. Balottin[2], A.L. Berardinelli.[2], M. Camia[2], M.C. Motta[2], G. Vita[3], S. Messina[3], M. Sframeli[3], G. Vita[3], M. Pane[5], M.E. Lombardo[4], R. Scalise[4], A. D'Amico[5], M. Catteruccia[5], G. Colia[5], C. Angelini[6], A. Gaiani[6], C. Semplicini, R. Battini[7], G. Astrea[7], M.G. D'Angelo[8], E. Brighina[8], F. Civati[8], M. Patalano[9], A. Sagliocchi[9], L. Magliano[9]

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This study described the complex experience of caregiving in families of patients with Muscular Dystrophy (MD) in Italy.

The study was carried out on 502 18-80 y key-relatives of 502 4-25 y patients with Duchenne, Becker, or Limb-Girdle MDs.

Most patients were male (96%), young (12.8 (5.6sd), and in school (86%). 39% of patients were in wheelchair. 84% of key-relatives were mothers, 88% had a partner, 56% were highly educated, and 53% employed. 73% of patients took drugs, 67% attended rehabilitation, and 14% had received a psycho-educational intervention. 66% of patients had social/welfare support, and 16% school support, 31 relatives received psycho-educational interventions, and 55 a social/welfare support, mainly (84%) by Family/Patients Associations.

In the previous two months, 59% of relatives had to neglect their hobbies for caregiving, 45% to awake during the night, and 45% had work/household difficulties. About 1 (/3 of relatives reported economic difficulties, while about three quarters reported feelings of loss, sadness/depression, and worries for the future. 88% of key-relatives acknowledged caregiving experience as a positive impact on their lives. 88% of the responders stated they could rely on friends in case of psychological stress; 92% felt their friends would help them and 97% to receive professional help in a crisis situation. Burden resulted higher among relatives: unemployed, singles; with lower support in emergencies; of patients not attending school, with higher disability and with DMD. Psychological benefits were more acknowledged by key-relatives with higher professional/social support.

These findings can be useful for the development of new strategies to sustain caregivers.

Social and economical burden associated with a family member disability condition

C. Giacobini

Not arrived

The CRPD approach for the caregiver and family activities to support persons with disabilities

G. Griffo

World Council member of Disabled Peoples International-DPI

The UN Convention on the rights of persons with disabilities (CRPD, 2006) move the attention to the respect of human rights of these persons. Defining the disability condition the interaction between individual characteristics and physical and social environment, CRPD has put in evidence the responsability of the states and the whole society to guarantee non discriminatory treatments and condition of equal opportunities into the access to goods, services and rights. This copernican revolution introduce a new forms of support that the caregivers and families should offer to these persons. Overcoming the medical model of disability, through appropriate training, it is necessary that the caregivers support empowerment of persons with disabilities, their capabilities, to process that beside the rehabilitation promote the habitation and the full participation to community life. New key words are human rights, inclusion, independent living, personal mobility. The slogan "nothing about us, without us" must become a guidelines for education and support to these persons.

Workshop 1
Spinal Muscular Atrophies

1.1 Spinal Muscular Atrophies (SMA): clinical features

A.L. Berardinelli
SC di NPI (Responsabile Prof U. Balottin), Centro di Riferimento Regionale per le Malattie Neuromuscolari in Età Evolutiva, Fondazione IRCCS Istituto Neurologico Nazionale C. Mondino, Pavia, Italy

The term spinal muscular atrophy (SMA) describes a diverse group of genetic disorders that all affect the spinal motor neuron. The different forms of SMA are associated with numerous gene mutations and significant phenotypic variability. The Autosomal Recessive proximal SMA or 5q-SMA, is the most common form of SMA, accounting in most series for up to 95% of cases.

Although very rare, non-5q SMA forms are clinically and genetically heterogeneous. This presentation will be focused on the main clinical features of 5q-SMA-well known to this audience- and will point out what is now acquired form a clinical point of view about some non 5q SMA, considering the most recent literature.

1.2 Genetic aspects of Spinal Muscular Atrophies

F.D. Tiziano
Istituto di Genetica Medica, Università Cattolica del "Sacro Cuore", Roma, Italy

Spinal muscular atrophies (SMA) are a heterogeneous group of neuromuscular conditions, generally classified on the basis of the muscular districts mainly involved. These conditions can be transmitted as recessive, both autosomal or X-linked, or autosomal dominant characters. The most common form of proximal SMA is due to mutations of the SMN1 gene, located in 5q13, independently of the phenotypic severity ranging from very severe to very mild forms. Aside from the classical form, other genetic defects may be responsible for SMA variants. Among these, IGHMBP2 mutations cause a distal form with diaphragmatic paralysis and respiratory distress (SMARD1). We have recently collaborated to the identification of the causative gene of a very rare condition, the SMA with myoclonic epilepsy, due to mutations in the ASAH1 gene, encoding for the acid ceramidase 1. The same gene is responsible for an allelic condition, the Farber lipogranulomatosis.

The genetics of distal SMAs is much more complex, since about 15 genes have been identified so far. Mutations in these genes are quite rare. Finally, spinal and bulbar SMA is a poly-glutamine disease, due to the amplification of a CAG triplet in the androgen receptor gene.

Among the conditions above, the pathogenic mechanisms of classical SMA and SMARD1 are the best characterized and will be discussed more in details.

1.3 Novel Therapeutic Approaches for Spinal Muscular Atrophies

S. Corti
"Dino Ferrari" Centre, Neuroscience Section, Department of Pathophysiology and Transplantation, (DEPT), University of Milan, Neurology Unit, IRCCS Foundation "Ca' Granda" Ospedale Maggiore Policlinico, Milan, Italy

Spinal muscular atrophy (SMA) is a motor neuron disease and the first known genetic cause of infant mortality. It is caused by mutations in the Survival Motor Neuron 1 (SMN1) gene, resulting in deficiency of SMN protein. No effective treatment is currently available. In recent years, the increasing understanding of SMA etiopathogenesis has brought to a historical turning point towards the development of new therapeutic approaches to this otherwise incurable disease. They include gene therapy, molecular therapy with antisense oligonucleotides (ASOs) and small molecules to promote exon 7 inclusion in the paralogous SMN2 gene. One of the most promising strategies under development is the use of ASO to redirect the splicing of SMN2, to increase the production of functional SMN protein. ASOs synthesized with Morpholino chemistry (MO) are particularly suitable for these applications due to their excellent safety and efficacy profile. We have already demonstrated that this strategy is able to rescue the phenotype in the SMA animal model. Phase II-III clinical trials, sponsored by ISIS using ASOs with phosphothiorate chemistry are on going in SMA patients. We have recently proposed to investigate a therapeutic approach to improve MO tissue uptake, delivery and its pharmacological profile by conjugation with cell-penetrating peptides. We aim to assess the feasibility of the conjugate to cross the blood brain barrier using non-invasive systemic injection and treat the disease in a symptomatic phase, expanding the therapeutic window. These results could be further validated in an in vitro SMA model using patient-specific induced pluripotent stem cells to provide a complementary strategy.

With regard to gene therapy, Adeno-Associated Virus serotype 9 (AAV9)–mediated SMN delivery has been shown to rescue the phenotype of SMA animal models and a Phase I clinical trial with this strategy is ongoing. Based on these successful data, we recently developed a new therapeutic approach for a subtype of SMA, the Spinal Muscular Atrophy with Respiratory Distress type 1 (SMARD1) using AAV9. We displayed rescue of the disease phenotype in the SMARD1 mouse model after a single systemic injection of the AAV9 encoding the wild-

type human IGHMBP2, the defective gene of the disease, supporting the translational potential of AAV-mediated gene therapies, opening the path for human clinical trials with this strategy also for SMARD1.

Lecture 1

New insights into the pathogenesis and treatment of spinal muscular atrophy

E. Tizzano
Head Clinical and Molecular Genetics and Rare Diseases Unit. Hospital Valle Hebron. Barcelona, Spain

Spinal muscular atrophy (SMA) is an autosomal recessive disorder that affects motor neurons (MNs). It is caused by mutations in the survival motor neuron gene 1 (SMN1). The SMN2 gene, which is the highly homologous SMN1 copy that is present in all patients, is unable to prevent the disease. The disease manifests itself according to the amount of protein that an individual may produce. This is directly related to the number of SMN2 copies that a patient may have. Most SMA patients have 2 to 3 copies from a possible range of 1 to 5 copies. The lack of both SMN1 and SMN2 genes has never been described indicating that the SMN protein is crucial for life although it is unknown why MNs are so sensitive to SMN depletion. The clinical characteristics of SMA vary widely. The disorder can appear soon after birth, or not until adulthood. Patients are clinically classified into three main subtypes. Type I, the severe form, affects infants before the age of six months and these children never sit unaided (usually type 0 SMA, the most severe congenital form, is included in this group). Type II is the intermediate form and it has an onset after six months; these children never walk unaided. Type III is the mild form and it affects patients after 18 months. Patients in this group are able to walk but they may later lose this ability (usually type IV SMA, the mildest form of the disease starting in the second or third decade of life is included in this group). This classification is useful to help doctors communicate with each other internationally to developing strategies for clinical trials.

Treatment for SMA is a major challenge because the clinical variations and complications between patients are extensive. To design suitable clinical trials we therefore need to take many factors into account. These include the type of SMA, the patient's age, the severity status of the disease, the type of therapeutic approach, the timing of the proposed intervention in relation to disease progression, the availability of reliable markers for prognostic and evolution of the disease and the relative homogeneity of the group under study.

The explanation for the neuromuscular phenotype in SMA is to assume that insufficient SMN protein causes motor neuron dysfunction and death, and that muscle atrophy is a consequence of denervation. However, the study of terminal peripheral nerves and neuromuscular junctions identify possible links between motor neurons and muscle in the pathogenesis of the disease. Moreover, other cell and tissue targets emerge that support the view of SMA of a disease beyond the motor neurons. In this context two main therapeutic strategies emerge as suitable in SMA. The first strategy directly addresses the genetic defect via SMN2 stimulation or via SMN1 replacement. Specific approaches include to increase the amount of complete SMN protein produced by the SMN2 gene by small molecules or antisense oligonucleotides and to deliver normal copies of SMN1 in MNs by gene transfer (gene therapy). The second strategy is an SMN-independent approach that aims to protect motor neurons from damage by neuroprotective agents or by cell therapy or to increase muscle strength and endurance by specific compounds. The pipeline of molecules under investigation is promising and several trials are ongoing in patients. In the meantime that these new strategies are proven to be effective, proactive measures regarding nutrition, physical therapy and respiratory care may alleviate clinical symptoms and improve quality of life of patients. All these results are eagerly awaited because is likely that therapy would be more effective if combinations of these approaches are used and if earlier SMA detection is implemented by neonatal screening.

Supported by SMA EUROPE and Fundación Mutua Madrileña

Workshop 2
Joint Workshop AIM-SIRN-SIMFER. Rehabilitative aspects in Muscular Dystrophies

2.1 Clinical rehabilitative aspects in Neuromuscular Disorders

T. Mongini
Center for Neuromuscular Diseases, Department of Neurosciences "Rita Levi Montalcini", University of Torino, Italy

Although the majority of neuromuscular disorders still lack a resolutive therapy, it is now widely recognized that appropriate and qualified 'rehabilitative' programs are able to modify significantly the natural history of severe progressive diseases, such as muscular dystrophies, congenital myopathies or metabolic myopathies. In this context, the term 'rehabilitation' acquires a wider significance, not only aiming to 'restore' a lost function but more importantly to prevent complications and to maintain a patient's quality of life.

Thanks to the many technological advances in the last decades, it is now possible to reduce the medical co-morbidity leading to a premature death, to prevent or limit the skeleton deformities and ameliorate maximize the patient's motor and psychosocial functions.

Among the most significant improvements, we must recall the non-invasive ventilation technology, the use of cough assist mechanical in-ex sufflator, the innovative cardiac devices, the advanced nutritional care, the advanced surgical techniques for scoliosis. Physical therapy plays a major role in the prevention of muscle retractions and joint deformities, and also in maintaining muscle strenght to prevent muscle deconditioning. Computer technology has also significantly improved the quality of adaptive devices such as wheelchairs and lifts, allowing independent mobility and greater social and vocational integration.

The comprehensive management of all 'rehabilitative' issues requires the organization of well-integrated multidisciplinary teams, with a strong relationship with territorial assistential setting.

2.2 Rehabilitative surgical aspects in early stages of Muscular Dystrophies

I. Riccio, G. Riccardi, G. Iolascon, F. Gimigliano, L. Politano, R. Gimigliano, V. Riccio
Dip. Multidisciplinare di Specialità Medico-Chirugiche ed Odontoiatriche; Cardiomiologia e Genetica Medica, Seconda Università di Napoli, Italy

Duchenne's muscular dystrophy is an X-linked disease with well defined evolutionary phases, characterized by degradation of the walking function, development of evolutive scoliosis and progressive decline of the respiratory function leading patients to premature death.

In 1985 Y. Rideau in France carried out a new global therapeutic strategy for treatment of lower limb deformities, scoliosis deformity and progressive restrictive syndrome.

The indication for surgery at the lower limbs is made very early, at the onset of the first signs of disease. The procedures are carried out at the same time and always bilaterally; they include: (i) hip section of superficial flexors; (ii) iliotibial band resection; (iii) subcutaneous tenotomy of semitendineous and gracilis; (iv) subcutaneous lengthening of Achilles tendons.

In the post-operative period, the patient begins exercises of active and passive mobility in few days and after three weeks recovers his performances; ambulation will remain almost normal for several years. A comparison of two groups of patients, the first precociously operated on the lower limbs, the other one not operated, shows better performances in the operated group.

Surgery rehabilitation associated with early treatment with steroids (DFZ) has shown in our patients an enhancement of the beneficial effects than the two treatments alone.

2.3 Mechanical ventilation in neuromuscular diseases

G. Fiorentino, A. Annunziata
Division of Respiratory Physiopathology and Rehabilitation; AORN "Dei Colli", Monaldi Hospital, Naples, Italy

The alteration common to neuromuscular patients is respiratory muscle weakness, which varies greatly according to the underlying disease. Weakness may affect inspiratory, expiratory muscles and muscles that innervate the upper airways.

The end result of inspiratory muscle deterioration is alveolar hypoventilation, with corresponding hypercapnia and hypoxemia; expiratory muscle impairment leads to an inefficient cough and retention of secretions; upper airway muscle impairment affects swallowing, leading to aspiration of saliva and food in the presence of an ineffective cough and to recurrent respiratory infections.

Neuromuscular patients may present apneas and hypopneas during sleep due to the combination of respiratory muscle weakness and upper airway obstruction. As a result, nocturnal hypoventilation leads to increased $PaCO_2$ and CO_2 retention. The mechanical ventilation (MV) is used to restore balance and support the work of breathing in these patients.

MV allows muscles to rest and recover, with a consequent improvement in inspiratory muscle function, ventilation and arterial blood gases during the day. Nocturnal MV prevents hypoventilation during sleep, resetting central response to CO_2 and improving ventilation and gas exchange during the day. MV has also been said to improve pulmonary function by recruiting atelectasic zones, increasing pulmonary distensibility and improving ventilation-perfusion ratios. The preponderance of evidence supports the use of MV. However the success of MV depends on selection of an appropriate interface, selection of an appropriate ventilator and ventilator settings, the skills of the clinician, the motivation of the patient, and not least, the support of the family.

Workshop 3
Laminopathies: clinical and molecular update

3.1 Early Onset laminopathies

A. D'Amico
Unit of Neuromuscular and Neurodegenerative Disorders, "Bambino Gesù" Children's Hospital, Rome, Italy

Laminopathies are an expanding group of hereditary diseases caused by mutations of genes that encode proteins

of the nuclear envelope. The most frequent neuromuscular forms are related to defects in LMNA gene and comprise a large spectrum of conditions ranging from severe congenital muscular dystrophies (L-CMD) to a limb-girdle muscular dystrophy with adult onset and much milder weakness (LGMD1B). L-CMD include different phenotypes that can be classified as: i) severe phenotype with generalized muscular weakness and contractures by birth; ii) 'dropped head' phenotype with prominent involvement of axial muscles that generally evolves to rigid spine phenotype; and iii) early Emery-Dreifuss phenotype. All these condition generally lead in the first 2 decades to cardiac disturbances, respiratory insufficiency, orthopedic complication and metabolic disorders. The clinical management requires a multidisciplinary and rigorous approach focused on early medical and rehabilitative interventions with the main aims to prevent 'fatal heart event', to cure co-morbidities (pulmonary insufficiency and spinal and joint contractures) and to improve the quality of life of these children

3.2 Atypical phenotypes in laminopathies.

N. Carboni
Division of Neurology, Hospital "San Francesco" of Nuoro, Nuoro, Italy

Laminopathies are an heterogeneous group of disorders caused by alterations on genes coding for proteins of the nuclear envelope. The genes most often mutated in these disorders are the LMNA and the STA gene. Mutations on the LMNA and STA genes are associated to well characterized phenotypes of the heart and skeletal muscles and, in the case of the LMNA gene, also to disorders affecting the peripheral nerves, the bone, the skin or causing premature ageing or metabolism disorders. Not rarely, have also been described atypical phenotypes due to mutations on the STA and LMNA gene. These phenotype do not fit the diseases classically related to STA or LMNA gene mutations; they may show an unusual, incomplete phenotype or even the concomitant presence in the same subjects of different clinical manifestations related to the same gene. We describe the clinical features, genetics and possible pathophysiology of the atypical cases in laminopathies.

3.3 Role of cytokines in the pathogenesis of laminopathies

P. Bernasconi[1], C. Evangelisti[2], P. Cavalcante[1], C. Cappelletti[1], N. Carboni[3], L. Politano[4], G. Boriani[5], G. Ricci[6], L. Vercelli[7], L. Ruggiero[8], A. Gambineri[9], A. D'Amico[10], C. Rodolico[11], L. Maggi[1], L. Morandi[1], R. Mantegazza[1], G. Lattanzi[2]
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Emerging evidences suggest that nuclear lamina defects dysregulate the NF-κB signalling pathway, causing an abnormal secretion of proinflammatory cytokines, which might contribute to the pathologic alterations observed in laminopathies. However, it is still unclear whether there is a different pattern of cytokine expression that could discriminate a laminopathy with skeletal and/or cardiac muscle involvement from muscular dystrophies (MD) due to other causes with or without cardiomyopathy. In collaboration with the Italian Network for Laminopathies, by Luminex technology we analysed the cytokine profiles of sera collected from 54 patients with genetically defined laminopathy with or without skeletal and/or cardiac muscle involvement, 11 MD patients, and 27 healthy individuals. Cardiopathic and non-cardiopathic patients' sera showed a cytokine profile differentially expressed compared to healthy controls' sera. A difference of cytokine expression was also observed in laminopathies with muscle and cardiac involvement versus laminopathies with only cardiomyopathy and versus MD. TGF-beta2 serum levels were higher in the LMNA patients than in healthy controls and MD, suggesting a direct link between LMNA mutations and dysregulation of TGF-beta2 pathway. To identify the signalling pathways triggered by TGF-beta2 in laminopathic cells, we used an experimental model expressing various LMNA mutants: wild-type lamin A was able to modulate TGF-beta2 expression, whereas pathogenetic LMNA mutants failed to negatively regulate TGF-beta2 secretion and caused increased cytokine levels. AKT1 and mTOR activity was up-regulated, an effect that could be inhibited by drugs able to modulate lamin A, mTOR activity or TGF-beta2. These data suggest to explore these drugs as potential therapeutic tools for laminopathies.

Lecture 2

New emerging phenotypes in LGMD

E. Pegoraro
Neuromuscular Center, Department of Neurosciences, University of Padova, Italy

The term Limb Girdle Muscular Dystrophy (LGMD) comprises muscular dystrophies with autosomal domi-

nant (AD) or autosomal recessive (AR) inheritance (as opposed to X-linked muscular dystrophies), predominantly proximal distribution of muscle weakness and wasting (as opposed to distal myopathies), and onset ranging from childhood to adulthood, after a substantially normal motor development (as opposed to congenital muscular dystrophies [CMDs] and congenital myopathies). The nosographic entity of LGMD has had a relevant historical role in the classification of muscle diseases, and remains a useful clinical concept in the diagnostic approach to patients presenting with muscular weakness.

In the molecular era, mapping of LGMD loci, identification of their protein products, and genotype-phenotype correlation studies have shown that LGMD shows extensive locus heterogeneity, i.e. there are multiple genetic loci responsible for this phenotype. A recent classification counts at least 8 AD and 23 AR loci. Moreover, most if not all of these loci show allelic heterogeneity, i.e. different mutations leading to different phenotypes within the LGMD spectrum, or beyond (e.g. CMD, myofibrillar myopathies, distal myopathies).

This complexity is taken to extremes by next generation sequencing approaches, which empower us for the discovery of new disease genes and unsuspected genotype-phenotype correlations, but at the same time pose the challenge of interpreting the pathogenetic significance of multiple identified genetic variants.

Workshop 4
Autosomal Dominant and Recessive LGMD new phenotypes

4.1 Insights and phenotypes of limb-girdle muscular dystrophy type 1F

C. Angelini[1], M. Fanin[2], G. Cenacchi[3], E. Pinzan[1], V. Pegoraro[1], E. Tasca[1], V. Nigro[4]
[1] Fondazione Ospedale "San Camillo" IRCCS, Venice, Italy; [2] University of Padua, Italy; [3] University of Bologna, Italy; [4] TIGEM, Naples, Italy

We report muscle histopathological, ultrastructural and radiological features of a large Italian-Spanish family with autosomal dominant LGMD, previously mapped to 7q32.2-32.2 (LGMD1F).

We collected the DNA, clinical history, muscle biopsies histopathology of one LGMD1F kindship. Biopsy of two affected patients mother and daughter was studied (in the daughter two consecutive biopsies at 9 and 28 years and in the mother at 48 years).

In LGMD1F patients the age of onset varied from 2 to 35 years, weakness occurred either in upper or in lower girdle; in 14 cases there was hypotropy both in proximal upper and lower extremities in calf muscles. Muscles MRI showed hyperintensity in proximal limb muscles. The daughter has a severe clinical course and the fiber athropy was more prominent in the second biopsy at 28 years. The mother has a relatively compromised histopathology and many small muscle fibers, and autophagic changes by acid-phosphates stain. Immunofluorescence against desmin, myotilin, p62 and LC3 showed accumulation of myofibrils, ubiquitin binding proteins aggregates and autophagosomes. Ultrastructural analysis revealed myofibrillar disarray, vacuolar changes, granular material and dense subsarcolemmal bodies deriving from cytoskeleton-myofibrillar proteins. We hypotize that the pathogenetic mechanism in LGMD1F might lead to disarrangement of desmin-associated cytoskeletal network.

Transportin-3 (TPNO3), which was found by NGS to be the causative gene in LGMD1F, is suggested to mediate the nuclear inport-export. The non-stop mutation identified in this family encodes for a longer protein which is expected to be unable to move to the nucleus. Clinical phenotype penetrance in this family correlates at 92% with mutation presence.

4.2 Tubular aggregate myopathies (TAMs): Clinical, histological and genetic features

M. Mora
Fondazione IRCCS Istituto Neurologico "C. Besta", Milano, Italy

Tubular aggregate (TA) myopathies are hereditary muscle disorders pathologically characterized by the presence of tubular aggregates. These are abnormal structures in the muscle fibers, consisting of regular arrays of tubules derived from the sarcoplasmic reticulum. Recently autosomal recessive and dominant mutations in stromal-interacting molecule 1 (STIM1) encoding a $Ca2^+$ sensor that controls $Ca2^+$-release-activated $Ca2^+$ (CRAC) channels, have been identified to cause tubular aggregate myopathy (TAM). Heterozygous missense mutations in the ORAI1 gene, encoding the CRAC channel itself, have also been found in families affected by dominantly inherited TAM with hypocalcemia. Dominant mutations in STIM1 are as well responsible for the York and Stormorken syndromes associated with myopathy, and recessive STIM1 and ORAI1 mutations cause immunodeficiency of variable severity. Common histological and ultrastructural features of TAMs are tubular aggregates positively stained with Gomori trichrome and NADH-TR, in both fiber types and often accompanied by increased endomysial connective tissue, central nuclei and fiber size variability. TAM clinical features are heterogeneous encompassing three major and distinct phenotypes: a first phenotype characterised

by slowly progressive weakness predominantly affecting proximal muscles, a second phenotype primarily involving myalgia with or without cramps, and a third phenotype referred to as limb-girdle myasthenia which associates a myopathic pattern with myasthenic features. Furthermore, occasional associations of TAs with other hereditary myopathies, such as myotonic myopathy, or acquired conditions such as alcoholic myopathy are known. Although the mechanism of formation of TAs has not been clarified, altered Ca^{2+} homeostasis related to a disordered sarcoplasmic reticulum is suggested to be a main contributing factor.

4.3 Fatal early onset dilated cardiomyopathy caused by mutations in FKTN gene

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Fukuyama congenital muscular dystrophy (FCMD) is frequent in Japan, due to a founder mutation of the fukutin gene (FKTN). Outside Japan, FKTN mutations have only been reported in a few patients with a wide spectrum of phenotypes ranging from Walker-Warburg syndrome to limb-girdle muscular dystrophy (LGMD2M). We report two new Caucasian related (brother-sister) patients, born from non-consanguineous parents, first observed in the 1970's, at the age of 7 and 5 years respectively, for the presence of raised serum CK, signs of mild muscular involvement and no mental retardation. A diagnosis of congenital muscular dystrophy was made. The course of the disease was mild in both siblings. A muscle biopsy, performed on the brother at the age of 17, revealed a pattern consistent with a diagnosis of limb-girdle muscular dystrophy. He developed at the age of 19 y 6 m a sudden severe dilated cardiomyopathy, and died in 1988, at the age of 20 from an intractable heart failure. He was still ambulant. The sister – now 45 years old – became chair-bound at the age of 31,7 years and until the age of 35 presented no heart involvement.

The analysis of the genes more frequently causing LGMD was negative. A compound mutation (c.139C>T and c.1304A>G) in FKTN gene has been recently identified by NGS. The first variant c.139C>T (Arg47X) is a nonsense mutation, inherited from the father, frequently associated with FCMD in Japan; the second variant, inherited from the mother, is novel. Why the same mutation can cause a different phenotypic presentation is not clear. However gender differences in AR-LGMDs (LGMD2A, 2B) are not rare.

Workshop 5
Advances in the treatment of lysosomal disorders

5.1 Pharmacological chaperone therapy for lysosomal storage diseases

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Lysosomal storage diseases (LSDs) are a group of inborn metabolic disorders caused by mutations in genes encoding proteins involved in different lysosomal functions, in most instances acidic hydrolases. Storage of different substrates in multiple organs and systems results in the variable association of visceral, ocular, hematologic, skeletal, and neurological manifestations that substantially impact patients' quality of life, health, life expectancy, and physical and intellectual performance, and are often responsible for major physical and neurological handicaps.

Different therapeutic approaches have been developed or are under pre-clinical evaluation to treat these disorders, including enzyme replacement therapy, hematopoietic stem cell transplantation, substrate reduction, gene therapy, and others. At present none of the therapeutic approaches that are already approved for clinical use have proved to be suitable to treat all LSDs, or all patients with a specific disorder.

Pharmacological chaperone therapy is an emerging approach based on small-molecule ligands that selectively bind and stabilize mutant enzymes, increase their cellular levels, and improve their lysosomal trafficking and activity. Compared to other approaches, pharmacological chaperone therapy shows advantages, particularly in terms of bioavailability of drugs, oral administration and positive impact on the quality of life of patients. After preclinical in vivo and in vitro studies pharmacological chaperone therapy is now being translated in the first clinical trials, either as monotherapy or in combination with enzyme replacement therapy for some of the most prevalent LSDs. For some LSDs the results of the first clinical trials appear encouraging and warrant further development. Future research in the field of pharmacological chaperone therapy will be directed towards the identification of novel chaperones, including new allosteric drugs, and the exploitation of synergies between chaperone treatment and other therapeutic approaches.

5.2 Effects of ERT on muscle tissue from patients with Pompe disease

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Pompe disease (OMIM 232300) is an autosomal recessive lysosomal storage disorder resulting from a deficiency in the glucosidase alpha acid (GAA) enzyme. The disease is characterized by progressive accumulation of lysosomal glycogen in various tissues, primarily heart and skeletal muscle, and it is clinically classified into three forms: infantile, juvenile, and late-onset.

The histopathological hallmarks in muscle tissue are fiber vacuolization and autophagy.

Recombinant human GAA is the only approved enzyme replacement therapy (ERT) available for disease treatment. It is effective in improving life expectancy and in preventing cardiomyopathy in infants, whereas the improvement is quite variable in adults.

Our project aimed at studying muscle biopsies from 18 late onset patients at molecular, biochemical, and histopathological level before and after ERT. All patients clinically improved or remained stable after therapy. Regarding the morphological aspect, we evaluated the following parameters: number of vacuolated fibers, percentage of vacuolization in type I and II fibers, degree of glycogen accumulation, CSA.

Pre-treatment muscle biopsies showed marked histopathological variability ranging from almost normal morphology to severe vacuolar myopathy. Post-treatment muscle biopsies morphologically improved in 11 patients, worsened in 3 patients and were unchanged in the remaining 4 subjects.

GAA enzymatic activity, tested by a fluorimetric assay in both lymphocytes and muscle tissue from all patients, was mildly increased in skeletal muscle after ERT compared with pre-treatment levels. Also, GAA expression assessed by immunoblotting slightly increased in a few patients.

In conclusion, this study shows positive effects of ERT in late onset patients with Pompe disease in terms of clinical, morphological and biochemical outcome.

5.3 ERT in adult onset Glycogenosis type II (Pompe disease)

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The adult form of type II Glycogenosis (Pompe disease) is a slowly progressive disease with prominent muscle symptoms caused by the deficiency of acid alpha-glucosidase (GAA). The clinical forms are quite variable ranging from a severe and rapidly progressive infantile form (IOPD) with muscle hypotonia and cardiac and respiratory involvement to the late onset form (LOPD), characterized by a milder and more heterogeneous phenotypes. Since 2006, Enzyme Replacement Therapy (ERT), with recombinant human alglucosidase alpha, is available and several studies, on treated patients, have been published, focusing on efficacy, safety, immune tolerance and adequate outcome measures.

ERT efficacy in IOPD has been demonstrated and a number of treated infants are still surviving ventilator-free, standing or walking unaided. Nowadays, some of the survivors have reached the age of 16 years.

As regards LOPD, ERT has demonstrated a milder effect compared to IOPD results. However, in 2012, a systematic literature review on 21 studies on ERT efficacy/safety, has illustrated that, at least two-thirds of patients, had an improved muscular and/or respiratory function. Some other studies have evidenced later that therapeutic responses seem to be more efficient during the first 2 years of treatment than thereafter, although, in some studies, the ERT effect was maintained up to 36 months. It has been suggested that LOPD responders have shown some favorable prognostic factors as female gender, younger age, better clinical status and early ERT start.

In conclusion, we should reconsider the recommendations for the therapy start, maybe also considering not only the clinical assessment but, even, laboratory data (i.e. MRI, enzyme activity, morphological findings) that may contribute to timely identify when to initiate the ERT treatment.

At the mean time, new drugs are under development and have shown promising results and new therapeutic options to improve the quality of life of patients.

Workshop 6
Advances in the treatment of Muscular Dystrophies

6.1 Ataluren in DMD: Results and Perspectives

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Approximately 13% of boys with Duchenne muscular dystrophy (DMD) have a nonsense mutation in the dystrophin gene, resulting in a premature stop codon in the corresponding mRNA and failure to generate a functional protein. Ataluren (PTC124) enables ribosomal readthrough of premature stop codons, leading to production of full-length, functional protein. Moreover Ataluren was shown to be

active in multiple cell-based and animal models. Phase 1 studies in healthy volunteers established the initial ataluren safety profile and defined dosing regimens for achieving target trough plasma concentrations known to be active in preclinical models. These results were followed by a phase 2a open-label in 38 DMD patients treated with different regimens with Ataluren and assessed by immunostaining in pre- and post-treatment muscle biopsy specimens as the primary endpoint, showing activity and safety and demonstrating increases in post-treatment dystrophin expression in a quantitative and qualitative analysis assessing the ratio of dystrophin/spectrin. A multicentre Phase 2b study was thus designed as a randomized, double-blind, placebo-controlled international study that evaluated the efficacy and safety of dose ranging study with 2 doses of Ataluren in 165 patients with nonsense mutation dystrophinopathy. Patients received either a high or low dose, or matching placebo, daily for 48 weeks. The study comprised a 6-week screening period and a 48-week blinded study treatment period.

This trial showed some efficacy in dystrophinopathy patients and used the 6-Minute Walk Test as the primary outcome measure.

6.2 Antisense therapy for DMD: the lesson from exon skipping approach

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Most mutations in the dystrophin (dys) gene are deletions that disrupt the open reading frame. The length and structural dys characteristics, with repetitive domains, suggest the possibility of excluding disruptive exons from mRNA during splicing, partially preserving protein function. Residual dystrophin levels of 29 to 57% have been hypothesized to ensure the preservation of muscle strength. Based on this evidence, clinical trials were designed to promote exon 51 skipping in DMD patients, expecting an improvement in their clinical phenotype to at least a BMD-like phenotype. The choice of exon 51 was based on the observation that in-frame deletions of this portion of the gene are generally associated with mild BMD phenotypes. 3'-exon 50 deletions account for at least 20% of DMD mutations. The Antisense OligoNucleotides (AON) used in clinical studies are 2'-O-methyl-modified ribose molecule with a full-length phosphorothioate backbone (2OMePS) or phosphorodiamidate morpholino oligomers (PMO). A phase III double blind trial with 180 DMD patients to assess drug efficacy and safety and collect pharmacokinetic, molecular, and clinical data has been concluded. Patients were subcutaneously treated with 6 mg/kg GSK2402968 versus placebo for 48 weeks, followed by a 2-year open label study. Difference in 6MWD (mean (CI) was 10.33m (-14.65, 35.31), p = 0.415) between drisapersen and placebo groups. Further analysis suggested that subgroup ≤ 7 years showed potentially clinically meaningful treatment difference of 21 meter. Extension data from those participating in the phase III DMD114044 study showed a 49 meter difference between those on continual treatment (n = 52) and those who had been on placebo for 48 weeks followed by active drug (n = 31). Skipping exon 44 could restore the reading frame in approximately 5% of patients and is currently being investigated.

The morpholino oligomer Eteplirsen was studied in an open-label phase II study that tested systemic administration with a dose-escalating model. Administration was safe and tolerable. Dys expression was proportional to the administered dose. Patients receiving high doses exhibited an increase in dys+ fibres.

Overall, exon skipping using AONs is a feasible approach. However, clinical trials demonstrated that the production of dystrophin, even if detectable in the muscle biopsy, could be inadequate for determining effective clinical improvement and varies widely among different muscles. Furthermore, neither PMO nor 2OMePS generate significant amounts of dystrophin in the heart. In general, these chemicals have poor cellular uptake and rapid renal clearance. Several strategies for improving the efficiency of this approach are currently being investigated, and various adjustments have been tested with promising results.

Workshop 7
Neuromuscular Junction Diseases: an update

7.1 Infantile myasthenic syndromes: clinical and molecular characterization

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Congenital myasthenic syndromes (CMS) are a heterogeneous group of inherited disorders in which the safety margin of neuromuscular transmission is compromised. CMS are classified on the basis of the located defect into presynaptic, synaptic basal laminin-associated and postsynaptic subgroup. Mutations involving the gene encoding cholyne acetyltransferase (CHAT) has been associated with presynaptic CMS, while synaptic basal-laminal-associated defects are due to mutations in the collagen tail subunit of the acetylcholinesterase (COLQ) or in laminin β2 subunit (LAMB2) genes. The postsynaptic subtypes of CMS are the most common form, they are due to mutations involving the acetylcholine receptor (AChR) subunit genes (CHRNA1, CHRNB1, CHRND, CHRNE), rapsyn (RAPSN), muscle specific tyrosine kinase (MUSK), Dok-7 (DOK7), skeletal muscle sodium

channel (SCN4A) genes. More recently several postsynaptic forms have been linked to congenital disorders of glycosylation with presence of tubular aggregates in skeletal muscle (CMSTA1, CMSTA2, CMSTA3).

Typically the clinical features vary according to the underlying molecular defect, but almost constant symptoms are ptosis, weakness, dysphagia, dysphonia. Onset of symptoms usually is at birth, in the first year of life and in infancy, rarely there are some patients who don't present any symptoms until childhood or adult life. Severity and course are highly variable, so may be difficult to differentiate CMS from other syndromes and today they are frequently misdiagnosed or undiagnosed. A correct clinical, neurophysiological and morphological setting is crucial for their molecular characterization and management, since CMS can be effectively treated.

7.2 Lambert-Eaton myasthenic syndrome (LEMS) management

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Lambert-Eaton myasthenic syndrome (LEMS) is a rare autoimmune disease of neuromuscular transmission due to antibodies to presynaptic P/Q-type voltage-gated calcium channels (VGCC). The autoimmune attack causes a decrease in the action potential-evoked neurotransmitter release, which underlies muscle weakness and autonomic dysfunction. In 50% of patients LEMS is a paraneoplastic disorder, generally associated with small-cell lung carcinoma (SCLC). Upon the diagnosis of LEMS, the search for a possible associated tumour is crucial; if the first screening is negative, tumour surveillance should be continued for at least two years. In patients with paraneoplastic disease, tumour treatment can significantly alleviate neurological symptoms.

Therapeutic options include symptomatic drugs and immunosuppressive therapies. Symptomatic treatment, currently based on 3,4 diaminopyridine (3,4-DAP), is the first approach in all LEMS patients. 3,4-DAP proved effective and well tolerated in clinical trials. Its therapeutic effect is basically achieved through voltage-gated potassium channel inhibition that prolongs nerve action potential. From in vitro and passive transfer studies, GV-58, a selective agonist of P/Q- and N-type VGCCs, appears to be a promising symptomatic agent, especially in combination with 3,4-DAP.

In patients whose symptoms are not adequately controlled on symptomatic treatment, long-term immunosuppression is considered. Oral steroids (prednisone/prednisolone) are used in all patients with disabling disease, often in association, mainly in non-cancer LEMS, with azathioprine or other immunosuppressants. Plasma-exchange and intravenous immunoglobulin induce significant albeit transitory improvement. Rituximab is a promising treatment in patients with refractory disease.

7.3 European database for myasthenia gravis: a model for an International disease registry

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MG subcategories, AChR-MG, MuSK-MG, Double Negative-MG, Ocular-MG, Generalized-MG, Thymoma-MG, make clinical studies challenging. MG Registries can facilitate these studies in several potential ways, including as a source of clinical, biological and immunological data on large numbers of patients, and as a source of patients for clinical trials.

Recently, the European-MG database (European MG Network, Grant EU-2005105, DG SANCO) has been developed, and the MGFA is developing a patient-driven registry, to be used in support of research, advocacy and public awareness.

We may present, as an example a comparison between two large physician-derived registries: the Duke MG Patient Registry (US) and the INNCB MG Registry (Italy), and our efforts toward developing and implementing a common platform for MG Registries: this platform could be the basis for the development of independent MG-DBs over a core of Common Data Elements (CDE) for an easy dialogue. The National Institute of Neurological Disorders and Stroke has an ongoing project to establish MG-specific CDEs. By analyzing data on disease progression and patient responses to different disease management strategies, registries may help to improve disease outcomes.

A European Network on MG, supported by EU funds (EuroMyasthenia Network, EU-2005105, DG SANCO, and Fight-MG project, HEALTH-F2-2010-242210), offers the best opportunity to establish a multidisciplinary team focused on this disease. The development of a database specific for patients with MG living in Europe (EuroMG-DB) was among the objectives. A working group defined the database structure along with the mandatory clinical and laboratory CDE that should be used to diagnose MG.

Specifically, physician-derived registries have the advantage of incorporating diagnostic and treatment data that may allow comparison of outcomes from different therapeutic approaches, and should be merged with self-reported data from patients. Registries have inherent ethical issues about privacy and use of data that must be clearly discussed and presented to the patient via informed consent. MG Patient Associations should play a pivotal role in disseminating information about registries and encouraging patient participation.

ABSTRACTS OF ORAL COMMUNICATIONS

(in alphabetical order of the first Author)

Muscle Lipids Characterization in Lipids Storage Myopathies

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Lipid Storage Myopathies (LSM) are commonly characterized by altered fatty acid (FAs) metabolism and intracellular triglyceride degradation causing a marked fat deposition and damage in various tissues, mainly in skeletal muscle cells. The most known categories of LSM are represented by primary carnitine deficiency, Carnitil-Palmitoil-Transferase II deficiency (CPT II), neutral lipid storage disease (NLS), and multiple acyl coenzyme A dehydrogenase deficiency (MADD). These disorders are all progressive often causing exercise intolerance, rhabdomyolysis and disabling muscle weakness.

Triacylglycerols (TAGs) and Fatty Acid MethylEsters (FAMEs) composition was evaluated by using a new approach combining two different methods: liquid gas chromatography and an electrospray ionisation–tandem mass spectrometry, 10 muscle biopsies from selected and unrelated patients affected by CPT II deficiency (4 pts) and MADD deficiency (6 pts) were studied. Our results showed an increase of TAGs and FAMEs, in the skeletal muscle of MADD compared to CPT II muscle samples. Furthermore, the composition of FAs showed an abnormal accumulation of Very long-chain, long-chain and medium chain fatty acids in MADD. This analysis approach offers certain advantages over other procedures used to characterize and compare lipid samples with regard to the disease. In addition, this results evidence the FAs profile distribution in different LSM, not only, demonstrating the nature and composition of the altered lipid, but also suggesting the potential enzymatic deficiency.

Ion channels gene expression analysis in myotonia congenita patients carrying ClC-1 chloride channel mutations

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Myotonia congenita is characterized by impaired muscle relaxation after contraction, resulting in muscle stiffness. It is caused by mutations in the *CLCN1* gene encoding the ClC-1 chloride channel. Here we report the functional study of new missense mutations found in patients with recessive MC: the T82A mutation found in compound heterozygosis with the known G190S, and the G270V mutation expressed in homozygosis. Recombinant hClC-1 channel mutants were expressed in tsA201 cells for patch-clamp recording. Both G270V and G190S induce dramatically shift the activation voltage-dependence toward more positive potentials, resulting in nearly zero chloride current at physiological voltage. The effect of G270V fully explains MC in the homozygous patient. Conversely, the T82A chloride currents were similar to WT currents. Looking for potential disease modifiers, we analyzed the gene expression of various ion channels using RTPCR in *Vastus Lateralis* muscle biopsies from these patients compared to two age-matched control individuals. No difference in ClC-1 mRNA expression was found, thus excluding alterations in *CLCN1* expression as a contributor to MC. On the other hand, we observed an increased mRNA expression of the sodium channel β1 auxiliary subunit and of the ATP-sensitive, inward-rectifier K^+ channel Kir6.2 in patient muscles. In addition, the potassium channel auxiliary subunit KCNE3 was totally lacking in patient muscles. If confirmed, alteration of these genes may contribute to the symptoms. These results improve our understanding of the myotonic phenotype, and the altered channels may constitute appealing druggable targets. Supported by Health Ministry (grant GR-2009-1580433).

Drug discovery for dystroglycanopathies via LARGE promoter activation screening

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A-dystroglycanopathies are congenital muscular dystrophies due to loss of α-dystroglycan (α-DG) functional glycosylation. Genetic overexpression of LARGE induces highly glycosylated α-DG, improving the defective phenotype of myoblasts derived from patients and murine models with distinct dystroglycanopathies. However, pharmacologically-active compounds able to increase endogenous LARGE protein levels are currently unknown. Our goal was to develop and validate a cell-based high-throughput screening assay aimed at identifying compounds able to activate LARGE promoter regions. In skeletal muscle LARGE is expressed as a ≈4.500 base-pair (bp) transcript. The 2,795 bp region at the 5' end of the gene encompassing exon1 and 200 bp of intron1 is GC-rich and contains a predicted transcription start site located in exon1 first 10 bp. The region is well-conserved and includes 2 regions of transcription factor binding. We subcloned a 1.3 kb sequence covering exon1 first 243 bp and the first region of transcription factor binding into the pEZ/PGO2 luciferase (luc) vector (pPr-1.3). In C2C12 cells pPr-1.3 displayed strong basal promoter activity as compared to HEK cells which do not express LARGE. C2C12 transfected with pPr-1.3 and differentiated for 4 days displayed a 7-fold induction of luc activity, thus indicating that the 1.3 kb region is functional and responsive to the transcriptional machinery which physiologically activates LARGE transcription in differentiating myoblasts. Hence, we generated pPr-1.3-C2C12 stable clones.

To generate alternative longer promoter constructs, the 2.7 kb region (+543 ® -2150, pPr-2.7) and a 2.4 kb region (+243 ® - 2.150, pPr-2.4) were transfected in C2C12 cells and compared to pPr-1.3: while pPr-2.7 displayed a 85% reduction of relative luc, pPr-2.4 showed only a 40% reduction suggesting that the region comprised between nt +543 and +243 could contain a transcriptional silencer.

Neuroimaging correlates of behaviour in DM1: VBM analysis and fMRI study of self-awareness brain networks

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The adult-onset form of myotonic dystrophy (DM1), has a wide phenotypic spectrum and potentially may affect any organ including CNS with mild to severe involvement.

We enrolled 63 patients with established clinical-genetic diagnosis of DM1, that underwent neurological assessment, psychological and neuropsychological evaluation and quality of life interview. A subgroup of 20 patients underwent 3T-MRI protocol including morphologic and functional investigation. Gray matter (GM) atrophy was measured with Voxel-based morphometry (VBM) and calculating Parenchymal Brain Fraction (PBF). fMRI examination investigated cortical BOLD-response during a self-awareness task. RESULTS. Neurological examination showed mild muscle involvement (MIRS mean 2.98 ± 0.92). Patients had frontal and visuo-spatial dysfunctions respectively in 47 and 42%; illness-unawareness was found in 52.1% and was smoothly associated to executive impairment ($p = 0.075$). VBM showed several clusters of reduced GM, in DM1 patients *vs.* controls, in fronto-temporal areas. PBF correlates with impaired cognitive performance for visuo-spatial and executive functions (TMT-A,TMT-B, REY-figure and Wisconsin Card Sorting Test). Within-group activation maps showed in patients higher activation in frontal and midline regions during self awareness task. fMRI revealed frontal and midline brain hypoactive regions in patients with reduced illness-awareness.

Our data indicate the existence of a correlation between brain atrophy, expressed with PBF, and impairment in typical cognitive domains for adult DM1 patients. Moreover, a high percentage of the sample showed decreased illness-awareness, associated to specific brain regions hypoactivation; this could be an interesting issue to study in deep, in view of future clinical trials and for proper planning of patients' management.

Longitudinal functional measures in Becker muscular dystrophy: implications for clinical trials and Duchenne exon skipping outcomes

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We aimed to evaluate natural history and genotype-phenotype correlations in Becker muscular dystrophy (BMD) in a monocentric longitudinal study, using functional measures currently adopted in clinical trials for dystrophinopathies.

We especially focused on BMD deletion groups modeling successful skipping of exons 51 and 45 in Duchenne patients.

We recruited 68 patients with molecularly defined *DMD* mutations and altered dystrophin quantity and/or Molecular weight, assessed by Western Blot of diagnostic muscle biopsies, and evaluated them at baseline and after 12 months with North Star Ambulatory Assessment (and NSAA) 6 Minute Walk Test (6MWT).

Baseline 6MWT and NSAA correlated with age ($r = -0.35$ and -0.38) and dystrophin levels ($r = 0.38$ and 0.38).

Baseline NSAA and 6MWT were positively correlated ($r = 0.86$), with a "ceiling effect" for NSAA.

Patients with deletions bordering exon 51 ($n = 10$) had higher dystrophin levels (Mann-Whitney $p < 0.01$), walked longer at baseline in the 6MWT (t-test $p < 0.01$), and had higher NSAA scores at baseline (Mann-Whitney $p < 0.01$).

At re-evaluation after 12 months, 6MWT distance was stable (± 50 m) in almost all patients, independent of age and mutation.

NSAA at 12 months also showed stable scores in most patients, but a greater decrease was observed in patients with deletions bordering exon 45 ($n = 29$, Mann-Whitney $p < 0.01$).

These findings are relevant for the design of clinical trials for BMD, for which stratification by *DMD* mutation appears to be crucial, and for prediction of phenotypes resulting from successful skipping of exons 51 and 45 in DMD.

Distal spinal muscular atrophy and ataxia with cerebellar atrophy in two unrelated patients; a new phenotypic variant of HRD and recessive KCS syndrome related to TBCE

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Hypoparathyroidism-retardation-dysmorphism (HRD) is a rare AR inherited condition, reported in children born to consanguineous parents of Middle Eastern origin (Saudi and Israeli pedigrees). Kenny-Caffey syndrome (KCS) is another rare AR dysmorphologic syndrome similar to HRD differing from the HRD phenotype owing to the additional presence of medullary stenosis of the long bones, calvarial osteosclerosis, and susceptibility to bacterial infection. These 2 phenotypes have been related to a single founder effect mutation deletion of 12 bp (c.155-166del12) in the *TBCE* gene. The pmn (progressive motor neuronopathy mouse) has been originally described as a model for a fast developing motoneuron disorder, and is caused by a missense mutation p.Trp524Gly in the tubulin specific chaperone E (TBCE) gene.

By WES we identified a novel homozygous mutation in *TBCE* in a 4 year-old-boy who was born from related parents. The patient had a peculiar phenotype of early onset, slowly progressive distal motor neuropathy with bilateral foot drop associated to spasticity and cerebellar ataxia. The brain MRI at age 4 years showed a global cerebellar atrophy. This phenotype has never been related to a mutation in *TBCE*. A second 3-year-old boy, originating from the same island of Ischia was subsequently recruited and found to harbor 2 hetero compound mutations in *TBCE,* one of which corresponded to the same mutation found in the first detected patient. This boy had a spastic ataxia and distal muscle atrophy in his legs. The MRI showed mildly thin corpus callosum and mild cerebellar atrophy in both. The proximal muscle biopsy confirmed a neurogenic atrophy in both. In fibroblasts, we detected a reduction of the TBCE protein in both patients by WB.

Recent observations have disclosed relevant pathogenetic mechanisms linking a TBCE defect to microtubule defects or protein misfolding/mislocalization as the primary cause of a Golgi fragmentation and atrophy, which is well known to be among the earliest pathological features of degenerating motor neurons.

We are describing a novel phenotype of a complex neurodegenerative disorder that involves the motorneurons associated with a spastic ataxia syndrome in two unrelated patients. We are developing an experimental study that recapitulates the motor neuron disease and the cerebellar involvement by the comparative analysis of cellular models, particularly from fibroblasts of our patients and from the *pmn/pmn* mouse in an international collaboration.

Evaluation of prelamin A-BAF protein complex as chromatin modifier protein platform involved in Emery Dreifuss Muscular Dystrophy (EDMD)

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Lamin A is a component of the nuclear lamina a proteinaceous mesh underlining the inner nuclear membrane. Because of its peculiar localization the nuclear lamina provides a molecular link between nuclear wall and genome. In particular, it has been demonstrated that nuclear lamina components directly interact with DNA and with proteins able to influence the accessibility to genetic information. Recently, it has been described that during human muscle differentiation, a physiological event that require a deep chromatin rearrange, lamin A decreases with concomitant increase of its protein precursor named prelamin A. Interestingly, one of main effect of prelamin A accumulation in the nucleus is the modification of chromatin organization. In our study we demonstrate that prelamin A affects chromatin organization through a molecular interaction with a DNA binding protein named Barrier-to-Autointegration Factor (BAF).

Interestingly, we observed that during muscle differentiation BAF localizes in the nucleus where co-localizes with prelamin A in addition, we observed that emerin and lamin A/C and gene mutations affecting prelamin A and BAF proper localization, prevent prelamin A-mediated chromatin organization effects. Our preliminary findings suggest a possible implication of prelamin A-BAF protein complex in chromatin remodeling during muscle differentiation and chromatin defects observed in EDMD.

Opa1 overexpression ameliorates the clinical phenotype of two mitochondrial disease mouse models

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Increased levels of the mitochondria-shaping protein OPA1 improve respiratory chain efficiency and protect from tissue damage, suggesting that it could be an attractive target to counteract mitochondrial dysfunction. Here we show that OPA1 overexpression ameliorates two mouse models of defective mitochondrial bioenergetics. The offspring from crosses of a constitutive knockout for the structural complex I component Ndufs4 (Ndufs4$^{-/-}$), as well as of a muscle-specific conditional knockout for the complex IV assembly factor Cox15 (Cox15$^{sm/sm}$) with Opa1 transgenic (Opa1^{tg}) mice were clinically and biochemically improved. Whilst the amelioration was significant but limited in Ndufs4$^{-/-}$::Opa1^{tg} mice, mitochondrial ultrastructure and respiration correction, motor performance improvement and survival prolongation were remarkable in Cox15$^{sm/sm}$::Opa1^{tg} mice. Mechanistically, respiratory chain supercomplexes containing active complex IV were increased in Cox15$^{sm/sm}$::Opa1^{tg} mice, and residual monomeric complex IV was stabilized. In conclusion, amelioration of cristae shape by controlled Opa1 overexpression improves two mouse models of mitochondrial disease.

Longitudinal follow-up and muscle MRI pattern of two siblings with polyglucosan bodies myopathy due to Glycogenin-1 mutation

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Polyglucosan bodies (PBs) are amylopectin-like deposits, constituting the hallmark of myopathies due to mutations of genes involved in glycogen metabolism, as the recently discovered GYG1 (Glycogenyn-1).

To enlarge the clinical spectrum of GYG1 mutations, additionally reporting muscle MRI pattern of involvement.

Two sisters, born from consanguineous Italian parents, had asymmetric impairment of arm abduction and pelvic girdle weakness. The onset in the elder sibling was at age 30 years with slow progression leading to wheelchair-dependency at age 71 years; the younger sibling, now aged 64, is still able to walk, though not to run, after 11 years of disease.

At hip/shoulder muscle MRI deltoids and glutei were the most affected muscles in the younger sister, all muscles being almost substituted in the elder; pseudohypertrophy of gracilis/sartorius/rectus femoris was a feature of both. Skeletal muscle biopsy showed PBs and genetic analysis of GYG1 revealed the homozygous c.143+3G>C mutation in both siblings.

We describe intra-familial variability of GYG1 mutations regarding both onset age and disease progression. Early impairment of arm abduction correlates with the selective fatty replacement of deltoid muscles seen at shoulder MRI. The c.143+3G>C mutation is confirmed to recur also in Italian population.

miRNAs as serum biomarkers for Duchenne muscular dystrophy: correlation analysis in a multicentric study between miRNAs levels and clinical status of DMD patients

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Currently no treatment is available for Duchenne Muscular Dystrophy (DMD) but in the last few years several promising experimental strategies are emerging. One of the main issue in the design of clinical trials is the lack of non-invasive and reliable biomarker. Specific muscle miRNAs (dystromirs) have been proposed as potential non-invasive biomarkers for monitoring the outcome of therapeutic interventions and disease progression We quantified miR-1, miR-133 and miR-206 in serum from 84 patients with DMD. MiR-1, miR-133 and miR-206 were upregulated in DMD. Ambulant patients had higher levels of dystromirs than non-ambulant patients. miR206 is statistically higher in patient < 7 years than in patients older than 7. A weak correlation was found with functional motor abilities assessed with North Star Ambulatory Scale and 6 minute walk test. Patients in daily steroid therapy had high levels of dystromiRNA than patients without steroid therapy. Longitudinal studies are needed to demonstrated if dystromiRNA can be considered as exploratory biomarkers for monitoring the disease progression or a predictive biomarkers indirectly represented the remaining muscle mass.

LysoPlex, a novel strategy to dissect the role of autophagy in the muscle

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The autophagic vacuolar myopathies (AVMs) are an heterogeneous group of muscular disorders, characterized by the development of autophagic vacuoles in the muscles. To date, although different myopathies with these features have been described, only the molecular mechanism of the Danon disease has been identified. In fact, this disorder is caused by mutations in LAMP2 gene, that encodes a glycoprotein involved in the chaperone-mediated autophagy. Similarly, other genes encoding for proteins related to lysosomal function and/or autophagy may be involved in the remaining AVMs. Lysosomes, in fact, are important in maintaining cellular processes in skeletal muscle and autophagy acts as contributor to disease pathogenesis and progression.

We have developed LysoPlex, a NGS workflow to sequence at high coverage 12,786 human exons of 891 genes, predicted to be involved in lysosomal function, endocytosis and autophagy pathway. Most of them are not yet associated to known genetic disorders. We designed the enrichment probes using a Haloplex custom platform targeting 99.48% of exons and we validated it, proving its high sensitivity and specificity. By using LysoPlex, we have had a complete view of sequence variants in the genes involved in the lysosomal-autophagic pathway: in particular, we identified disease causing mutations in 70 patients with neuronal ceroidlipofuscinoses and we pointed out novel putative causative genes of these diseases

We are recruiting samples from prescreened and undiagnosed patients affected by autophagic vacuolar myopathies, to study the role of lysosomes and autophagy in these muscular disorders.

A clinical and enzyme functional study in a novel *ASAH1*-linked adult spinal muscular atrophy phenotype

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ASAH1 gene encodes for the N-acylsphingosine amidohydrolase (acid ceramidase) that is involved in the degradation of ceramide into sphingosine and free fatty acids within lysosomes. Loss of function mutations causes Farber disease, an early-onset severe neurological disease, while milder homozygote mutations are responsible for very rare cases of spinal muscular atrophy (SMA) with progressive myoclonic epilepsy (SMA-PME) which is characterized by childhood onset of proximal muscle weakness and intractable myoclonic seizures. The disorder is progressive and patients usually died in the teenage years.

We studied a family in which two members, a 30-year-old pregnant woman and her 17-year-old sister, were affected with very slowly progressive proximal muscle weakness since childhood. Electromyography and muscle biopsy analyses suggested a chronic neurogenic process as usually seen in SMA but the search for deletions or point mutations in the Survival Motor Neuron gene (*SMN)* resulted negative. No history of seizures or myoclonus has been reported and EEG was unremarkable.

The molecular study of *ASAH1* gene showed the presence of the homozygote nucleotide variation c.124A>G that causes the amino acid substitution p.T42A.

Biochemical functional evaluation on cultured fibroblasts by mono–dimensional HPTLC and mass spectrometry analysis showed reduction in enzyme activity and accumulation of ceramide, thus confirming the pathogenic role of the mutation.

This study describes for the first time the association between *ASAH1* mutations and an adult SMA phenotype with no myoclonic epilepsy, thus expanding phenotypic spectrum of *ASAH1*-related SMA.

ASAH-1 molecular analysis should be considered in the study of *SMN*-negative adult SMA patients.

MYH7-related myopathies: clinical, histopathological and imaging findings in a cohort of Italian patients

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The Myosin 7 gene (*MYH7*) encodes slow/beta-cardiac myosin heavy chain, a class II myosin expressed primarily in the heart, but also in skeletal muscles. *MYH7* defect was typically associated with distinct muscle disorders on the basis of site of mutations and the following clinical phenotype have been shown: Familial Hypertrophic Cardiomyopathy (FHCM) associated with mutations in the globular head of the protein, Laing Distal Myopathy (LDM) with mutations in the proximal rod, and Myosin Storage Myopathy (MSM) with changes in the distal rod. Recently the number of cases presenting mutations in *MYH7* has increased significantly, adding further phenotypes to the list and making more complex the differential diagnosis on a clinical ground only.

In this work we describe 16 cases, 8 of which are familial, carrying reported or new mutations in *MYH7*. Patients displayed a broad phenotype including atypical picture, such as infantile dropped head and "bent" spine, which cannot be classified in previously described clinical categories. Patients with overlapping manifestations were also identified. Half of patients had congenital or early infantile weakness with predominant distal involvement of upper and lower limbs. Patients with later onset presentation had a prevalent proximal weakness. Scoliosis, calf hypertrophy and nasal speech were common findings. Serum CK levels were normal or mildly elevated (< 1000 U/L). Six of 16 patients manifested cardiac involvement including non-compacted myocardium even at early onset. Muscle biopsy was consistent with minicores myopathy in 8 cases and, in one case, a picture of fiber-type-disproportion (FTD) was noted. Muscle MRI was meaningful in delineating a shared pattern of selective damage of glutei and tibialis anterior muscles, with relative sparing of quadriceps. In most severe cases, involvement of gastrocnemii was also present.

This work adds to the genotype-phenotype correlation of mutations in *MYH7* confirming the complexity of the disorder.

Therapeutic potential of miR-21 inhibition in the treatment of muscle fibrosis

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Excessive extracellular matrix deposition progressively replacing muscle fibres is the endpoint of most severe muscle diseases. Recent evidence indicates that microRNAs (miRNAs) play fundamental roles in pathological processes. MiRNAs are small non-coding RNA molecules, whose main function is to downregulate gene expression by various mechanisms. Aberrant expression of miRNAs has been related to development of fibrosis in various tissues through regulation of anti- and pro-fibrotic genes. In particular, miR-21, is one of the most highly upregulated miRNAs during tissue injury, and its persistent overexpression disrupts tissue repair and contributes to tissue fibrosis in heart, kidney,liver and lung. In a previously study we found that miR-21 expression was significantly increased both in DMD muscle biopsies and DMD muscle-derived fibroblasts and that its inhibition in vitro decreased the expression of profibrotic molecules. To assess the therapeutic potential of miR-21 inhibition, we treated mdx mice with antagomiR-21.

AntagomiR-21 or scrambled locked nucleic acid oligonucleotides were administered intraperitoneally to three-month-old mdx mice with three subsequent injections. Four weeks after the first injection, the diaphragm muscle showed significant reduction of the extent of fibrosis, significantly increased transcript expression of PTEN and SPRY-1, both targets of miR-21, and significantly reduced collagen I and VI expression. Our findings indicate that miR-21 inhibition contrasts muscle fibrosis and suggest that pharmacological modulation of miR-21 expression has therapeutic potential for reducing fibrosis in muscular dystrophies.

An Italian cohort of patients with mutation in Glycogenin-l gene

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The 3 main enzymes involved in biosynthesis of glycogen are glycogenin-l, glycogen synthase and branching enzyme Glycogen synthase and branching enzyme allow further elongation and branching of the glucose polymer primer while Glycogenin-l is a glycostyltransferase that forms a short glucose polymer of approximately 10 glucose residues by autoglucosylation. We studied with NGS methodology a cohort of 14 patients, with undiagnosed glycogenosis presenting with crams and muscle pain and PAS-positive materials at the muscle biopsy.

We identified mutations in Glycogenin l gene in three Italian patients presenting with adult onset of proximal muscle weakness associated with cramps and myalgias; CK level was normal or mild increased. No family history for neuromuscular diseases. The muscle biopsy showed the presence of PAS-positive. PAS-diastase resistant materials in numerous muscle fibers: the electron microscopy confirmed the presence of amylopectin in muscle tissue. We identified in two patients the common c.143-3g>c homozygous mutation. In one patient a single heterozygous, p.Arg324* mutation was discovered; we are studying RNA to search for the second mutation. No mutations were identified in the others 11 patients. Recently Malfatti et al. described in 7 patients a new polyglucosan body myopathy. We confirm that Glycogenin l gene has to be tested in adult polyglucosan body myopathy and represent the 23% of our undiagnosed patients.

A new gene associated with Progressive External Ophtalmoplegia

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Progressive External ophthalmoplegia (PEO) is a common phenotype characterizing mitochondrial disorders, and can be associated with multiple mtDNA deletions. The onset is typically in adulthood and affected subjects may also present general weakness of skeletal muscles. The underlying genetic defects comprise autosomal dominant or recessive mutations in several nuclear genes with a role in replication of mtDNA. We report here the first patients carrying mutations in *RNASEH1*, encoding the ribonuclease H1.

All these patients started off with PEO and exercise intolerance in their twenties, then developed limb hyposthenia, coordination deficits, dysarthria, dysphagia. Two unrelated patients showed severe balance impairment with progressive loss of autonomous gait. Four sibling developed progressive respiratory impairment that led one of them to non-invasive ventilation, and one to die because of it. Ragged-red and COX negative fibre were observed in patients' muscle biopsies together with impaired activity of various mitochondrial respiratory chain complexes. The electron microscopy showed peculiar enlarged mitochondria. Next generation sequencing approaches lead to the identification of compound heterozygous *RNASEH1* mutations in two sporadic patients and a homozygous mutation in four siblings. Western blot analysis showed the virtual absence of the *RNASEH1* protein in total lysate from patients' fibroblasts. By an in vitro assay, we demonstrated that mutant *RNASEH1* has reduced capability to remove the RNA from RNA/DNA hybrids, confirming the pathogenic role of the identified variants. Since increasing evidences indicate the presence of RNA primers during mtDNA replication, this result also explains the presence of mtDNA deletions and confirms the importance of *RNASEH1* for the maintenance of mtDNA.

Managed habilitation in McArdle's disease: pilot experience for guided patients' empowerment

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There is still no cure for McArdle's disease, the most common muscle glycogenosis characterized by exercise intolerance and recurrent exercise induced myoglobinuria, but several indications have emerged in recent years as effective ways to ease disease impact and improve patients' functioning. Regular aerobic exercise, dietetic manipulation and use of sugar prior to acute efforts are among the most established indications. In spite of this, at a recent check with the McArdle's patients followed in our Clinic, the adherence to these indications was at most inconsistent. Reason for the lack of adherence were the fear of pain and muscle breakdown, the failure to perceive immediate benefit, the inability to properly limit effort and training, the difficulty in translating into daily routine the dietetic indications.

Building upon an established and successful experience of the British glycogen storage association, we devised a one week program of intensive strictly supervised introduction to proper management of McArdle in which clinical and physiology evaluations were alternated with personalized sessions of supervised exercises. The intensive part of the program was concluded by a 3 h outdoor uphill walk and was followed by detailed indications for home-work checked by active weekly telephone monitoring.

8 molecularly defined McArdle's patients were recruited in the first round of the program. The intensive session was well tolerated by all but one patient who complained of crural pain and was later found to be suffering for an herniated lumbar disc. No rise in ck was observed after 45 min exercise sessions repeated twice a day for 4 days. The functional evaluations confirmed the significant reduction in aerobic power but also the inappropriate response to even mild exercise (e.g. 12 min walking test). Psychological evaluation of the recruited patients revealed different and mostly malfunctional coping strategies towards exercise and eating.

The first post training evaluation at 3 months is available for 3 subjects, and provides evidence for mild but consistent improvement in most functional measures. Most importantly, the program was successful in modifying patients' behaviour and improve attitude towards regular exercising and more appropriate dietary habits. The chance that each patient had of confronting her/his experiences with fellows patients suffering the same disease was highly appreciated.

Even apparently simple indications may require direct supervised testing and appropriate personalization to translate in accepted behaviour. Better informed and more proactive patients result in improved functioning, effectively realizing empowerment of our McArdle's subjects.

Predictors of adaptation to non-invasive ventilation in neuromuscular disorders

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Cognitive impairment has been described in several NMD, including amyotrophic lateral sclerosis (ALS), Duchenne muscular dystrophy (DMD) and myotonic dystrophy type 1 (DM1) with distinctive patterns. Several studies have demonstrated that non-invasive ventilation (NIV) improves survival and quality of life in NMD. Scanty literature investigated which are the predictors of NIV tolerance.

The aim of this study was to evaluate the impact of cognitive profile, bulbar symptoms and dyspnoea on NIV tolerance in patients with ALS, DMD and DM1, highlighting possible differences among these disorders.

We included 50 patients with ALS, 10 with DMD and 10 with DM1 and retrospectively evaluated clinical data (onset, neurological, psychological and pneumological status) of pa-

tients trained and started on NIV during hospitalization at Nemo Sud Clinical Center over the last year.

Neuropsycological evaluation (PM38, ECAS, FAB) and neurobehavioral assessment (neurobehavioral rating scale revised (NRS-R)) showed an impairment in all the executive functions in ALS and DM1. Whereas DMD patients showed only a specific deficit of verbal fluency. All the cohorts had an involvement of visuo-spatial ability. Neurobehavioral abnormalities were more prominent in DM1 cohort with emotional and behavioural hyperactivation and this could account for the higher percentage of patients with low compliance in DM1 (> 70%) than in DMD and ASL cohorts (< 10%).

Negatives predictors of adaptation were presence of severe bulbar symptoms, asymptomatic status with a low score at Borg dyspnoea scale (< 4), and presence of behavioural abnormalities associated to cognitive impairment.

This study highlights the need of a neuro-rehabilitative patient-tailored approach to optimize patient's training and compliance.

Clinical and genetic aspects in 20 Italian patients with glycogenosis type V (McArdle disease)

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McArdle disease (Glycogen Storage Disease type V) is an autosomal recessive disorder due to the mutation of the gene encoding the muscle isoform of glycogen phosphorylase (PGYM).Patients usually present exercise intolerance, often associated to the "second wind" phenomenon, rhabdomyolysis, myoglobinuria and acute renal failure. High values of serum CK are also present, even at rest.

We present clinical, morphological, biochemical and genetic data of 20 McArdle patient (13 M, 7 F), age range (11-86 mean 39,5), diagnosed and followed in our Unit over the last 20 years.

The diagnosis was achieved by means of biochemical assay and/or genetic analysis.

The onset of symptoms ranged from 5 to 75 years old. At onset, the presenting manifestations were: massive rhabdomyolysis in 4 patients, easy fatigability and exercise intolerance in 11 patients, myalgia and muscle weakness at lower limbs in 2, only presymptomatic hyperckemia in 3 pts. Serum CK was persistently elevated also at rest in all patients (range 279-9589 UI/L). EMG showed a myopatic pattern in 5 patients, whereas in the others was unremarkable. Forearm ischemic test performed in 8/20 showed no lactate rise. Muscle biopsy evidenced absence of phosphorylase staining in all patients but one whereas a mild glycogen storage was found in all cases. The residual enzymatic activity of phosphorylase was virtually absent in all. Molecular genetic analysis confirmed the diagnosis in all but two patients.

A complete molecular and clinical characterization of patients with McArdle disease is important because it will allow to identify selected subgroups of patients to be differentially recruited in case of new therapeutic strategies.

A data base model for neuromuscular disorders

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In the era of multicentric experimental studies, the role of patient registries and databases are essential.

In our Department, the combined work of clinicians and IT experts allowed the design of an application able to collect, extract and analyze either a single patient or a homogeneous group of patients data.

Starting from historical data, collected in spreadsheets, we designed a data structure, with specific form and coding for each single information according to standard types, allowing to aggregate and collect data.

Two types of data sets are managed: demographic/historical data (time independent) and clinical data/exams recorded at any evaluation (time dependent). A web application able to organize different types of information and to allow data entry, search and modification, has been built with a user-friendly interface. Data export and analysis on patients and parameter, with a quick visualization of graphs tracking the evolution during time, is possible.

Data management is respecting Privacy rules and ensures anonymous treatment of personal data.

At present the system collects the clinical history of 120 patients (mainly with Duchenne/Becker and Limb Girdle muscular Dystrophy) with a mean of 10 complete clinical evaluations for each patient. This initial data base has been an excellent tool for longitudinal evaluation and diagnostic.

Mitochondrial neuropathies: data from the Italian Network

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The Italian Network of Mitochondrial Diseases

Involvement of the peripheral nervous system in mitochondrial disorders (MD) has been previously reported. However, the exact prevalence of peripheral neuropathy in MD is still unclear. Based on the database of the "Nation-wide Italian Collaborative Network of Mitochondrial Diseases", we reviewed the clinical data of the ≈1200 histologically, biochemically and/or molecularly defined patients present in our database, with special regard to peripheral neuropathy. The detailed clinical picture was available for 1100 patients (mean age at onset 24.4 ± 20.5 years; age at last evaluation 39.8 ± 22.3 years; females 51.0%; childhood onset [before age 16-yrs] 43.8%), and peripheral neuropathy was present in 131/1100 patients (11.9%), being one of the ten most common signs and symptoms. In 35 of them, neuropathy was one of the presenting features at the onset of the disease.

Some genotype-phenotype relationship data are also provided, and our study supports the variability of the clinical expression of MD.

Italian Registry of NLSDs. Clinical and genetical characterization

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Some cases of Neutral Lipid Storage Diseases, due to mutation of PNPLA2 and CGI58, are described in different ethnic group in the world, but there are few study regarding clinical characteristic, phenotypic variability and natural history in a large specific population. We have collected data of 21 Italian patients with NLSD, type M and type I, with long-term follow up. They received the diagnosis between 1 and 66 years, in 9 Neuromuscular Centers, in a collaborative study that involved neurologists, geneticists, and expert of lipid metabolism, starting 2013. Every center signalized the patients with diagnosis genetically confirmed. All, but two of patients, are alive after a median time of disease of 40 years, but frequently they had severe motor disability, that frequently start with asymmetric proximal deficit. Two patients with NLSD-I dead with epatic failure, one after a liver transplantation tentative. No patient is update mechanically ventilated. No patient received cardiac transplantation, but one NLSD-M received PM implantation. This study highlights some peculiar aspects of a specific population, in fact Italian NLSD patients differ partially from other patients, like Japanese, that had prevalent cardiac compromission. Critical aspects for disability and life expectancy in Italian patients are arytmic cardiopathy and scheletric muscle atrophy in NLSD-M and liver disease in NLSD-I. Some factors, like diet and life style, can influence clinical characteristics of disease, because patients with the same mutation in the same family have different clinical involvement. This observation is important for the clinical application of the therapeutic strategies.

Expanding the array of mutations in *GMPPB*. A multicenter study

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Alpha-dystroglycanopathies include a broad group of congenital (CMD) and limb-girdle muscular dystrophies (LGMD) associated with reduced glycosylation of alpha dystroglycan (a-DG) in skeletal muscle. To date, mutations in about 20 genes have been associated with a *continuum* of clinical and molecular features. Mutations in six genes (*FKRP, POMT1, POMT2, POMGnT1,FKTN,* and *LARGE*) are by far the most common in larger Italian and UK cohorts but additional genes have been detected with the use of Next Generation Sequencing (NGS) technologies.

GMPPB, coding for GDP-Mannose Pyrophosphorylase B, has thus far been identified in 10 patients in association with complex phenotypes ranging from classical CMD to a less severe LGMD.

Using a targeted NGS approach focused on dystroglycanopathies (DystroPlex) in 44 as yet undefined children with low a-DG and crossing our data with those emerging from a core panel of genes that cause all known forms of nonsyndromic muscle disorders (MotorPlex), we identifiedpredictably pathogenic *GMPPB* mutations in five patients who presented a broad array of clinical manifestation ranging from severe CMD with early death, to CMD and epilepsy or eye defect to young adult with LGMD.

Clinical, imaging and genetic features in new *GMPPB*-related patients confirm the complexity of genotype-phenotype correlations in dystroglycanopathies. However, our data suggest that few mutations are more frequently found in children who also show epilepsy.

Sardinian cluster of GYG1 mutation

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Glycogenin-1 (GYG1) is a glycosyltransferase involved in biosynthesis of glycogen. Recently, a new form of polyglucosan body myopathy due to mutation in GYG1 was described.

We describe five adult patients with polyglucosan body myopathy, all of Sardinian origin, caused by the same homozygous intronic mutation in GYG1 gene. Clinically and pathological features were reported.

The cohort of patients comprised two males and three females. Two patients were sisters while other patients were unrelated. All referred non consanguinity but three patients had the same place of origin in the center of Sardinia. Age at onset ranged from 40 to 60 years old. All patients developed a slowly progressive muscle weakness involving pelvic and scapular girdle. Two patients showed cardiac involvement. CK levels were normal in all patients except one. Electromyography founded evidence of myopathic pattern in four patients; one patient had neurogenic findings indicating radiculopathy. Patients underwent needle muscle biopsy. Morphological examination by light microscopy showed partially alpha- amylase resistant PAS positive inclusions. Molecular analysis showed the same homozygous mutation in GYG1 gene (c.143+3G>C) in all five patients.

The genetic peculiarity of Sardinian population and the long history of isolation probably eased the diffusion of this rare mutation and the disease advent. Our data increase literature cases, contribute to a better definition of this new disease and indicate the presence of a disease cluster, suggestive of a founder effect, probably originated in the Centre Sardinia.

Combined cell and gene therapy to treat merosin deficient Congenital Muscular Dystrophy

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Merosin Deficient Congenital Muscular Dystrophy type-1A (MDC1A) is a severe disorder caused by homozygous mutations in the LAMA2 gene, encoding the laminin alpha2 chain of laminin211. It is characterized by progressive muscular dystrophy and dysmyelinating neuropathy leading to motor deficits, join contractures and hypotonia, which in about 30% of cases lead to death in early childhood. MDC1A has no available treatment so far. Muscle and nerve degeneration are direct consequence of lack of interaction of muscular and Schwan cell receptors to laminin211.

Mesoangioblasts (MABs) are vessel-associated progenitors that can form skeletal muscle and have been shown to restore defective protein levels and motor skills in animal models of recessive muscular dystrophies. However, a preclinical study in MDC1A mouse models by intramuscular delivery of MABs failed to ameliorate the disease. We observed that MABs, unless formed skeletal muscle and engrafted MDC1A dystrophic models, synthetized only minimal amount of laminin211, thus could not rescue the disease. To overcome this problem, we engineered MABs to synthesize and secrete mini-agrin, a synthetic protein that can reconnect orphan laminin211 receptors to other laminin isoforms present in the basement membrane of MDC1A muscles and nerves. MABs engineered to deliver mini-agrin and injected in muscles of MDC1A mice showed amelioration of muscle histology, increased expression of laminin receptors in muscle, and attenuated deterioration of motor performances. Our study demonstrates the potential efficacy of combining cell with gene therapy to treat MDC1A.

FGF21 is a reliable biomarker for mitochondrial diseases

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FGF21 (fibroblast growth factor-21) is a member of the FGF family of proteins that exerts complex metabolic effects. It is predominantly synthesized by the liver, although it is also significantly expressed in skeletal muscle. Recent studies showed that FGF21 transcription in muscle is controlled by mitochondria-driven signals involving the production of ROS and its concentration is increased in serum of patients affected by mitochondrial myopathy. We analyzed serum from 50 juvenile-adult patients with mitochondrial disorders (MD) recruited consecutively in our neuromuscular center (35 PEO, 8 MERRF, 5 MELAS, 3 MNGIE and 1 Leber), 20 disease controls (8 Glycogen storage disease type II, 6 LGMD, 3 OPMD, 3 DM1) and 20 healthy controls. FGF-21 concentration was significantly elevated ($p < 0.0001$) in patients with mitochondrial disease compared with normal and diseases controls, including those with autophagy deregulation and/or secondary impaired mitochondrial dysfunction such as GSD II and OPMD. In MD FGF21 concentration had a much higher sensitivity and specificity than conventional serum biomarkers, as lactate and creatine kinase. More important FGF21 showed an inverse correlation with age of onset of symptoms ($p < 0.001$) and, accordingly, positively correlated with severity of the disease. In contrast to what reported by previous studies, instead, there was no correlation with proximal muscle weakness score, both on clinical ground and on muscle MRI. These data confirm that FGF21 is a sensitive and specific biomarker for MD, independently from the presence of muscle weakness, and it represents an useful diagnostic tool to go together with or justify muscle biopsy or genetic investigations.

New *DNAJB6* genotypes delineate new DNAJB6 myopathy phenotypes

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DNAJB6 myopathy is a limb girdle, proximal myopathy in the vast majority of cases, except in an African-American family, where all affected members have a distinctly distal myopathy. We report on another Italian family with invariant onset exclusively as distal leg weakness and wasting, in which, by exome sequencing, we found a novel *DNAJB6* mu-

tation. In all five affected members of this family the disease was characterized by variable age of onset and severity, and weakness later spread from distal to proximal muscles of upper and lower extremities, eventually involving most muscle groups including facial and bulbar muscles. We also report on four sporadic patients in whom we found three novel and one previously reported mutation in the *DNAJB6* gene, including a splicing mutation. At disease onset three of the sporadic patients showed proximal lower limb involvement, associated in one case with concomitant distal lower limb muscle weakness, and one patient showed pure distal myopathy. Dysphagia was observed in four familial and in one sporadic case. Respiratory involvement was observed in two familial and two sporadic cases, requiring nocturnal mechanical ventilation in one of these. None of the sporadic cases presented facial involvement or tongue atrophy. Pathologically, all patient muscle biopsies were characterized by protein aggregates, autophagic vacuolation, myofibrillar degeneration and fiber atrophy. Genetically, all mutations were in the hot spot region of the *DNAJB6* gene affecting the G/F domain, including the splicing mutation that completely abolished it. Our findings further broaden the clinical and molecular spectrum of *DNAJB6*-related myopathies.

Peripheral neuropathy is a common manifestation of mitochondrial diseases

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Peripheral neuropathy in mitochondrial diseases (MD) may vary from a subclinical finding in a multisystemic syndrome to a severe, even isolated, manifestation in some patients.

To investigate the involvement of peripheral nervous system in MD we performed an extensive electrophysiological studies including nerve conduction velocities, late response and concentric needle electromyography in 106 patients with morphological, biochemical and genetic diagnosis of MD (12 A3243G-PEO/MELAS, 14 MERRF, 3 MNGIE, 67 PEO with single or multiple deletions of mitochondrial DNA, 10 other).

We found a neuropathy in 44 patients (41,5%). The incidence was very high in MNGIE (100%), MELAS (92%) and MERRF (71%), while 25% of PEO patients had evidence of peripheral involvement. The most frequent abnormality was a sensory axonal neuropathy found in 27/44 patients (61%). A sensory-motor axonal neuropathy was instead detected in 18% of the patients and sensory motor axonal demyelinating neuropathy in 13%. Finally two had a polyneuropathy with predominant demyelinating aspects and one Leigh patient had a motor axonal neuropathy. It is interesting to note that the great majority had preserved tendon reflexes and no sensory disturbances.

In conclusion peripheral involvement in mitochondrial disease is frequent even if often mild or asymptomatic. The correct identification and characterization of peripheral neuropathy through electrophysiological studies represents another tile in the challenge of mitochondrial diseases diagnosis.

Improvement of genetic diagnosis of late onset Pompe disease by an innovative next-generation sequencing screening

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Pompe disease is a rare neuromuscular disorder, caused by a deficiency of acid alpha-glucosidase (*GAA*) and resulting in a progressive lysosomal glycogen accumulation.

An adult onset form, presenting with a slowly progressive proximal lower limb and/or paraspinal muscle weakness, characterized by different degrees of severity and different ages of onset, has been described. For the maximum clinical effect of enzyme replacement therapy (ERT), an early diagnosis is required. However, the lack of a universal test and the clinical overlap with other recessive myopathies with limb-girdle presentation hamper an early and rapid diagnosis.

We included the *GAA* gene in a NGS panel of 93 genes causing neuromuscular disorders. Up to now, we have tested, by this targeting approach, 503 prescreened patients affected by unclassified forms of LGMD or myopathy. Nine patients with a late onset Pompe disease and a complete molecular characterization have been identified: all of them have the most common c.-32-13T>G mutation and a second causative mutation on the other allele.

Moreover, in a further patient, bearing a single already known heterozygous variant, the biochemical test confirmed the *GAA* diagnosis: an extensive mRNA study is still on going to detect the second causative mutation. Finally, another patient shows two never described variants, needed a further characterization.

In our screening, *GAA* represents the fourth most common cause of recessive myopathy with Limb Girdle presentation, suggesting that the prevalence of adult onset Pompe disease is likely underestimated. Moreover, the biochemical analysis for atypical patients with a single genetic heterozygous variant, is strongly recommended to confirm or exclude the disease.

Widening the clinical and mutational spectrum of CASQ1-related myopathy

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Recently a common mutation, c.731A>G (p.Asp244Gly), in the calsequestrin-1 gene (*CASQ1*) has been reported in patients presenting with a proximal myopathy or fatigue. Mus-

cle histopathology showed the presence of large vacuoles containing aggregates of sarcoplasmic reticulum proteins. CASQ1 is a Ca^{2+} binding protein and functions as a luminal sarcoplasmic reticulum calcium sensor in both cardiac and skeletal muscle cells.

Objective of this study was to identify and characterize *CASQ1*-mutated patients among patients diagnosed with a vacuolar myopathy with histopathological features resembling the original description by Rossi et al.

Twelve patients (10 M, 2 F) from 6 families were identified (age 55.1 ± 16.5). Four patients were asymptomatic, in the others first symptoms occurred at an age of 49.9 ± 13.6 and included exercise intolerance (4), muscle pain (3), proximal weakness in lower limbs (3) and frequent falls (2). A mild to moderate proximal muscle weakness was detected in 5/12 patients. Muscle strength was normal in 7/12 patients. Myalgia and exercise intolerance were frequent. CK level was elevated in all patients (1456 ± 887 U/L). No cardiac or respiratory abnormalities were observed.

CASQ1 gene directed sequencing identified the common p.Asp244Gly mutations in 11/12 patient and a novel CASQ1 missense mutation in a single patient. Haplotype analysis in the *CASQ1* carriers showed a shared haplotype suggesting linkage dysequlibrium.

Muscle histopathology showed vacuoles, mainly in type II fibers, that appeared empty with ematoxylin and eosin, Gomori and NADH-TR staining but were SERCA1, CASQ1, and RYR1 positive.

In conclusion, *CASQ1*-related myopathy is an aggregate myopathy characterized by either myalgia and exercise intolerance or a moderate, proximal myopathy with retained ambulation till late age. CASQ1 c.731A>G is a common mutation due to a founder effect, but in presence of typical histopathological features *CASQ1* gene sequencing is recommended.

Clinical and genetic spectrum in a large cohort of patients with a genetic diagnosis of Congenital Muscular Dystrophies in the UK and differences with the Italian population

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Congenital muscular dystrophies (CMD) are a highly heterogeneous group of conditions clinically characterized by muscle weakness from birth or shortly after, and variable involvement of eyes, heart and central nervous system. There are important geographic variations regarding the incidence and prevalence of different CMDs.

This study reports the relative frequency and clinical and genetic spectrum of CMD in the entire cohort of UK patients in whom the genetic analysis for the CMD genes was requested between 2001 and 2013. Overall 1042 DNA samples were referred during this time interval and genetic diagnosis was reached in 366 of them. Detailed clinical information was available for 346 patients; 230 fulfilled a clinical diagnosis of CMD while the others had milder forms. The most common type of genetically confirmed CMD was laminin-α2 related dystrophy (MDC1A) accounting for 40% of cases, followed by dystroglycanopathies (25%), Ullrich-CMD (22%) and CMD related to mutations in *SEPN1* gene (13%). Fifteen patients carried pathogenic mutations in the newly discovered *ISPD*, *GMPPB* and *B3GALNT2* genes and here we also describe the associated phenotypes.

In the Italian cohort, the point prevalence of CMDs was 0.563 per 100000 and the most common forms were those with α-DG deficiency (40.18%).

Comparing the two populations, we found many differences in terms of prevalence of different forms of CMDs. In both cohorts, the number of cases without known genetic mutations remains significant.

Immune system abnormalities in Italian patients with Myotonic Dystrophy type 1 and type 2

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In myotonic dystrophy, serological alterations related to the immune system are known, including low levels of IgG. Noteworthy, an association between myotonic dystrophy type 2 and autoimmune diseases has been recently described in the Dutch population. The aim of this study was to investigate the presence of serological immune system abnormalities in Italian patients with myotonic dystrophy type 1 and 2 (DM1 and DM2). Twenty-five DM1 and sixteen DM2 patients were tested for the presence of a wide panel of autoantibodies (anti nuclear (ANA), anti parietal gastric cells (APCA), anti intrinsic factor (FI), anti smooth muscle (ASMA), anti mitochondria (AMA), anti pancreatic insula (ICA), rheumatoid factor (FR), anti citrullinated peptide (CCP), anti endomysium (EMA), anti Saccaromyces cerevisiae (ASCA), anti neutrophil cytoplasmic (ANCA), anti cardiolipin). Serum levels of Immunoglobulins A, G and M, C-reactive-protein and circulating immune complexes were also measured in all patients. Twelve DM1 (48%) and eleven DM2 patients (69%) showed at least one positive antibody. DM1 showed a higher frequency of CCP and ASCA antibodies, as opposed to DM2 that displayed a higher frequency of ANA, ASMA, APCA, FI and FR antibodies. Around 40% of patients in both groups presented low levels of IgG, three patients in both groups showed low IgM, and two DM2 patients had low IgA levels. Among DM1, the majority of 'autoantibody positive' patients had a low CTG expansion (E1 subclass) and normal IgG values. In conclusion, Italian DM1 and DM2 patients seem to have an enhanced predisposition to develop autoimmune diseases and this tendency might be underestimated due to the low levels of serum immunoglobulins found in many patients. The underlying mechanisms of the immune system abnormalities found in DM need to be investigated.

Analysis of X chromosome inactivation in carriers of Becker muscular dystrophy

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Becker muscular dystrophy (BMD) is an X-linked recessive disorder affecting about 1:18,000 male births. Female carriers are usually asymptomatic, but about 7% of them may present clinical symptoms, in particular myalgia, muscle weakness or more frequently cardiomyopathy.

Aim of the present work was to evaluate whether a) X-chromosome inactivation (XCI) plays a significant role in the onset of cardiomyopathy in BMD carriers, as observed in DMD carriers, and b) the pattern of XCI is transmitted from mothers to daughters.

To this aim, the XCI pattern was determined in the lymphocytes of the following two groups: manifesting carriers (Group 1, 7 subjects) and non-manifesting carriers (Group 2, 22 subjects), using the AR methylation-based assay. The non manifesting group was in turn subdivided into further 2 subgroups, over 50 y (Group 2a, 9 subjects) and under 50 y (Group 2b, 13 subjects), because symptoms, and in particular cardiomyopathy, usually starts after 50 y.

We also compared the XCI pattern in 8 mother–daughter pairs within groups 1, 2a and 2b, to test the inheritance of the XCI pattern. Pearson χ^2 test was used for statistical analysis.

The results showed that 5/7 manifesting (71,4%) and only 1/22 non-manifesting (4,5%) BMD carriers present a skewed XCI pattern, with a preferential inactivation of the X chromosome carrying the normal allele. However, no significant difference was found in the XCI pattern between mothers and their daughters.

These results suggest that: a) cardiomyopathy in BMD carriers is likely related to the skewed XCI; and b) the pattern of XCI is not transmitted.

MUSCLE CLUB SESSION

MC1. Nemaline myopathy mimicking bulbar-onset motor neuron disease

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Dysphagia, dysphonia and tongue weakness in adult patients may suggest motor neuron disease. We describe a 58-year-old woman, complaining of dysphonia, dysphagia for solid foods, weight loss and fasciculations, and presenting with an electromyography pattern of chronic denervation, who had been diagnosed with amyotrophic lateral sclerosis in a reference center, one year prior to our observation.

Nine months after receiving this diagnosis, she showed marked enlargement of the base of the tongue. A deep localized hypodensity with dishomogeneous contrast enhancement was demonstrated in a tongue CT scan; a needle biopsy of this area revealed amorphous material and scattered proliferating fibroblasts. Serum tumour markers were negative.

At our observation, severe macroglossia and tongue paralysis were assessed. Needle electromyography showed absent activity in the tongue and signs of chronic denervation in shoulder and pelvic girdle muscles; motor evoked potentials were normal. Serum creatine kinase was > 300 U/L. At deltoid biopsy, predominance of type I fibers, some of which hypertrophic, and atrophy of both fiber types were seen; most type I fibers contained subsarcolemmal rods which stained red in Gomori trichrome, thus suggesting a diagnosis of adult-onset nemaline myopathy. Pathological and MRI evidences of tongue involvement in nemaline myopathy patients have been reported; furthermore, tongue paralysis has been described in a patient with nemaline myopathy and bulbar sarcoid encephalopathy. These experiences suggest that nemaline myopathy should be considered in the differential diagnosis of motor neuron disease.

MC2. A novel *AIFM1* mutation expands the phenotype to an infantile motor neuron disease

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AIFM1 is a gene located on the X chromosome, coding for AIF (Apoptosis Inducing Factor), a mitochondrial flavoprotein involved in caspase-indipendent cell death. *AIFM1* mutations have been associated with different clinical phenotypes: a severe infantile encephalopathy with combined oxidative phosporylation deficiency (COXPD6) with or without ventriculomegaly, and the Cowchock syndrome, an X-linked Charcot-Mary-Tooth disease (CMTX4) with axonal sensorimotor neuropathy, deafness and cognitive impairment. In two male cousins with an early-onset mitochondrial encephalopathy we identified a novel *AIFM1* variant. Their phenotype was characterized by global developmental delay with marked hypotonia and early onset, sub-acute weakness of limbs followed by very poor spontaneous motility, epilepsy and lactic acidosis. Both muscle biopsies showed signs of severe denervation that was particularly severe in one of them, where the presence of large groups of markedly atrophic fibers and clusters of hypertrophic fibers resembled the picture of spinal muscular atrophy (SMA); COX deficiency was present in fibroblasts and muscle. Our patients manifested a phenotype that included signs of both cortical and motor neuron involvement; the severe neurogenic pattern at muscle biopsy emphasizes the role of AIF in development and function of motor neurons.

MC3. Dropped head syndrome as first manifestation of scleromyositis

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Dropped head syndrome (DHS) is a rare clinical entity which occurs in patients with severe weakness of neck extensor muscles. It may represent the first clinical manifestation in several neuromuscular diseases such as myasthenia gravis, motoneuron disease and non-inflammatory myopathy. Scleromyositis, an overlap syndrome characterized by the presence of symptoms of scleroderma and myositis, belongs to the latter group.

Three female patients came under our attention with a difficulty in maintaining their neck erect. Neurologic examination showed atrophy and weakness of the extensor muscles and mild weakness of proximal muscles of arms; in two patients a mild dysphagia was also present.

In all three patients there were presence of symptoms of scleroderma and myositis. The only laboratory test abnormalities detected were serum iperCKemia and high levels of lactate dehydrogenase. Electromyography showed a spread myopatic process and signs of muscle inflammation and necrosis. Neuroimaging studies revealed no abnormalities. Muscle biopsy showed a non-specific myopathic features with perivascular inflammatory infiltrates. All patients received therapy with immunomodulatory: glucocorticoids and immunoglobulin in two of them while in third patient, because of the coexistence of rheumatoid arthritis, tocilizumab and steroid therapy were started. After 4 months, improvement of muscle strength and of dysphagia was observed. Laboratory tests showed lower CK.

Scleromyositis has to be considerated a treatable cause of DHS and set up an early treatment with immunosuppressive/immunomodulatory drugs can reverse muscular weakness and muscle fibers damage.

MC4. Mutations in *GMPPB* gene presenting with pseudometabolic myopathy

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Mutations in guanosine diphosphate mannose (GDP-mannose) pyrophosphorylase B (GMPPB), a key enzyme of the glycosilation pathway, have been recently described in families with congenital (CMD) and limb girdle (LGMD) muscular dystrophy with reduced alpha-dystroglycan (alpha dystroglycanopathies).

Affected individuals typically display a combined phenotype of muscular dystrophy, brain malformations and generalised epilepsy. However, a wide spectrum of severity has been described ranging from classical CMD presentation to children with mild progressive LGMD with or without intellectual disability, to patients with isolated episodes of rhabdomyolysis. Feature of cardiac involvement, including a long QT interval and left ventricular dilation by age 10 years have been also described in four cases.

Here we report a 21 years-old Italian male presenting with elevated serum CK levels without overt muscle weakness. Major complains included exercise intolerance with limb myalgia, and few episodes of myoglobinuria suggestive of a possible metabolic myopathy. Muscle biopsy showed only minimal alterations, whereas a marked reduction of glycosilated alpha dystroglycan was present. Extensive genetic analyisis of genes associated with alpha dystroglycanopathies identified two missense mutations in the *GMPPB* gene, one novel and one previously reported.

This case further confirms the peculiar pseudometabolic presentation of this disorder and highlights the importance of exhaustive molecular characterization of patients with reduced glycosilation of alphadystroglycan at muscle biopsy.

MC5. Unusual pR471H Laminopathy

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An only child of healthy consanguineous parents was born at 37 weeks of gestation after an uneventful pregnancy. By 5 weeks of age he had failed to thrive and was noted to have dysmorphic facial features and muscle hypotonia. A brain MRI showed bifrontal atrophy and delayed myelination. Muscle biopsy at 8 weeks revealed a myopathic pattern with an increased fiber diameter spectrum and minicores by electron microscopy. Suspecting "precharacteristic" infantile neurogenic atrophy genetic testing revealed a normal *SMN1* gene, but a mutation R471H in the *LMNA* gene. The patient's health continued to deteriorate until death after 5½ months of age.

MC6. Neuromyopathy with vitamin E deficiency

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We observed a 51 years old man with a ten years history of muscle cramps, myalgia and increased CK. His medical history also included cholecystectomy complicated by pancreatitis, HCV hepatopathy and defect of the coagulation's factor X.

At 42 years he underwent EMG that was normal and muscle biopsy that disclosed dystrophic changes with increased acid phosphatase activity. Immunoblot for dystrophin, dysferlin and calpain and genetic tests for myotonic dystrophy type 1 and 2 were negative.

When we observed the patient neurological examination showed hypotonia, areflexia, vibration sense impairment and ataxic gait.

Laboratory analysis showed increased CK (2260 U/L) and lactic acidosis; EMG revealed a sensorymotor polyneuropathy. A new muscle biopsy disclosed myopathic changes with cytoplasmic granules strongly reactive with acid phosphatase and autofluorescent; electron microscopy showed granular dense bodies similar to lipopigments.

These findings suggested us to search for serum vitamin E level that was 0.5 micromol/L (normal values 11.5-46.5).

A fat malabsorption of unknown origin was detected. Abetaliproteinaemia, coeliac disease and autosomal recessive ataxia with vitamin E deficiency were excluded. Oral supplementation with high doses (1200 mg/day) of vitamin E was ineffective and a multivitaminic e.v. therapy was started. After 3 months serum level of vitamin E was 17 micromol/L with a clinical improvement of myalgia and fatigability.

Our patient has developed neuromuscular symptoms due to a chronic vitamin E deficiency revealed by muscle biopsy. We underline the role of an early diagnosis and therapy in this potentially treatable disease.

ABSTRACTS OF POSTER COMMUNICATIONS

(in alphabetical order of the first Author)

Spontaneous breathing pattern in children with Spinal Muscular Atrophy: correlation with motor function assessment

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Impairment of respiratory function can be variable in patients with SMA. The assessment of pulmonary function usually requires cooperation and can be consistently performed from the age of 5 years, while different indirect assessments (i.e. nocturnal pulse oximetry and blood gas) are usually performed in younger children with SMA. Rapid and shallow breathing (RSB), tidal volume (VT), and its ribcage percentage contribution (%VRC, index of intercostal action) were measured in 5 SMA1 (median age 2.6 y), 26 SMA2 (median age 3.8 y), 6 SMA 3 (median age 5.4 y), and 14 healthy control (HC; median age 5.2 y) children during seated (HC, SMA 2 and 3 only) and supine quiet breathing by Opto-Electronic Plethysmography (OEP). Motor function was assessed by means of the CHOP INTEND Scale (in SMA 1), the Hammersmith Functional Motor Scale-Expanded (HFMSE) and the Upper Limb Module (ULM) (in SMA 2 and 3). SMA 1 children showed the highest RSB, the lowest VT and negative %VRC compared to the other groups. SMA 2 children showed higher RSB compared to HC in both postures. In seated position, their VT and %VRC were significantly lower than SMA 3 children and HC. In supine position, no differences were found between SMA 2, SMA 3 and HC children in VT and %VRC. SMA 3 children showed a breathing pattern comparable to HC in both postures. Linear regression showed a weak correlation of the HFMSE with RSB and VT, and a good correlation of the CHOP INTEND and HFMSE with %VRC. No correlations were found either with ULM and with age. These data provide initial support to the usefulness of OEP as a valid and reliable method to measure the efficacy of new therapies on respiratory function, and more specifically, to test the effect of respiratory rehabilitation aimed to improve intercostals weakness and, consequently, lung restriction and ineffective cough even in young children with SMA.

Early-onset cerebellar ataxia due to novel mutations in *ACO2*

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Inherited ataxias are a group of heterogeneous disorders affecting children and adults. In almost half of the patients, the genetic cause of the disorder is unknown. *ACO2* mutation have been reported in individuals from two unrelated families who presented at 2 months of age with truncal hypotonia, seizure and ophthalmologic abnormalities. The course was severe with profound psychomotor retardation and progressive visual loss.

We report a 3 years old boy who presented with difficulties in sitting, he was not able to sit until 14 months. Walking ability is impaired for the presence of motor dyspraxia and he is able to walk only with bilateral support. He also present delayed speech and language development.

Neurological examination evidenced a prominent cerebellar involvement with oculomotor dyspraxia, truncal unsteadiness and disequilibrium, gait ataxia, mild limb dysmetria and reduced muscle tone. Brain MRI showed no cerebellar anomalies. Hearing tests showed a mild auditory neuropathy. WES analysis found two novel mutations (p.P712L and p.R607C) in *ACO2*.

Aconitase activity in patient fibroblasts was 60% of controls. Respiratory chain enzymes activities in fibroblasts were normal, while high-resolutionrespirometry showed 50% spare capacity comparing with controls.

Defect in mitochondrial aconitase was associated with an infantile neurodegenerative disorder affecting the cerebellum and retina. We report a case of aconitase deficiency with milder phenotype confined to cerebellum, thus suggesting that aconitase mutation must be considered in child onset cerebellar ataxia differential diagnosis, even when retinal involvement is not present.

Selective short-term verbal memory involvement in two siblings carrying centronuclear myopathy due to *DNM2* gene mutations

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DNM2 gene encodes a ubiquitously expressed large GTPase which is involved in endocytosis and intracellular membrane trafficking. Mutations in *DNM2* gene are associated with autosomal dominant centronuclear myopathy (ADCNM), as well as with Charcot-Marie-Tooth neuropathy. Cognitive involvement with borderline mental retardation in patients with *DNM2* mutations was previously reported only in one family. We report two siblings who presented with ADCNM due to *DNM2* mutation with selective short-term verbal memory involvement. The older patient, 64-year-old man, firstly experienced symptoms in the second decade with difficulty in running and climbing stairs. Clinical examination showed: ptosis, hyperlordosis and proximal weakness with distal involvement particularly in the lower limbs. Electromyography confirmed a myopathic disorder. Muscle biopsy showed increased central nuclei. His sister, 57-years-old, had a similar phenotype with a slowly progressive childhood-onset myopathy. Both had normal language development. Molecular analysis found a heterozygous mutation in *DNM2* gene (c.1105C>T, p.R369W). At variance with previous description, neuropsychological examination including Mini Mental State Examination and Mental Deterioration Battery showed a specific involvement of verbal memory function with the Immediate Recall Rey Auditory Verbal Learning Test Score of 27.40 in male and 17.70 in female (cut-off 28.53). Both patients had a normal IQ (107 and 100).

This report further expands the clinical spectrum of CNS involvement associated with DNM2-CNM. We postulate that the role of dynamin-2 in CNS should be further studied and patients with DNM2-CNM should be carefully evaluated with specific neuropsychological tests.

Genetic modifiers of ambulation in the CINRG Duchenne Natural History Study

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We studied the effects of *LTBP4* and *SPP1* polymorphisms on age at loss of ambulation (LoA) in a multiethnic Duchenne muscular dystrophy cohort (DMD).

We genotyped *SPP1* rs28357094 and *LTBP4* haplotype in 283/340 participants in the Cooperative International Neuromuscular Research Group Duchenne Natural History Study (CINRG-DNHS). Median ages at LoA were compared by Kaplan-Meier analysis and log-rank test. We controlled polymorphism analyses for concurrent effects of glucocorticoid corticosteroid (GC) treatment (time-varying Cox regression), and for population stratification (multidimensional scaling of genome-wide markers).

Hispanic and South Asian participants (n = 18, 41) lost ambulation 2.7 and 2 years earlier than Caucasian (p = 0.003, < 0.001). The TG/GG genotype at *SPP1* rs28357094 was associated to 1.2-year earlier median LoA (p = 0.048). This difference was greater (1.9 years, p = 0.038) in GC-treated participants, whereas no difference was observed in untreated. Cox regression confirmed a significant effect of *SPP1* genotype in GC-treated participants (HR 1.61, p = 0.016). *LTBP4* genotype showed a direction of association with age at LoA as previously reported, but not statistically significant. After controlling for population stratification, we confirmed a strong effect of *LTBP4* genotype in Caucasians (2.4 years, p = 0.024). Median age at LoA with the protective *LTBP4* genotype in this cohort was 15.0 years, 16.0 if GC-treated.

In conclusion, *SPP1* rs28357094 acts as a pharmacodynamic biomarker of GC response, and *LTBP4* haplotype modifies age at LoA in the CINRG-DNHS cohort. Adjustment for GC treatment and population stratification appears crucial in assessing genetic modifiers in DMD.

Brachio-cervical inflammatory myopathy: a distinct phenotype among inflammatory muscle diseases

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Brachio-cervical inflammatory myopathy (BCIM) is an immune-mediated myopathy characterized by skeletal muscle inflammation and progressive weakness in the proximal regions of the arms and posterior neck, occasionally associated with extramuscular manifestations. Here we report three patients (one 80-year old man and two 66 and 75-year old women) with about one year history of discomfort and progressive weakness of neck extensor muscles associated with severe dysphagia and dyspnea. Moreover, the two women had respectively Myasthenia gravis and undifferentiated connective tissue disease. All three patients came at our observation with a suspected diagnosis of motoneurone disease. We performed extensive laboratory tests, screening for autoimmune diseases, electromyography (EMG), musculoskeletal MRI and muscle biopsy. Clinical examination associated with elevated myonecrosis markers and a myopathic "irritative" pattern on EMG (fibrillations, short duration and early recruited motor unit potentials) suggested a diagnosis of BCIM. Muscle MRI showed the presence of muscle degeneration with only mild, not specific hyperintensities in T2 weighted images with short inversion recovery, while biopsy demonstrated unambiguous signs of inflammation characterized by mononuclear cell infiltration with prominent CD4 positive cells. Treatment with oral corticosteroids (prednisone) and immunosuppressive agents (azathioprine, methotexate or tacrolimus) led to a progressive improvement in swallowing and muscle strength. Insofar, although it is still debated if BCIM may constitute either a clinical phenotype or a distinct class of immune-mediated myopathies, it is mandatory to make the correct diagnosis of a potentially treatable disorder in such patients with sometime misleading history of a chronic myopathic with scarce signs of inflammation on muscle MRI.

Scapuloperoneal spinal muscular atrophy due to TRPV4 mutation: a rare neuromuscular condition to be considered

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Scapuloperoneal spinal muscular atrophy (SPSMA) is characterized by progressive scapuloperoneal atrophy and weakness caused by heterozygous mutations in the TRPV4 gene. Additional features such as vocal cord paralysis, scoliosis and/or arthrogryposis are likely to occur. The pathogenic mechanism underlying the mutant TRPV4–mediated peripheral neuropathies is not yet clear. Herein, we report two cases of SPSMA in a family carrying the R269H mutation in TRPV4 gene. In particular a 23-years-old male, presented, since birth, with bilateral congenital clubfoot and, later on, developed progressive shoulder girdle muscles and lower limb muscle weakness and atrophy. At visit screening he was accompanied by his father, a 67-years-old man, who occasionally disclosed a slight clinical involvement, with winged scapula, pectoral muscle wasting and difficulty to walk on heels. Neurophysiological analysis and muscle biopsy studies were performed on the proband, confirming a motor axonal neuropathy. Molecular analysis showed in both the R269H mutation in TRPV4 gene. An early diagnosis is mandatory in this rare form of SPSMA, in order to consider and identify the more severe congenital onset characterized by distal and proximal lower limb weakness, possible vocal cord paralysis and arthrogryposis. In summary, we describe a SPSMA, a rare neuromuscular disorder, in an Italian family harbouring the p. R269H mutation in TRPV4 gene, confirming the importance of an early diagnosis together with the clinical heterogeneity of this disease. Therefore, TRPV4 mutations should be considered in scapuloperoneal syndromes presenting with an autosomal dominant inheritance and a neurogenic pattern.

Clinicopathological features and disease course in three patients with focal myositis

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Focal myositis is a rare, tumor-like lesion that affect a single muscle, frequently in lower limb. Muscle biopsy is gold standard for diagnosis, even if the aspect of the lesion may vary. Focal myositis is reported to improve spontaneously or after a short-term steroid therapy, with rare case of relapse. We present 3 patients diagnosed with focal myositis in our center, and describe their morphological features and disease course.

Three patients with a diagnosis of focal myositis were included. Histochemical and immunohistochemical data, obtained from muscle biopsy of the affected muscle, were collected. All 3 patients underwent serial MRI imaging examination and we performed needle EMG in one of them. 2 patients received high dose steroid treatment.

Age ranged from 39 to 60 years. All of them presented with a solitary and often painful mass affecting a single muscle (anterior tibial in two and anterior forearm in one). None of them had systemic symptoms and lacked antecedent trauma. CK levels were slightly elevated (mean 500 U/L). Muscle biopsies revealed relevant myopatic changes (also with dystrophic features) with various rate of inflammation. Follow up after steroid therapy revealed slowly regression in two cases and severe recurrence in the other one.

Focal myositis is considered a benign condition, but in our experience it may cause significant disability, even in 'responder' patients. Muscle MRI seems to be the most reliable exam to monitor treatment response. The complementation with physical therapy may be of help, especially in most aggressive cases.

Expression in zebrafish of mutated human DNM2 produces defects similar to those in human centronuclear myopathy and Charcot-Marie-Tooth 1B neuropathy

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Mutations in the dynamin-2 gene (*DNM2*) cause autosomal dominant centronuclear myopathy (CNM) and dominant intermediate Charcot-Marie-Tooth neuropathy type B (CMTD1B), a motor and sensory neuropathy. Since the relation between *DNM2* mutations and these two diseases is poorly understood, we used the zebrafish as a new animal model to investigate and compare the effects of two different *DNM2* mutations. In this study we identified also a new alternatively spliced zebrafish *DNM2* mRNA (*DNM2a*) with greater similarity to human *DNM2.*

First we knocked-down the zebrafish *DNM2a* producing defects in morphology, causing an increased number of central nuclei, disproportion of fiber diameter and a lower number of fibers. Next we overexpressed human *DNM2* in zebrafish, injecting mutated human mRNAs (R522H causing CNM, and G537C causing CMT). Defects arose especially in secondary motor neuron formation, with incorrect branching in embryos injected with mRNA CNM-mutated, and total absence of branching in those injected with mRNA CMTmutated.

Morphological injuries mimicked knock-down experiments, resulting in defects in muscle organization more evident in embryos injected with mRNA CMT-mutated compared to those injected with mRNA CNM-mutated.

Our data demonstrate that the zebrafish *DNM2a* knockdown is a valuable model for dynaminophaties. In addition, overexpression of human *DNM2* mRNAs, containing disease-causing mutations, results in defects that are similar to those present in human dynaminopathies, making this a suitable model to understand the mechanisms underlying *DNM2*-associated conditions.

A new sodium channel myotonia (SCM) mutation in the Nav1.4 DII-S4S5 linker

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Mutations in the voltage-gated sodium channel Nav1.4 may cause myotonia or periodic paralysis depending on the different gain of function effects. Usually mutations in the DII-S4S5 linker of Nav1.4 are associated with episodic weakness through a disruption of slow inactivation. The aim of our study is to describe the functional effects of a new *SCN4A* mutation located in the DIIS4S5 linker with a pure myotonic phenotype.

The patient, a 39 year-old-man, presented with a 29 year history of muscle stiffness exacerbated by cold and fasting. No episodes of weakness have been reported. Neurological examination showed lid-lag and upper limb percussion myotonia. A variant c. G2095A (p.A699T) was found on *SCN4A* gene.

The biophysical properties of the mutant Nav1.4 channels were evaluated by whole-cell voltage-clamp analysis of HEK293 cells transiently transfected with WT or A699T Nav1.4 channel.

Preliminary data showed a small depolarized shiZ of the Vmid of the voltage dependence of inacyivation (WT = -64.3 ± 1.0 mV, n = 7; A699T = -61.8 ± 0.5 mV, n = 10; $p < 0.05$) and a slowing down in the time constant between WT and A699T ($p < 0.05$) (WT n = 7, A699T n = 10) when measured at -10 mV.

Steady state of the voltage dependence of slow inactivation was enhanced in mutant A699T (WT = -43.8 ± 2.1 mV, n = 5; A699T = -56.9 ± 1.8 mV, n = 3; $p < 0.005$).

In conclusion A699T mutation likely causes a myotonic phenotype through an alteration of fast inactivation.

Other mutations in the same domain often disrupt slow inactivation and predispose the patients to episodic weakness. Our data support the hypothesis that an intact slow inactivation may prevent a periodic paralytic phenomenon.

A challenging acute encephalopathy of the temporal lobes

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MELAS syndrome (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) is a rare genetic condi-

tion whose differential diagnosis is often posed with juvenile stroke, but more rarely even with inflammatory/infectious encephalitis, causing diagnostic challenges.

A man of 45 years with episodes of confusion and headache in last 3 weeks came to the Emergency Room of our Hospital because of two generalised seizures followed by coma. Brain MRI showed temporal lobes T2 hyperintensity with diffusion restriction and contrast uptake and bilateral globus pallidus and caudate nucleus T1 hyperintensity. Proton spectroscopy of the brain showed a lactate peak with reduction of N-Acetyl-Aspartate. In suspect of herpes encephalitis, antiviral and antibiotic therapy was immediately started. CSF presented increased proteins, glucose and lactate but not white cells. Increased lactate was also present in serum. In the hypothesis of a mitochondrial disorder, 2 gr endovenous carnitine and 600 mgs of coenzyme Q10 were administered, with rapid clinical improvement (GCS 13) and regression of the lactic acidosis.

Genetic testing showed the 3243A>G mtDNA mutation in urine, compatible with MELAS syndrome. A one-month later brain MRI showed regression of cerebral edema and marked lactate reduction.

The clinical presentation of the 3243A>G mtDNA MELAS mutation is markedly variable. Here we describe the case of a MELAS syndrome mimicking the clinical and neuroimaging features of herpes encephalitis.

Parkinsonism and mitochondrial myopathy in a calcium metabolism syndrome

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Mitochondria are ubiquitous organelles that play a crucial role in energy metabolism. Mitochondrial myopathy manifests with exercise intolerance and ragged red fibers on muscle biopsy. Mitochondrial dysfunctions, both congenital and acquired, have also been implicated in Parkinson's disease and in a number of cardiac and endocrine disorders. A 75 year-old female was admitted to our ward for hyperCKemia (with peak levels of 1047 U/l). She was already being treated for parkinsonism, with good pharmacological response. Her medical record was notable for a partial thyroidectomy occurred at 45 years old; bilateral cataract surgery at 50 years old; persistent hypocalcemia with CT evidence of basal ganglia calcifications, as in Fahr syndrome. Clinical examination showed a bradykinetic-rigid syndrome (UPDRS III: 27/108), without weakness. Blood tests revealed high serum creatine kinase (536 U/l), increased lactate (both basally and after ischemic forearm exercise), normal calcium, low vitamin D and parathormone; urinary excretion of both calcium and phosphate was low. A deltoid muscle biopsy showed numerous COX-positive ragged red fibers with moderate lipid accumulation, as in mitochondrial myopathy; ring-fibers, moth-eaten and franky necrotic fibers were also observed, along with sparse inflammatory infiltrates and myophagocytosis. This case is peculiar for the association of metabolic myopathy, parkinsonism and disendocrinopathy. We believe that mitocondrial dysfunction is a common pathogenetic pathway.

Topic: myology, mitochondria.

Clinical and biomolecular findings in Italian patients with myotonic dystrophy type 2 premutation

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Myotonic dystrophy type 2 (DM2) is caused by an unstable [CCTG] tetranucleotide part of a polymorphic complex repeated motif [TG]n[TCTG]n[CCTG]n in the CNBP gene. In DM2 patients the pathological number of repeats range from 75 to 11.000, however, recently few patients with DM2-linked myopathy and a [CCTG]55 expansion have been reported. Here we describe two Italian patients with clinical and histological muscular abnormalities associated with DM2 premutated uninterrupted alleles. Patient A, a 51-year-old woman, showed progressive proximal leg weakness and pains and no myotonia was evident. Muscle biopsy revealed an increase of central nuclei and of fiber size variation, type I and type II fiber atrophy and nuclear clumps. Patient B, a man of 26-year-old, was a paucisymptomatic patient presenting iperCKemia, severe myalgia and no myotonia. Muscle histopathology showed a mild fiber size variation. Analysis of CCTG expansions revealed 36 and 53 repetition number in patients A and B respectively. FISH in combination with MBNL1-immunofluorescence performed on muscle sections did not show the presence of nuclear accumulation of mutant RNA or of MBNL1 and alternative splicing of CLCN1, MBNL1 and INSR were not altered. Haplotype analysis is currently in progress to assess whether these CNBP premutated alleles derive from the same founder origin as the European DM2 mutation. Further studies are necessary to understand if the myopatic phenotype described in these two Italian patients is linked to the DM2 locus or to other still undefined genetic mutations.

Clinical and biological significance of elevated cardiac troponin T (cTnT) serum levels in patients with myotonic dystrophy type 1 and type 2

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Myotonic dystrophy type 1 (DM1) and 2 (DM2) are autosomal dominant multisystemic disorders characterized by skeletal muscle and cardiac involvement. Since an elevation of circulating cardiac troponin T (cTnT) in patients with neuromuscular diseases without myocardial injury was reported, the

aim of this study was to determine the clinical and biological significance of elevated cTnT in DM patients. 60 DM patients (46 DM1 and 14 DM2) were analysed. Each patients underwent full clinical and cardiac assessment and routine blood tests. Cardiac investigations comprised ECG, 24h ECG-Holter and 2D-echocardiogram. Serum levels of cTnT, cTnI, and other cardiac biomarkers were performed. To verify if circulating cTnT was released by injured skeletal muscle, protein expression was analyzed by western blot (WB) in skeletal muscle biopsies using the antibodies present in hs-cTnT assay. 53/60 DM patients showed elevated serum levels of cTnT not accompanied by an increase of cTnI values. ECGs and echocardiograms revealed little or no cardiac involvement in both DM1 and DM2 patients. WB revealed a positive immunoreaction by one antibody in both DM and healthy skeletal muscle. No correlation is found between increased levels of cTnT and cardiac manifestations observed. Moreover the serum increases of cTnT do not seem to be caused by the release of cTnT from injured skeletal muscle. Thus, it is possible that a cardiac involvement below our ability to detect it with conventional measures might be present in these patients. Further cardiac studies with more accurate and reproducible technique such as magnetic resonance image will be needed.

Longitudinal follow-up of six adults with late-onset glycogenosis type 2 undergoing enzyme replacement therapy for over 60 months

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Enzyme replacement therapy (ERT) has shown some effectiveness, either in stabilization or improvement of respiratory and motor function, in a few adults with type II glycogenosis. Reported follow-up of treated patients has been no longer than 4 years. We extend the observation period by describing 6 subjects undergoing ERT for over 60 months. Disease onset was 29.5 years, mean delay between disease onset and start of ERT being 12.5 years. A series of parameters were analyzed at both baseline and at 60 months follow-up. At 6-minute walking test the mean distance walked changed from 417.9 m (± 87.3) to 459 m (± 91.8) ($p = 0.3$) whereas mean Walton Scale score changed from 2.4 to 3 ($p = 0.5$). Mean sitting forced vital capacity increased from 64.8% (± 19.2) to 74.5% (± 21.5) of predicted values ($p = 0.4$). All patients were ambulant at baseline and maintained this ability with the exception of one case. The number of subjects requiring nocturnal non-invasive ventilation changed from two to five along the observation period. Only one patient, presenting with progressive scapular girdle weakness, remained both fully ambulant and ventilator-free. In 5/6 antialglucosidase alfa IgG antibodies were tested, being positive in 4/5. These results provide further evidence of ERT variable effectiveness and outline the need to analyze larger cohorts of patients in order to clarify both ERT long-term outcomes and factors predictive of a better response.

A novel dynamin-2 gene mutation associated with centronuclear myopathy

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A 54-year old woman and her two daughters manifested slowly progressive distal muscle weakness with walking difficulties since childhood. They came at our observation with a diagnosis of Charcot-Marie-Tooth neuropathy (CMT).

Neurological exam revealed bilateral ptosis, facial weakness, distal muscles weakness, particularly in lower limbs with foot drop, pes cavus and reduced tendon reflexes. CK were normal. EMG was myopathic with normal nerve conduction velocities, muscle MRI demonstrated fatty infiltration of paraspinal muscles, posterior compartment of the thigh, mild involvement of quadriceps, prominent affection of anterior and posterior compartments of the legs. Muscle biopsy documented nuclear centralization, sometimes in group of 3 or 4, and alterations of the intermyofibrillary network consistent with centronuclear myopathy (CNM). The mother had also moderate leucopenia and thrombocytopenia.

Based on clinical and laboratory data a dynamin-2 gene (DMN2) mutation was suspected and a novel heterozygous mutation c.1934T>G (p.M645R) in a highly conserved domain of the protein was found in all three patients.

Mutations in DNM2 are associated with autosomal dominant CNM and CMT. DNM2-CMT patients may have neutropenia that instead is reported in only one DNM2-CNM patient.

This report confirms that distal weakness associated with ptosis and facial weakness are common features in DNM2-CNM and such clinical picture may give rise the suspicion. Moreover the presence of leuco-thrombocytopenia in one of our patients suggests that this association may be also a hint for the search of DNM2 mutations. Furthermore we describe a novel mutation in a conserved domain of the gene expanding the genetic and clinical spectrum of DNM2- CNM.

Effects of functional electrical stimulation in myotonic dystrophy type 1

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Functional Electrical Stimulation (FES) is a new rehabilitative approach and refers to the process of pairing the electrical stimulation with a functional task, as cycling or walking, in persons unable to perform actively these movements. In the last years, the important role of FES is emerging in neurological diseases with severe muscle weakness, as stroke or spinal cord injury. The role of FES in neuromuscular disorders has never be evaluated. The aim of the study was to evaluate the effects of FES in improving lower limbs muscle strength, endurance and gait speed in Myotonic Dystrophy type 1 (DM1).

Five DM1 patients were enrolled in the study. Three patients performed FES training while the other two carried out strength exercises. The modified Medical Research Council (MRC) scale, the Six Minutes Walking test (6MWT), the time to cover 10 meters (10mWT) and muscle MRI were used for the assessment at the baseline and at the end of the treatment.

A statistically significant improvement of muscle strength ($p = 0.0008$) and of the 6MWT ($p = 0.02$), emerged only in those patients who performed FES training. No significant changes in gait speed was observed. Muscle MRI showed a reduction of intramuscular fatty infiltration after FES training.

This study suggests that, in DM1, FES can be considered a valid and safe method to improve endurance and muscle strength, even in those muscle with severe weakness in which no other rehabilitative options are otherwise available. It also highlights the need to perform future controlled trials in this field.

Genetic counselling in Becker muscular Dystrophy: should we change standards of approach?

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Becker muscular dystrophy (BMD), first described by Becker in 1953, is a benign variant of Duchenne muscular Dystrophy (DMD), that may present with only myalgia and muscle cramps, exercise intolerance and myoglobinuria, or asymptomatic elevation of the serum CK.

The mean age of onset is about 12 years (range 1-70 years). Patients who present before the age of 5, are indistinguishable from those with DMD. BMD is usually transmitted by mothers – carriers of the gene mutation – to 50% of males who will be affected and to 50% of females who will be carriers.

We report the case of a 4-year old boy who was molecularly diagnosed as BMD, following a chance discovery of hyperCK-emia. After the diagnosis, a genetic counselling was offered to his mother and grand-mother, to investigated the carrier status. The mother was found to be carrier, the grand mother not. Some years later, a female second cousin of the case index came to us because a mild hyperCKemia (2x), questioning the pattern of inheritance in this family.

A careful reviewing of the pedigree, allowed us to understand that in this family the mother of the index case had inherited the mutation from her father, who is currently 68 years old and totally asymptomatic. The increased values of the serum CK and the molecular analysis confirmed this hypothesis showing that grandfather and grandson share the same mutation (del exons 45-55).

This case report suggests to extend the molecular analysis to maternal grandfathers at least in families with young BMD boys with attenuated phenotype.

Two novel compound heterozygous mutations in ACAD9 in a patient with infantile-onset hypertrophic cardiomyopathy, hypotonia, and lactic acidosis

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Complex I deficiency is the most common inherited mitochondrial respiratory chain defect and has been linked to pathogenic mutations in nuclear and mitochondrial genes. Nevertheless, the molecular diagnosis remains unknown in many patients.

Clinical data were collected. Biochemical, histochemical, and molecular studies in muscle and cultured fibroblasts from the patient were performed. Next generation sequencing techniques were applied to identify the molecular defect.

A 3-month-old boy was diagnosed with hypertrophic cardiomyopathy and failure to thrive. Family history is notable for a brother who had died in infancy of cardiac insufficiency due to hypertrophic cardiomyopathy. Neurological examination revealed hypotonia, and the metabolic workup showed increased lactic acid in plasma. The patient died at the age of 8 months of cardiac insufficiency. Western blot analysis showed reduced level of complex I protein and mitochondrial respiratory chain enzyme activities showed severe complex I deficiency. Whole exome sequencing revealed two novel heterozygous variants in ACAD9 gene (p.R518C and p.E564K), both confirmed by Sanger sequencing and predicted to be pathogenic by PolyPhen, SIFT, and Provean software tools. Parents were each heterozygous for one ACAD9 variant.

Since the initial identification of complex I deficiency due to ACAD9 mutations, 15 patients have been described. Clinical presentation ranges from severe fatal infantile forms with hypertrophic cardiomyopathy to mild encephalomyopathy. Here we describe two novel mutations in ACAD9 associated with complex I deficiency and a fatal infantile phenotype. This report confirms the clinical heterogeneity of ACAD9 mutations and its importance as complex I assembly factor.

X-linked myotubular myopathy in females

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X-linked myotubular myopathy (XLMTM) is a rare congenital muscle disorder due to mutation in *MTM1*, which encodes myotubularin, a key protein of muscle cell differentiation and intracellular organelles dynamics.

Affected males present at birth with severe generalized hypotonia, associated with feeding difficulties and respiratory insufficiency. Most of them die in infancy or early childhood.

Female carriers are usually asymptomatic but seldom show muscle weakness possibly due to skewed X-inactivation. A characteristic morphological alteration described as "necklace fibers" has been reported as the histological marker of manifesting *MTM1* carriers.

Here, we report the clinical, pathological and genetic findings of two girls who manifested XLMTM and harbored mutations in *MTM1*. Interestingly, upon extensive genetic analysis both girls also showed a second variant in *LMNA* and in *DNM2*, respectively..

Both girls who are age 16 and 7 years, had onset of mild distal weakness, generalized hypotonia, ptosis and opthalmoparesis in early infancy. Muscle biopsy showed internalized nuclei, radial strands, typical "necklace fibers" and neurogenic-

like features. In both, serum CK levels were normal or mildly elevated. There were no involvements of heart and respiratory muscles.

The eldest patient harbored a *de novo* heterozygous splice-site mutation in *MTM1* already described in XLMTM and a heterozygous variant of unknown significance in *LMNA* on the paternal allele. The youngest child harbored a novel heterozygous missense change in *MTM1*, a variant located in a critical protein domain and deemed to be predictably deleterious by in silico investigations. She also carried a heterozygous missense of uncertain significance in *DMN2* on the maternal allele. Study on different tissues in the patients did not suggest preferential X-inactivation.

Our results suggest that other muscle disease associated gene could play a role in the clinical severity of manifesting carriers in XLMTM. Further molecular and cellular studies are sought to confirm this hypothesis.

NGS target re-sequencing approach for undiagnosed persistent hyperckemia

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Asymptomatic or mildly symptomatic persistent hyperckemia albeit extremely common represents a diagnostic challenge for being a subtle and unspecific manifestation of different muscular dystrophies, metabolic conditions and several other neuromuscular disorders.

The diagnostic work up of hyperckemia is complex and requires a growing list of investigations, gene testing and accurate analysis of muscle biopsy. Still in a high percentage of patients the specific diagnosis remains pending despite a comprehensive medical effort.

In this study we applied a NGS approach to screen 20 genes (ANO5, LMNA, CAPN3, FKRP, FKTN, CAV3, RYR1, AGL, ENO3, GAA, LDHA, PFKM, PGAM2, PGK1, PGM1, PYGM, LAMP2, ACADVL, CPT2, LPIN1) which are a known cause of hyperckemia either because associate with muscular dystrophy or with other genetically determined metabolic conditions, all of which are not easily spotted at muscle biopsy because of unspecific presentation.

Ampliseq/Ion Torrent PGM technology was used. Analysis of variants was performed using IonReporter and CLC softwares setting a minimum coverage of 20X. Validation of selected rare variants was obtained by Sanger.

Eighteen patients have been fully analyzed. 38 variants were validated and classified into three categories: 13 likely pathogenetic (34%) - 7 uncertain significance (18%): - 18 likely benign (47%).

We were able to reach a definite genetic diagnosis in 4 cases (22%) including two patients with two mutations in ANO5, a patient with homozygous mutation in CPT2 and a patient with dominant clinically-associated mutation in RYR1. In addition we identified 5 cases with a single variant in either ANO5, ENO3 and FKTN recessive genes which need further investigations.

A new approach on muscular involvement in DM1 patients: EMD, MMG and force combined evaluation

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Electro-mechanical delay (EMD), mechanomyogram (MMG) and force combined registration, during contraction and relaxation, were applied on a cohort of eleven DM1 male patients (age: 38 ± 15 yrs; body mass: 75 ± 14 kg; stature: 1.78 ± 0.07 m; onset: 28 ± 11 yrs; CTG expansion: 428 ± 305; mean ± standard deviation) and eleven age- and body-matched healthy controls.

DelayTOT and R-DelayTOT electrochemical and mechanical components were calculated from *Tibialis anterior* and *Vastus lateralis* muscles, and compared with commonly used scores for clinical evaluation in DM1 patients such as MRC, MIRS and Rivermead.

We found significant differences between patients with DM1 and HC both in DelayTOT and R-DelayTOT components: torque output was significantly lower in DM1 than in HC (-39%, -29%, -52%, and -48% in TA and VL muscles, under electrically-evoked and voluntary contractions, respectively; $p < 0.05$); delayTOT and R-DelayTOT components were significantly longer in DM1 compared to HC in both muscles and under both contraction regimes.

These findings suggest that an EMD, MMG and force combined approach may be used as a valid tool to assess neuromuscular involvement and impairment degree. Furthermore given the strong correlation with clinical evaluation score this method could be also used as a follow up to test the efficacy of pharmacological or non-pharmacological interventions.

Our purpose is to further extend this study by evaluating the correlation between *Tibialis anterioris* EMD and the atrophy degree shown in needle biopsies from the same muscle.

Role of swallowing-breathing coordination on oropharyngeal dysphagia in patients with Myotonic Dystrophy Type 1 (DM1)

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Although dysphagia is seldom a complaint in DM1, oropharyngeal and oesophageal abnormalities are present early in the disease and may be responsible for pneumonias, the most common cause of death in adult DM1.

To determine the existence and frequency of subclinical physiological abnormalities in oropharyngeal swallowing and (ii) to explore the role of coordination between respiratory and swallowing pattern abnormalities.

Combined Fiberoptic Endoscopic Evaluation of Swallowing (FEES), respiratory phase and submental S-EMG recordings were analyzed in fifteen patients with adult-onset DM1 (mean age 48 ± 11.31). The severity of swallowing dysfunction

was determined using the Penetration Aspiration Scale (PAS) and the Dysphagia Outcome and Severity Scale (DOSS). For each patient manual muscle strength (modified MRC) and respiratory parameters were collected. Data were compared to 15 age- and sex-matched controls.

FEES detected mild to moderate dysphagia in 10 apparently asymptomatic patients (66.6%). None required non-oral feeding but diet counselling and swallowing exercise training were provided. In 74% of swallows, deglutition was followed by an expiration phase. Percentage of inspiration-deglutition-inspiration patterns correlated to viscosity and bolus consistency. Mean swallowing apnea duration was 2.66 sec depending on bolus viscosity and size. Mean number of swallows/bolus was 2.8. A strong correlation between FVC/FEV1 and swallowing apnea duration was found ($r = 0.881/r = 0.952$).

We recommend to evaluate oropharyngeal motility early in the disease process so that adequate nutritional counselling and management can occur. Our data also suggest that factors other than muscle weakness and myotonia may be involved in oropharyngeal dysphagia in DM1.

Congenital (CDM) Myotonic Dystrophy: a retrospective observational study in an Italian cohort

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The natural history of congenital (CDM) myotonic dystrophy has been described mostly in the UK, US and Canadian cohorts but data in Italy are scanty especially regarding mortality and long term respiratory outcome.

A retrospective chart review was performed in 39 CDM patients from 4 neonatal intensive care units and 5 neuromuscular units. Antenatal information, gestational age, birth weight, CTG expansion size, Apgar scores, oxygen saturimetry, timing and duration of assisted ventilation were obtained. Mortality at birth, number of children requiring tracheostomy, non-invasive ventilation or autonomous breathing at follow-up were recorded (mean follow-up: 17 years, range 1-31). Motor function, developmental and nutritional assessments were also included. 35 patients showed respiratory distress at birth (90%). Nineteen (54%) were weaned off ventilation; 6 patients required tracheostomy (17%); 12 patients (34%) required non-invasive ventilation for 10-14 hours/day while two 18-months old children (6%) are still on IV. All patients had delayed motor milestones. Only 1 child required gastrostomy tube at birth. All children were discharged to their homes. Two patients < 12 months were readmitted for acute respiratory failure.

Our data confirm and strengthen previous reports demonstrating that respiratory failure is a major concern in CDM. There is remarkable clinical heterogeneity and prognosis is variable. How ventilation affects outcome is still to be clarified. Natural history data on larger cohorts are mandatory to provide physicians, caregivers and families with preliminary information on critical care management, family planning and disease burden and to interpret results of potential interventions.

STIM1 mutations at a common amino acid residue (p.340) identified in two individuals with a predominant muscle disease phenotype

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Dominant mutations in STIM1 have been identified in the complex phenotype Stormorken Syndrome (SS) and in non-syndromic tubular aggregate myopathy (TAM). All reported individuals with SS have a common p.R304W gain of function mutation in the coiled coil domain 1 of STIM1. In contrast mutations in TAM are restricted to the EF hand domain. We performed exome sequencing on patient 1 and identified a *de novo* STIM1 mutation. We subsequently selected a cohort of patients based on clinical phenotype and/or presence of tubular aggregates in the muscle biopsy and performed immunostaining for STIM1 and direct sequencing of the STIM1 gene. Two patients with STIM1 mutations were identified. Patient 1 has the common SS mutation (p.R304W), and exhibits features in keeping with this (thrombocytopaenia, miosis, aspenia, hypocalcaemia). Tubular aggregates were present in muscle biopsy and showed accumulation of STIM1. Patient 2 has a novel mutation at the same amino acid residue (p.R304G), and presents with a strikingly similar pattern of neuromuscular phenotype but, aside from miosis, no additional features of SS. The neuromuscular phenotype in both patients comprises myalgia, muscle stiffness, and reduction in range of joint movement, with mild weakness on examination. Our results show that the use of STIM1 for immunoanalysis in patients with tubular aggregates can be applied to screen for patients with STIM1 mutations. In addition, we report a novel mutation at the common SS amino acid residue in a patient with TAM and miosis.

Tibialis Anterior needle biopsy: a minimally-invasive tool in Myotonic Dystrophy type 1 clinical trial

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Myotonic Dystrophy type 1 (DM1) is a neuromuscular disorder resulting from expansion of CTG repeat in 3'UTR of DMPK gene. This mutation causes the accumulation of toxic RNA in nuclear foci that leads to alteration of alternative splicing mechanisms.

The ongoing therapeutic strategy for DM1 is targeting toxic RNA through administration of Antisense Oligonucleotides. According to this, it is important to develop new sensitive and minimally-invasive methods for monitoring the clinical trial. Tibialis Anterior (TA) needle biopsy is a minimally-invasive procedure that allows to obtain small tissues samples and that can be repeated on the same patient and on the same muscle over the time. Here we show that the very small muscle samples obtained by TA biopsies are enough to investigate several DM1 muscular biomarkers.

Two pieces of TA muscle were taken from 6 DM1 patients and 4 healthy subjects. Serial sections obtained from one piece (40 mg) were used for: i) histopathological evaluation; and ii) evaluation of the number of MBNL1/RNA foci. The other muscle piece (20 mg) was used for RNA extraction to assess: i) alternative splicing of 8 genes involved in the multi-systemic phenotype of DM1 (INSR, CAMK2G, CACNA1S, CLCN1, NFIX, PDLIM3, LDB3 and cTNT); and ii) DMPK expression levels.

Our work underlines the feasibility and the test-retest reliability of TA needle biopsy in combination with sensitive biomolecular methods as a useful tool for investigating the pathomolecular mechanisms in DM1 and for monitoring the efficacy of a therapeutic intervention in a clinical trial.

Natural ClC-1 mutations causing myotonia congenita reduce sensitivity to 9-AC

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Loss-of-function mutations of ClC-1 are responsible for dominant and recessive myotonia congenita (MC). We recently identified two novel ClC-1 mutations associated to MC in Italian families, the F484L located in helix N and the G270V located in helix G. Interestingly both mutations resides close to residues taking part to the putative binding pocket for 9-anthracenecarboxylic acid (9-AC).

The later is one of the more potent organic compound inhibiting heterologously expressed ClC-1 channels. Interestingly, none of the other ClC channels tested so far are as sensitive to 9AC as is ClC-1. It is also known to reduce the macroscopic resting Cl conductance of skeletal muscle (gCl) that is mostly carried by ClC-1. The drug acts strictly from the intracellular side of the channel and its action is strongly voltage-dependent, reducing currents mostly at negative voltages. Residues in the ion conducting pore have been shown to be pivotal for drug block, forming a putative hydrophobic binding pocket.

By whole-cell patch clamp, here we tested the sensitivity to 9-AC applied from the outside of MC mutant channels expressed in tsA201 cells.

Our preliminary results show that both F484L and G270V pore mutants are insensitive to 9-AC inhibition. Interestingly, 9-AC slows the kinetics of G270V channels activation, thus suggesting that this mutation might hamper 9-AC reaching its blocking binding sites.

It is expected that such structural and pharmacological information would contribute to the rational design of much-needed therapeutic agents.

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Severe rhabdomyolysis in a patient with "Heat Stroke"

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We reported a case of a 42 year-old patient with a severe rhabdomyolysis (CPK > 250.000UI, myoglobin > 4000UI), not related with exercise or anaesthesia.

The patient worked as roofing felt layer and reported a negative family history for neuromuscular disease and, since 3 days before the hospital admission, a normal working day activity with a mild

pyretic status.

Neurological examination at admission showed a mild dysarthria and a weakness of lower limb muscles (MRC3), he was pyretic (38.4°C). His brain MRI showed extend colliquative necrosis areas in cerebellar dentate nucleus, related to ischemic lesion due to heat-stroke.

A muscle biopsy was performed but any significant abnormalities were detected at morphological and biochemical analysis.

Due to the presence of acute renal failure, dialysis was started. After 36 hours since the hospital admission, the patient became lethargic, dyspneic and was transferred to intensive care unit. A multiorgan failure was present and the patient died three days later.

Rhabdomyolysis often occurs in "heat stroke" syndrome, but its cause has not been established yet.

In our case different conditions could have a role in the induction of massive muscles necrosis (acid-base disorder, acute temperature increase, muscle ischemia, etc.); the normality of the biochemical muscle profile lead to exclude the metabolic genesis. This case brings to attention to investigate previous exposure to heat source in patients with rhabdomyolysis, hyperthermia, cerebellum or other focal neurological signs.

Longitudinal assessment of respiratory function in Duchenne muscular dystrophy (DMD)

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We have been monitoring the respiratory function in 95 DMD patients (aged between 6 and 24 years) who were evaluated once or twice a year in relation to age and clinical conditions, making a total of 597 evaluations. During every evaluation, forced vital capacity (FVC%), nocturnal oxygen saturation and respiratory muscle action during quiet breathing at rest in supine position were respectively measured with spirometry, pulse oximetry and opto-electronic plethysmography. The impact on respiratory function of steroid therapy, scoliosis and spinal fusion have been evaluated.

Data showed that FVC% was significantly higher ($p < 0.05$) in patients in the age range 10-13 years who currently took steroids compared to the peers not under steroid therapy; higher

values of FVC% were recorded also in older treated DMD compared to untreated ones. Moreover, FVC% significantly decreased with the severity of scoliosis (Spearman correlation coefficient < -0.49) and worsened after spinal fusion that is not an ameliorative treatment of the restrictive DMD lung. Steroids and scoliosis did not show any effect and/or correlation with nocturnal oxygen saturation and the ventilatory pattern at rest.

A unique myopathy syndrome in a patient disclosing clinical, laboratory, and genetic findings of late-onset Pompe disease, together with a lack of dysferlin on muscle biopsy

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Autosomal recessive late-onset Pompe disease (LOPD) is caused by compound mutations of the acid-alpha-glucosidase gene (GAA) which lead to deficiency of GAA enzyme activity and accumulation of glycogen within the autophagic vacuoles. Clinical hallmarks are a limb–girdle-like myopathy and ventilator failure due to diaphragm involvement. Dysferlinopathy is an autosomal recessive myopathy due to a mutation in the DYSF gene which causes deficiency of function of the protein dysferlin, involved in muscle repair. Symptoms manifesting in early adulthood primarily affect the limb–girdle skeletal muscles, and leave the heart and diaphragm spared. The unusual case of a patient with a clinicopathologic pattern of both LOPD and dysferlinopathy is described.

A 39-year-old male came to our observation complaining for 2 years of progressive ventilator insufficiency and fatigability, moderate myalgia, and difficulty walking. Blood muscle enzymes, ventilatory function, electrophysiology, and total body muscle MRI studies were performed. Glycogen storage as well as GAA activity were assessed by peripheral blood smears and muscle biopsy. Genomic DNA was extracted from blood leukocytes.

Mild weakness and atrophy mainly involved the anterior leg muscles; the thighs and hips were involved to a lesser extent; upright posture and gait were only possible with a double support. The upper limbs and shoulders were substantially spared. Serum muscle enzymes were increased by 1.5–2 times the norm; EMG showed a myopathic pattern in the lower limbs and hip muscles and pseudo-myotonic discharges in the paravertebral muscles of the lower back. Forced vital capacity was reduced by 20% of the expected values when standing, and further decreased in the supine position. GAA activity was 2.54 μmol/h/L on DBS, suggesting the diagnosis of LOPD, which was further supported by the finding of numerous glycogen granules within anti-LC3II-positive autophagosomes in lymphocytes on blood smears. Mutation analysis of the entire gene disclosed only a very rare c.2276G>C genetic variant in exon 16 on one allele of GAA, which was confirmed by cDNA studies. Muscles microscopy revealed a mild dystrophic pattern without any evidence of PAS and acid phosphatasepositive vacuoles in the muscle fibers, and a rather markedly reduced expression of dysferlin confirmed by Western-blot.

Our data reinforce the advice that diagnostic protocols should be as complete as possible in LOPD, and stimulate discussion on the criteria for enzyme replacement therapy in heterozygous patients.

Effectiveness of neurorehabilitation on a child with Pompe disease receiving Enzyme Replacement Therapy

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Pompe disease is a rare, progressive and often fatal muscular disease. The underlying pathology is a deficiency of the enzyme acid alpha-glucosidase that hydrolyzes lysosomal glycogen. The advent of enzyme replacement therapy (ERT) for this condition will necessitate early diagnosis. An optimal disease management includes a multidisciplinary approach of evaluation, intervention and monitoring.

The authors describe a patient with non-classic form of infantile-onset Pompe disease. This 21-months-old male is the first child of a non-consanguineous parents. The first neurological examination revealed a moderate hypotonia with waddling gait, difficulties in postural changes and facial weakness. Oral communication resulted compromised and he showed also obstructive sleep apnea. Heart echocardiography were normal.

The diagnosis of Pompe disease was confirmed and he received ERT. Furthermore he started physiotherapy and speech-language therapy; nocturnal noninvasive positive pressure ventilation was also prescribed.

At follow-up at the age of 30-months and 40-months, patient developed progressive significant improvement of neuropsychological profile and a positive effect on muscular strength with more stable gait and advancement in postural changes; the respiratory involvement was sufficiently stable.

Rehabilitation management of Pompe disease should preserve motor and physiological function, prevent or minimize secondary complications and maximize the benefits of ERT. Aerobic exercise, in particular, may be used in conjunction with ERT to attenuate skeletal muscle wasting and loss of motor function. Cognitive function is usually thought to be normal in these patients, but it's possible that subtle impairments might become apparent if the natural history of infantile Pompe disease is preferentially modified by ERT.

Next generation sequencing analysis in a group of Limb Girdle Muscular Dystrophies patients

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Recently the application of new sequencing technologies to neuromuscular disorders has allowed a quicker detection of mutations. However this method produces a high amount of data and interpretation is often difficult.

We studied, through the dedicated Next-generation sequencing (NGS) MotorPlex covering 99 muscle genes, a group of 38 unresolved Limb Girdle Muscular Dystrophy (LGMD) cases belonging to a sample of 174 LGMD subjects. In these patients the best candidate genes as suggested by protein analysis or clinical presentation were previously ruled out.

We identified 164 variants, titin, dysferlin and calpain-3 being the most polymorphic genes.

Correlation with bioptical and clinical aspects allowed to reach a definitive diagnosis in 16 patients (42%). In 1 LGMD2B and 3 LGMD2A subjects with single heterozygous mutations in autosomal recessive genes at Sanger analysis, NGS detected the second mutation. Through NGS we also identified 2 LGMD2B, 1 BMD, 1 LGMD2I, 1 LGMD2D, 2 LGMD2L, 1 LGMD2K, 2 LGMD2A patients and 2 patients carrying mutations respectively in LAMA2 and GAA gene. The 2 LGMD2L patients were previously diagnosed by Sanger sequencing as carriers of heterozygous mutations in FKRP gene. Interestingly in 3 patients of this sample the second mutation was demonstrated only after Sanger sequencing.

A probable diagnosis was reached in 12 cases but must be further confirmed, while in the remaining patients the gene variants are not remarkable.

Overall NGS is a fast method of analysis and can be useful especially when protein study is not possible. The interpretation of the results is often complex and a correlation with clinical data and the confirmation by Sanger sequencing are essential.

Ischemic stroke after cardiac arrest in a young patient as a terminal stage of an unrecognized Danon disease

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Danon disease is an X-linked multisystem disorder, due to the primary deficiency of Lysosome-Associated Membrane Protein 2 (LAMP2). The classic clinical presentationis characterized by hypertrophic cardiomyopathy, skeletal myopathy and mental retardation, but unusual features have emerged in the last few years.

We describe a case of a 20-year-old man with long-term cognitive impairment who was admitted at 19 years of age to the Cardiological department for dyspnea and chest pain.

His ECG was suggestive of Wolff-Parkinson- White (WPW) syndrome,whereasecochardiography revealed a severe hypertrophic cardiomyopathy. During hospitalization, he experienced a cardiac arrest followed by an ischemic stroke with a prominent visual failure. Neurological examination evidenced visual loss but also diffuse muscle wasting and marked proximal muscle weakness at four limbs. Muscle biopsy revealed a vacuolar myopathy with glycogen storage.

Immunohistochemical studies evidenced LAMP-2 deficiency and amolecular genetic analysis confirmed LAMP-2 deficiency with the identification of a novel mutation c. 1057 C>T (p. Q353X) of the gene. Cerebrovascular complications have been rarely reported in Danon disease, but this case suggests that in young patients with vascular disorders after cardiac arrhythmias, Danon disease has to be included in the differential diagnosis, especially in presence of signs and symptoms of skeletal muscle involvement.

Intracranial arterial abnormalities in patients with Late Onset Pompe Disease (LOPD)

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Pompe disease is a rare inherited metabolic disorder due to lysosomal alpha-glucosidase (GAA) deficiency. It has been recently considered as a multi-systemic disorder since, although glycogen accumulation is largely prominent in skeletal muscle, other tissues and organs are also affected. As regards, the cardiovascular system, only few reports have documented cerebrovascular malformations in patients with Pompe disease.

Aim of this study was to better define the presence and type of intracranial arterial abnormalities in a cohort of Late Onset Pompe Disease (LOPD) patients. We studied 21 patients (age range 16-76 years) of whom 4 had a presymptomatic hyperCKemia and 17 mainly presented with proximal/axial muscle weakness. A cerebral CT angiography, using MIP and VRT for 3D image reconstruction, was performed in all patients.

An unruptured intracranial aneurysm was detected in 2/21 patients (respectively 2 mm and 4 mm) (9.5%), a basilar artery fenestration in 1/21, a vertebro-basilar dolichoectasia (VBD) in 10/21 (47%) whereas posterior circle anatomical variants were identified in 5 patients.

These data confirm that the occurrence of cerebral arteries abnormalities is a quite frequent finding in LOPD patients, more often involving the posterior circle. Consequentially, performing a CT angiography or a MR angiography in all LOPD patients is recommended for early detection of cerebrovascular malformations. In fact, although rarely symptomatic, these abnormalities, if not timely diagnosed and serially followed, could lead to life-threatening events as sub-arachnoid haemorrhage or brainstem compression.

Clinical and pathophysiological clues of respiratory dysfunction in late-onset pompe disease: new insights from magnetic resonance imaging

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Respiratory insufficiency commonly develops in patients with Late Onset Pompe Disease. Until now, pulmonary function in LOPD has been evaluated with standard pulmonary function tests which do not extensively provide accurate definition of respiratory muscles pathophysiology.

We studied 11 LOPD patients (4 females and 7 males) and 5 non smoking controls. Pulmonary function tests included FVC in sitting and supine positions, MIP and MEP. MRI protocol included a sagittal Balanced Turbo Field Echo (B-TFE) breath hold scan, passing through the centre of the right diaphragm, acquired both at maximum inspiration and maximum expiration using a respiratory trigger as control. Then we calculated, the differential values between inspiration and

expiration lung (lung delta), and to assess diaphragm activity, we defined the diaphragmatic movement area (DMA). We compared pulmonary function results with data obtained from the MRI study.

In this study, we found abnormal variations in the cranio-caudal lung height and of lung areas in inspiration and expiration (lung delta) as well as the area of diaphragmatic movement. MRI data well correlate with pulmonary function tests in LOPD patients. In particular, there was a strong correlation between pulmonary function tests and diaphragmatic movement area, as expression of the diaphragmatic failure in LOPD patients.

MRI data allowed us to confirm that development of respiratory insufficiency in LOPD is mainly due to diaphragmatic weakness with sparing of the antero-posterior chest expansion, due to spared activity of the intercostal muscles.

Testing the predictive value of D4Z4 methylation status in FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is considered as an autosomal dominant disorder, causally related to reduced number (≤ 8) of 3.3 kb tandemly arrayed D4Z4 repeats on 4q35. The current model explaining FSHD pathogenesis favours the possibility that D4Z4 array governs 4q35 chromatin conformation leading to aberrant overexpression of nearby genes. Consistent with this model it was reported that patients carrying 4q with a D4Z4 array less than 9 repeats (FSHD1) and contraction-independent patients (FSHD2), who carry D4Z4 array of normal size on both chromosomes 4q, display decreased level of D4Z4 methylation. In order to test the specificity and sensibility of this epigenetic signature we analyzed a cohort of 82 FSHD families (85 FSHD1 patients, 24 non-manifesting relatives and 35 FSHD2 patients), 49 healthy controls and 10 subjects affected with other muscular diseases. The study revealed that the methylation status of the D4Z4 region does not strictly correlate with the presence and severity of FSHD clinical phenotype as we detected both D4Z4 methylation profiles, hypomethylation (≤ 25%) and normal level of methylation (≥ 35%), in all subgroups. In conclusion, our screening indicates a low predictive value of D4Z4 methylation status in FSHD clinical and molecular practice.

Longitudinal assessment of Upper Limb function in DMD patients: 12 month changes

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While there have been considerable advances for outcome measures in ambulant children with Duchenne Muscular Dystrophy (DMD), no prospective longitudinal study has so far been devoted to the assessment of upper limbs. This information appears to be relevant for a better understanding of the disease progression in non ambulant patients. As a result of an international effort, a new tool, the Performance of Upper Limb (PUL) was specifically designed to assess upper limb function in DMD boys. The purpose of the PUL is to assess changes that occurs in motor performance of the upper limb over time both in ambulant and non-ambulant DMD boys and adults. A recent cross sectional study has demonstrated the ability of the PUL to assess a wide range of activities.

The aim of the present study was to use the PUL in a cohort of DMD children and young adults to assess the range of findings at different ages and their changes over 12 months.

We also aimed to focus on the non ambulant subgroup in order to establish the possible benefits of glucocorticoids (GC) after loss of ambulation

The results showed a progressive deterioration of scores with age, with early involvement of the proximal muscles that was more obvious after the age of 10 years in the proximal domain and after the age of 15 in the more distal ones. The PUL Scale appears to be a useful tool for upper limb motor disease assessment in DMD ambulant and non-ambulant patients, that may be used in clinical trials and in clinical settings.

Benefits of GC on upper limb function in non ambulant DMD patients

Of the 91 non ambulant pts in our total cohort, 48 were on GC (mean age:16,98) and 43 were not (mean age 16,97). The mean total scores at baseline were 47.92 in the group on GC, and 33.70 in the untreated group. The mean changes were -3,79 in the treated group, and -5,07 in those who were not on steroids.

The difference was more evident on the middle domain, especially in the GC treated patients between the age of 12 and 18. In contrast, at shoulder level, the treated group appeared to have more negative changes compared to the untreated one, as since the GC untreated all scored 0 at baseline they could not lose any further point. Our data suggest that continuing GC after loss of ambulation appears to have a beneficial effect on upper limb function.

Genetic and clinical features of symptomatic DMD carriers: a pediatric cohort

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Female carriers of Duchenne Muscular Dystrophy (DMD), usually asymptomatic, might developed muscle weakness up to 17%, and one third present cardiac abnormalities or cognitive impairment.

Cases with a clinical picture similar to dystrophinopathy males have also been described. The clinical features of DMD carriers during childhood is poorly known.

We describe a pediatric cohort of DMD carriers providing clinical, genetic and histopathological features as well as the disease natural history with a mean follow-up of 7 years.

Fourtheen female carrying DMD mutations (age 5-18 years) are included. The age at diagnosis ranged between 1-8 years, with mean diagnosis delay of 1 year. Six patients (43%) presented with symptomatic onset, characterized by limb girdle weakness or abnormal gait, associated with exercise intolerance. The other 8 patients (57%) came to evaluation because of incidental finding of elevated CK. CK was elevated in all, ranging from 392 to 13000 U/L. Calf hypertrophy was observed in 8 patients (57%). No patient developed respiratory or cardiac involvement. The most frequent complication over time was scoliosis (43%). Four patients (29%) developed cognitive impairment with attention deficit. We performed electromyography in half of patients, showing a myopathic pattern in 4 (57%). Muscle biopsy revealed a mosaic reduction of dystrophin in 9 available cases and histopathological features well correlated with disease severity. DMD gene mutations were mostly deletions (71%), located between exon 44-55 and resulting in loss of reading frame in 5 cases. The three patients experiencing the most severe course were affected either by a nonsense, missense or frameshift mutation.

Our retrospective analysis suggest that DMD gene mutations may be suspected in female child with persistent hyperCKemia. Evidence of calf hypertrophy and myopathic pattern at electromyography may also be helpful. DMD carriers should be specially followed for orthopedic and psychiatric complications during childhood.

Muscle biopsy suggests a case of infantile neuroaxonal dystrophy

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Infantile neuroaxonal dystrophy (INAD) is an autosomal recessive degenerative disease with onset in the first or second year of life. Mutation in the PLAG2G6 gene encoding iPLA-VI, a calcium-independent phospholipase, has been identified in affected children. Psychomotor regression is the most frequent presentation, usually with ataxia and optic atrophy, followed by the development of tetraparesis. We report a 4 years old male who was born from cousin-cousin marriage who regularly acquired stages of psychomotor development until 18 months of life, when he presented a regression of obtained skills, with loss of expressive language capacity, ability to maintain upright posture and head control. Moreover, he developed a progressive cerebellar atrophy, a sensorineural deafness and an absence of voluntary activity of lower limbs on electromiography. The challenge of his clinical picture could lay for a mitochondrial encephalomyopathy or a neuroaxonal dystrophy. A muscle and a skin biopsy were performed. Muscle biopsy only showed both fibre types atrophy and extensive aspects of type grouping suggesting a neurogenic damage to deepen. Ultrastructural examination of cutaneous nerves highlighted myelinated and unmyelinated axons with many electrondense neurogranules, aggregates of neurofilaments, mitochondria and unspecific dense bodies suggesting dystrophic axons. Preliminar results of the genetic analysis showed that the child is carrying a homozygous mutation in the PLA2G6 gene.

Blood film examination for vacuolated and PAS-positive lymphocytes as diagnostic screening test for patients with late onset Pompe disease (LOPD).

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Pompe disease is an inherited metabolic multi-system disorder resulting in glycogen storage in different tissues, caused by a deficiency of the lysosomal enzyme acid alfa-glucosidase.

Glycogen storage is often a morphological marker in muscle biopsy of Pompe patients but it could be also present in other tissues. Abnormal cytoplasmic vacuolation of lymphocytes, detectable on routine blood film examination, has been recently proposed as possible screening tool in these patients.

We examined blood smear of 16 LOPD patients, aged 14-71 years. The cohort phenotype encompasses 5 patients with presymptomatic hyperckemia and fatigue, and 11 with proximal muscle weakness. We collected also peripheral blood films from 20 healthy controls and from 12 patients affected by other muscle glycogenoses. Blood samples were collected and analyzed by preparation of four blood films: two of them were stainedby May-Grunwald/Giemsa (MGG) and the other two by PAS for each sample.

The number of vacuolated and PAS-positive lymphocytes, expressed as percentage of 100 lymphocytes examined, was significantly higher in LOPD patients than in healthy controls and patients with other muscle glycogenosis (21% *vs*. 4% *vs* 2%, respectively; $p = 0.002$).

In this group of patients, we have shown that PAS-positive cytoplasmic vacuolation of lymphocytes in peripheral blood films could be considered as a reliable screening tool to support an early diagnosis of Pompe disease.

Dysphagia in Myotonic Dystrophy type 1: preliminary results of an integrated europhysiological and swallowing protocol

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Dysphagia is under-diagnosed in Myotonic Dystrophy (DM1) patients, which are unaware about it, and may cause nu-

tritional derangement and respiratory failure, secondary to ab ingestis pneumonia. Early diagnosis and management would be relevant to reduce morbility and improve quality of life of patients. We propose an integrated protocol to evaluate swallowing function in adult DM1 patients with the aim to define the real prevalence of dysphagia and to understand underlying pathogenetic mechanisms. Our protocol includes: laryngeal accelerometric and submental and cricopharyngeal (CP) muscles EMG recordings to evaluate Dysphagia Limit (DL) and swallowing jitter; needle EMG of genioglossus muscle (G-EMG); fiberoptic endoscopic evaluation of swallowing (FEES), quantitatively scored through the PAS and DOSS, and Water Swallow test (WST), Eating Assessment Tool (EAT-10) and Mini-Nutritional Assessment (MNA) surveys. Our preliminary results in 10 DM1 patients (6 M, 4 F, mean age 46 ± 10), compared to 6 healthy subjects (HS) (3 M, 3 F, mean age 30 ± 3), showed normal MUAPs mean duration of GEMG, with steady presence of myotonic discharges (9/10 patients). All DM1 patients had reduction in DL compared to HS. 6/10 patients displayed abnormal swallowing jitter, 5 of them showed anomalies in other parameters of the swallowing reflex. Myotonia in CP muscle was present in 3 patients, it didn't seem to be correlated to other swallowing dysfunctions. None of the patient was at risk for malnutrition using the MNA; 5 patients failed the WST and 4 presented an EAT-10 score > 3; 2 patients did not show signs of dysphagia at FEES; 4 and 5 patients presented signs of penetration respectively with liquids and semisolids. Our preliminary data confirm that swallowing problems are very common in DM1 and show that DL and swallowing jitter seem to be the most sensitive altered parameters in these patients. We are extending this protocol to a larger court of Italian patients.

Italian Registry of NLSDs. Clinical and genetical characterization

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Some cases of Neutral Lipid Storage Diseases, due to mutation of PNPLA2 and CGI58, are described in different ethnic group in the world, but there are few study regarding clinical characteristic, phenotypic variability and natural history in a large specific population. We have collected data of 21 Italian patients with NLSD, type M and type I, with long-term follow up. They received the diagnosis between 1 and 66 years, in 9 Neuromuscular Centers, in a collaborative study that involved neurologists, geneticists, and expert of lipid metabolism, starting 2013. Every center signalized the patients with diagnosis genetically confirmed. All, but two of patients, are alive after a median time of disease of 40 years, but frequently they had severe motor disability, that frequently start with asymmetric proximal deficit. Two patients with NLSD-I dead with epatic failure, one after a liver transplantation tentative. No patient is update mechanically ventilated. No patient received cardiac transplantation, but one NLSD-M received PM implantation. This study highlights some peculiar aspects of a specific population, in fact Italian NLSD patients differ partially from other patients, like japanese, that had prevalent cardiac compromission. Critical aspects for disability and life expectancy in Italian patients are arytmic cardiopathy and scheletric muscle atrophy in NLSD-M and liver disease in NLSD-I. Some factors, like diet and life style, can influence clinal characteristics of disease, because patients with the same mutation in the same family have different clinical involvement. This observation is important for the clinical application of the therapeutic strategies.

Gender differences in the occurrence of cataract in Myotonic Dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is the most frequent muscular dystrophy in adult, affecting 1:8000 individuals. It is a multi-systemic disorder involving muscle, heart, endocrine and respiratory apparatus and eye. The lens is particularly affected in DM1 and an early appearance of cataract is one of the most common and reliable symptoms of the disease. In the general population cataract is most frequently associated with old age, with values of 80% in people over 75 years.

In DM1, however, cataract can occur at a much earlier age and can even appear in the lenses of teenagers. Aim of this study was to investigate the gender difference – if any - in the occurrence of cataract surgery in a population of 243 patients (128M;115F), regularly followed at our Service. We found that 69/243 (28,4%) had cataract surgery, at an average age of 44,7 ± 11,2 years. Forty were males (31,2%, mean age 42,8 ± 9,8) and 29 were female (25,2%, mean age 47,3 ± 12,6 years). The differences in mean age and percentages were not statistically different (p = 1,29, Student t test for non paired data). However when we examined the data according to the age – more or less 40 years – at which males and females had cataract surgery, we observed that 21/40 (52,5%) males had cataract surgery before the age of 40 y, compared with only 5/29 females (17,2%). The differences were statistically different ($p < 0.001$, chi-square test). The factors possibly influencing these differences are examined and discussed.

Difficulty in detecting Alpha dystroglycanopathy: a case report

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Alpha dystroglycanopathy due to POMT2 (Protein O-mannosyltranserase 2) mutations is an autosomal recessive disease that is characterized by a large spectrum of clinical manifestations. Cardiovascular involvement is a rare report in congenital muscular dystrophies (CMD) with reduced glycosylation of alphadystroglycan (α-DG).

We describe 2 brothers whit a progressive muscular weakness, mental retardation, severe dilatative cardiomyopathy

and retinitis pigmentosa. Patients had a first muscular biopsy performed in young years showed dystrophic changes with vacuoles with Desmin accumulation. A new muscular biopsy performed after seral years showed normal immunostaining for Desmin and α-DG (anti-α-DG IIH6C4 antibody). Next generation sequencing (NGS), in search for dilatative cardiomyopathies genes, detected compound heterozygous of two novel mutations in POMT2 gene (p.Asp451 and p.Trp570Leu). Using programs predicting the pathogenicity, both mutations seemed to be deleterious. Fibroblast were grown from skin biopsy and cultured. Flow cytometry for the analysis of α-DG glycosylation in fibroblast is ongoing.

Clinical phenotype with severe cardiovascular findings is an uncommon report in POMT2 CMD and difficulties in defining central nervous system involvement complicate the diagnostic challenge. We hope that flow cytometry, allowing the detection of slight reduction in the level of anti-α-DG antibodies which may be equivocal by IIC, can confirm the dystroglycanopathy. Our data, also in progress, lead us to think about the non-diagnosis, made in past years based on the established clinical and bioptical criteria and the possible misdiagnosis based on newer methods.

Myositis ossificans in lupus panniculitis treated with Rituximab: a case report

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We report the case of a 64 years old woman, diagnosed in 2002 with dermatomyositis and successfully treated with high-dose steroid, iv immunoglobulin and azathioprine by another Center. At that time, a scleroatrophic lesion on the left breast skin was observed, defined as a "perivascular dermatitis with lobular panniculitis", compatible with a lupus erythematosus. Over the following years, the cutaneous lesion spread to left shoulder, right gluteus and abdomen, but no specific therapy was set. She came to our attention one year ago for a relapse of muscle symptoms (lower limb proximal weakness) and the presence of calcific lesions that included most of the abdominal wall and right gluteus. Muscle MRI and Rx confirmed the diagnosis of myositis ossificans, Due to inefficacy of steroid therapy, we tried a therapeutic approach with Rituximab, on the basis of two similar cases reported in the literature. We started on January 2015 with the standard "induction protocol" (375 mg/m^2 weekly for four weeks, then 375 mg/m^2 one and two months after the last administration), followed by a "maintenance phase" (Rituximab 375 mg/m^2 bimonthly for three times). So far, the patient received five doses in total, with an initial improvement of muscular strength and a transient increase of the cutaneous lesions, treated with topical antibiotic with benefit.

Retrospective study of a cohort of 508 patients affected by myasthenia gravis: from diagnosis to management

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Myasthenia gravis (MG) is an autoimmune neuromuscular condition in which antibodies directed against neuromuscular junction targets cause symptoms of fatigable weakness. Disease-modifying therapies for MG include chronic immunosuppression; exacerbations of MG often requires plasma exchange or intravenous immunoglobulin (IVIg). The natural history of MG is unpredictable. In the first few years the disease course is worst with subsequent gradual disease stabilization. We retrospectively evaluated 508 patients, aged from 12 to 95 years old, affected by MG. 418 patients were AChR abs positive (221 females and 197 males), with onset < 40 years old in 118 patients. 25 patients were antiMusk abs positive (16 females and 9 males) and 65 were seronegative (38 females and 27 males). An atypical presentation of MG was evident in 3% of cases. Diagnosis was achieved through clinical evaluation, antibodies' dosage, neurophysiological analysis, thorax CT scan. We considered the different courses of the disease among the three different groups of patients and within the same group. In our cohort, the percentage of ocular myasthenia had lesser clinical worsening episodes and high chance of complete stable remission. Generalized disease had less chance drug free remission. The risk of episodes of worsening persisted at a steady rate over a period of time of about 9 months. The risk of exacerbations was unpredictable and occurred in some cases after prolonged clinical quiescence, often related to reduction of immunosuppression. 1% of patients developed a recrudescence of thymoma even 8 years later extended thoracic surgery.

Combined CLCN1 and SCN4A mutations in the same patients: clinical and neurophysiological phenotype

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Non-dystrophic myotonias include Myotonia Congenita (MC), either dominant or recessive, caused by skeletal muscle chloride channel-1 gene (CLCN1) mutations, as well as Paramiotonia Congenita (PC) and Sodium Channel Myotonia (SCM), caused by dominant mutations in the voltage-gated sodium channel alpha-subunit (SCN4A). Symptoms and clinical features may overlap, thus the pattern of transmission addresses the decision on which gene to screen first.

We here describe two relatives concomitantly harbouring mutations in SCN4A and CLCN1.

Genetic screening first detected a heterozygous CLCN1 mutation (F167L), previously reported as either dominant or recessive in different pedigrees. Since the electrophysiology (lack of cMAP decrement after exercise; pattern of myotonia) did not satisfy the genetic findings, SCN4A was sequenced, which detected a novel mutation in exon 21, with a SCM phenotype.

At least 10 CLCN1 mutations are more or less ambiguously associated with both recessive and dominant pedigrees, with various explanations: an attractive one is the existence of a modifying (protective or aggravating) genetic factor, strictly related to the channel function, able to modulate the expressivity of the CLCN1 mutation. Our report indicate that candidate genes as CLCN1 modifiers may be searched for among other genes related to muscle channels, including SCN4A.

We discuss the peculiar aspects, the expected additive effect of the combined mutations in delaying muscle repolarization, and the need for more extensive genetic investigation when the clinical picture does not fit the genetic background. These cases provide an example of the complexity, overlap, and unexplained features of non-dystrophic myotonias.

The clinical counterpart of abdominal muscle weakness in late-onese type II glycogenosys (GSDII)

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The severity of respiratory dysfunction in GSDII is not always proportional to the degree of skeletal muscle weakness. Respiratory parameters may be influenced by the complex interactions between the diaphragm and the other muscles involved in respiration: these include posterior trunk muscles, possibly participating in inspiration, and anterior abdominal wall muscles, contributing to forced expiration.

We analysed trunk muscles MRI and respiratory function in 19 adults with GSDII, and considered a postural drop in vital capacity (ΔVC) ≥ 30% as a sign of diaphragm weakness.

We found that ΔVC was, among respiratory parameters, the most closely related to both anterior and posterior trunk muscles atrophy. Although the correlation was stronger with muscles which show an early, selective damage (*Multifidus, Longissimus,* and *Internal Oblique*), this does not seem to reflect disease severity only, since the other respiratory parameters, and upright VC in particular, did not show the same correlation.

Thus, upright VC does not seem to be influenced by abdominal wall weakness. Abdominal weakness does not increase, as expected, diaphragm compliance in clinostatism, but rather contributes to postural drop. These findings underline the contribution of abdominal weakness to respiratory dysfunction, not only in conditions of functional overload such as forced expiration, but also in physiologic conditions, especially in clinostatism. Detection of abdominal weakness may suggest the need of more extensive respiratory assessment, i.e. by polysomnography, even when upright VC is still within normal ranges.

Clinical and molecular features of a *POLG*-related mitochondrial disease case

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POLG-related diseases have an overlapping clinical spectrum involving primarily central and Peripheral nervous system, liver and muscle but also other organs such as the gastrointestinal tract. In general, theage, specific organ involvement and rapidity of progression may be quite variable. We describe the case of a woman came to our attention at the age of 40, for an two years history of myalgias, muscle cramps, exercise intolerance and progressive upper and lower limb girdle weakness. She was born from consanguineous parents, both native of Lunigiana, a mountain area of north Tuscany. The family history was inconsistent for muscle disease; her younger son was affected by epilepsy and mild cognitive delay.

Neurological examination revealed a mild bilateral eyelid ptosis and a moderate-severe hyposthenia at the scapular and pelvic girdle muscle level.

Blood creatine kinase level was increased (up to 1000 U/L).

A delayed recovery in lactate kinetics after ischemic forearm exercise was detected.

Electromyography revealed a myopathic pattern. During the first year of follow-up, the clinical picture further worsened. The patient developed stress urinary incontinence due to pelvic floor muscle impairment. Moreover, she progressively complained a gastrointestinal dysmotility, with abdominal cramps, diarrhea alternating with constipation. Consistent with the syndromic clinical picture, we hypothesized a mitochondrial disorder, confirmed by the muscle histological investigations, showing numerous cytochrome c oxidase–deficient fibers.

Multiple deletions of mitochondrial DNA (mtDNA) in muscle tissue were detected and the sequence analysis of the *POLG* gene revealed the homozygous sequence variant c.1156C>T (p.R386C).

A very slowly progressive brachial amiotrophic diplegia

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The term "Brachial Amiotrophic Diplegia" has been used to identify a primary sporadic motor neuron disorder which remains largely restricted to the upper limbs over time. Only a few cases are described all over the world with a follow-up ranging between 2 and 11 years.

It is one of the causes of the clinically heterogeneous condition named "man in the barrel syndrome" (MIBS) characterized by brachial diplegia and preservation of motor function in the leg and facial muscles, giving to the patient the appearance of seeming constrained in a barrel.

We report on a 69-year-old patient having a 35-year history of slowly progressive bilateral weakness of the proximal segments of upper limbs and shoulders. Clinical evaluation showed severe weakness and atrophy of muscles of upper limb girdle while distal segments of upper limbs as well as lower limbs were preserved. Deep tendon reflexes were absent at the upper limbs and normal at the lower ones.

Electromyography showed chronic neurogenic abnormalities in the upper limb proximal muscles.

Cervical MRI and MRI of the roots, primary trunks, branches of division and cords of both brachial plexus were normal.

Interestingly, this patient has a 35-year history of disease with no evidence of clinical spreading to other muscle districts. This is the longest follow-up described of primary Brachial Amiotrophic Diplegia, thus strengthening the concept that this condition entails a very low mortality and a 2 favorable prognosis and needs to be distinguished from ALS variants such as upper limb onset ALS and flail arm syndrome.

Functional characterization of a C-terminal Nav1.4 mutation found in a patient presenting with myotonia and congenital myasthenia syndrome

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Voltage-gated sodium channels (Nav1.4) are essential for initiation and propagation of action potentials from the neuromuscular junction to the entire muscle. About forty mutations of Nav1.4 have been associated with myotonia; these mutations increase Nav1.4 activity, resulting in sarcolemma hyperexcitability, and consequent muscle stiffness. A few Nav1.4 mutations have been linked to myasthenia, but the molecular mechanism leading to myasthenic weakness is less clear, although a loss-of-function is expected. We report the functional characterization of a new Nav1.4 mutation, M1808I, found in a patient presenting with both nondystrophic myotonia and congenital myasthenia. The mutation was introduced in Nav1.4 cDNA and expressed in tSA201 cells used for whole-cell patch-clamp recording of sodium (INa) currents. The INa density and voltage-dependence of INa activation, fast inactivation, and slow inactivation were compared to those of WT. The only difference was found in the proportion of channels resistant to slow inactivation, which was greater for M1808I compared to WT. Interestingly, this effect was no more observable in presence of 0.5 μM intracellular Ca^{2+} or of the co-expressed β1 auxiliary subunit, suggesting that M1808 may be involved in the modulation of Nav1.4 by these factors. In conclusion, the impaired slow inactivation of M1808I likely contributes to sarcolemma hyperexcitability and myotonia; in contrast, the elucidation of molecular mechanisms underlying myasthenia warrants further investigation. Supported by Health Ministry (grant GR-2009-1580433) and Telethon-Italy (grant #GGP14096).

Identification of mutations in *TMEM5* in a child presenting Limb-girdle Muscular Dystrophy: is there room for an additional LGMD2 form?

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Syndromes associated with hypoglycosylated alpha-dystroglycan (a-DG) encompass a spectrum of clinical phenotypes ranging from severe muscular involvement with eye and brain abnormalities to adult-onset limb-girdle muscular dystrophy (LGMD) without central involvement. Recently, mutations in *TMEM5* have been described in families with severe forms of dystroglycanopathies.

We describe a 13-year-old boy with a LGMD phenotype presenting a mild psychomotor delay and a polymicrogyria-like syndrome at brain MRI. Having excluded mutations in genes related with the main forms of LGMD, we used a novel targeted gene array in next generation sequencing (NGS) (Dystroplex) to analyze the coding exons and flanking introns of 95 genes linked to CMD, LGMD or related diseases. We identified the homozygous c.139delG in *TMEM5* predictably associated with frameshift and early protein truncation (p.Ala47Argfs*42). The mutation in *TMEM5* had already been detected in a patient who presented with a muscle-eye-brain disease. Additional variants of predictable pathogenic significance emerging while analyzing the results of Dystroplex were the heterozygous c.1654-6A>G in *POMT2* and c.1079A>G/p.Y360C in *DOLK*.

To our knowledge, this is the first description of a relatively milder phenotype associated with mutations in *TMEM5*. Unlike previously described children, our patient did not show neural-tube defects, visceral malformations and gonadal dysplasia but shared with them mild mental retardation and structural brain abnormalities.

This case report offers additional evidence to the notion that mutations in genes of the a-DG glycosylation pathway can determine a wide phenotypic spectrum of disorders and corroborate the use of NGS methodologies as first-tier diagnostic approach in dystroglycanopathies.

Natural history and genotype-phenotype correlation in a large cohort of patients with Becker Muscular Distrophy (BMD): a retrospective study

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We studied the natural history of 97 patients with BMD, referred at the neuromuscular rehabilitative unit of UILDM Lazio and the Institute of Neurology of Catholic University in a long-term follow-up (up to 20 years). The diagnosis of BMD was based on muscle biopsy and/or identification of the mutation in the dystrophin gene. All clinical and functional markers of disease severity, including steady cardiopulmonary and muscular examinations, were collected. Clinical and disease characteristics as age at onset, motor impairment, ADL measures were analyzed. We found a wide spectrum of clinical phenotype, ranging from asymptomatic or paucisymptomatic patients (isolated elevated creatine kinase values, cramps, myalgia 38%, only tendon contractures 12%) to seriously compromised patients, non-ambulatory or needing ventilatory support (19%). Three patients underwent cardiac transplantation due to the presence of severe dilative cardiomyopathy. Data regarding neuro-rehabilitation program, orthopedic surgical intervention and use of orthesis were also collected.

The aim of our study was to establish clinical characteristics, natural history and genotypephenotype correlation in a large cohort of patients with BDM. In these years several novel therapeutic approaches in Duchenne Muscular Dystrophy (DMD) are ongoing in different steps of clinical trials, some of which with significant preliminary results. The differential progression of symptoms and the wider spectrum of clinical phenotype in BDM in comparison with DMD have important implications for the design of clinical trials. The definition of a detailed natural history of BMD and the identification of sensitive outcome measures are therefore mandatory for building up future clinical trials.

A preliminary protocol for management of chronic respiratory insufficiency in Myotonic Dystrophies: results of the 207th ENMC Workshop

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Respiratory problems are among the leading causes of death in adult Myotonic Dystrophy type 1 (DM1) and usually account for the high rate of mortality in the congenital form of DM1. Reports on a similar pattern of respiratory impairment in adult DM2 patients are growing.

We describe the results of the 207th ENMC workshop which was held in Naarden to discuss management of chronic respiratory insufficiency in Myotonic Dystrophies (DM).

A platform of 14 experts in DM and in respiratory care from across Europe, Canada and USA to share clinical pathways and to revise existing guidelines and recommendations for cough assistance and domiciliary non-invasive ventilation for a better management of chronic respiratory insufficiency in DM. Additional management issues including: peri-operative management, secretion management, weight control, physical exercise program and patient and carer education, training and acclimatization were also discussed.

Management and recommendations for respiratory involvement in spinal muscular atrophy (SMA) types I, II, III

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1st Italian SMA family association consensus meeting: January 30th-31st 2015, Rome, Italy

Data on respiratory assessments and management of SMA stratified by disease type and severity are still controversial.

The general aim of the workshop was to update existing recommendations for respiratory standards of care in SMA.

Twenty-three participants including 8 pulmonologists, 6 intensive care specialists, 2 pediatricians, 2 adult neurologists and 5 child neurologists in addition to SMA Family representatives and supported by the Italian SMA Family Association met in a dedicated workshop in Rome to revise management and recommendations for respiratory involvement in SMA.

Data were discussed and the panel of experts reached agreement on the following: (i) Description of the actual pathway of care in SMA I; (ii) Respiratory Assessment and Follow-up Recommendations in SMA II and IIIA; (iii) Criteria for NIV launching in SMA II and III; (iv) Secretion Management in SMA II and III.

Consensus was reached on a minimal set of requirements for respiratory assessment and management in SMA, according to disease severity and type. The necessity for further investigation on these preliminary results was emphasized as well as the mandatory need to explore delicate themes such as end-of-life care and ethical issues, especially related to SMA type 1.

A new double-trouble phenotype: fascioscapulohumeral muscular dystrophy and hereditary spastic paraparesis due to spastin mutation

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We describe a family with genetically confirmed overlapping diagnoses of hereditary spastic paraplegia due to *Spastin* mutation (SPG4) and facioscapulohumeral muscular dystrophy (FSHD).

Hereditary spastic paraplegia (HSP) is a disease group where the corticospinal tracts are involved causing lower limb weakness and spasticity. Mutations in the *spastin* gene are the most common cause of autosomal dominant HSPs. FSHD is the third most common muscular dystrophy characterized by an autosomal dominant mode of inheritance, facial involvement, selectivity and asymmetry of muscle atrophy and weakness.

The proband, a 56 year-old female, suffered from a progressive spastic gait first noticed at age of 20. Since the third decade, she developed a progressive, proximal, lower limb weakness and her ambulation changed from spastic into a waddling gait with bilateral foot drop. Molecular testing revealed a mutation in the *SPAST* gene. In the following years, a 24 year-old nephew was diagnosed as having FSHD based on the clinical phenotype and genetic analysis showing a deletion of 3.3kb DNA repeats. We therefore extended the molecular testing for both *SPAST* and FSHD and performed muscle MRI to all the willing family members, showing the coexistence of the two gene mutations in at least 3 family members.

This family adds to the increasing number of unique patients presenting with atypical phenotypes, particularly in FSHD. This "double trouble" overlapping syndrome produce a spasticity compensation and underline the need of further genetic testing in unusual clinical presentations even if a mutation in one disease gene has been found.

New mtDNA mutations causing mitochondrial myopathies

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We report on two novel mtDNA mutations in patients affected with mitochondrial myopathy. The first patient, a 56 year-old man,

had four limb muscle weakness and a m.4440G>A *tRNAmet* mutation; the second patient, a 44 year-old woman, had chronic progressive external ophthalmoplegia and a m.8305C>T *tRNA lys* mutation. Muscle biopsy from both patients showed RRFs and many COX-negative fibers. These mutations were present only in muscle tissue and were heteroplasmic with higher mutation load in COX-negative fibers (well above 80% and near to 50%, respectively). In the second patient long PCR showed also multiple deletions which were not confirmed by Southern Blot, and the analysis of the most common "PEO genes" did not reveal any variation. Both mutations satisfy the canonical rules required for pathogenicity. In the PEO patient the lower m.8305C>T mutation load in COX-negative fibers, associated with the presence of multiple deletions, suggests the possibility that the clinical phenotype could be due to a synergistic role of this mutation with a still unknown nuclear variation.

Our report further reinforces the notion that mutations in mitochondrial tRNAs represent hot-spots for mitochondrial myopathies in adults.

Expanding molecular and clinical spectrum of POLG1 mutations

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Mutations in the POLG1 gene compromise the stability of mtDNA and result in extremely heterogeneous phenotypes which often have overlapping clinical findings, making difficult to categorize patients into syndromes, and genotype-phenotype correlations are still unclear.

We screened for POLG1 mutations 206 patients presenting with multiple neurological and/or extraneurological signs suggestive of mitochondrial disease.

We identified 8 previously reported and 4 novel POLG1 mutations in 13 patients, whose clinical signs included PEO, spastic tetraplegia, myopathy, polyneuropathy, ataxia, parkinsonism and intellectual disability. Only 3 patients were found to be compound heterozygous, while in the other 10 patients we observed the heterozygous state. We think that the novel identified mutations may have a pathogenic role, since they were not found in a group of 200 unrelated control chromosomes and the effect of these mutations was predicted as possible damaging using the polyPhen software program. In other 3 patients we found 2 probably benign variants.

The present study further characterizes the spectrum and the genotype-phenotype correlation of identified POLG1 mutations in our patients cohort.

Can myoadenylate deaminase deficiency be considered a well proven muscle disease entity?

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In our Center for Neuromuscular Diseases, we routinely use forearm ischemic exercise testing for blood lactate and ammonium in evaluation of patients with exercise intolerance, myalgias, muscle cramps and/or hyperCKemia (average one hundred and fifty exercise testing for year). In the last year, we detected lack of an increase in the blood ammoniun concentration during exercise only in three unrelated patients, all affected by exercise intolerance with muscle cramps and myalgia. In these patients genetic test confirmed mutations in *AMPD1* gene, suggestive of myopathy due to myoadenylate deaminase deficiency (MAD). The spectrum of the MAD deficiency ranges from asymptomatic carriers to patients who manifest exercise-induced muscle pain, fatigue, idiopathic hyperCKemia and, occasionally, rhabdomyolysis. Notably, starting from the observation that it is the most common muscle enzyme defect, found in about 2-3% of all muscle biopsies, MAD deficiency is not currently considered a well proven disease entity. Nevertheless, in people with muscle symptoms, *AMPD1* gene mutations seem to be significantly more frequent than healthy population, suggesting the clinical significance of this defect. For this reason, it can hypothesize that clinical heterogeneity may be due to other molecular genetic factors or due to metabolic conditions such as pathways compensating for the defect. Importantly, the real basis for the high percentage of asymptomatic homozygous subjects remains to be revealed.

Circulating microRNAs as biomarkers of muscle differentiation and atrophy

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Aims. Previous studies on amyotrophic lateral sclerosis (ALS) have focused on mechanisms that appear to be toxic to motor neurons. The identification of circulating biomarkers is urgently needed to facilitate 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 the phenotype at onset, in bulbar or spinal onset.

Methods. In 24 sporadic ALS patients (13 bulbar, 11 spinal) we measured the levels of muscle specific miR-206, miR-1, miR-133a/b, miR-27a by real-time-PCR, and investigated the plasma expression of the growth factors myostatin and follistatin, which are mostly produced in skeletal muscle where they act as negative regulator of muscle growth. A morphometric analysis of muscle fiber size was used to correlate muscle atrophy with biochemical and molecular parameters.

Results. We found a significantly increased expression of miR-206 and miR-133 in ALS patients compared to controls and also between spinal and in bulbar ALS. MiR-27a was also found to be significantly reduced in ALS patient than in controls. Myostatin/follistatin ratio was significantly higher in ALS than in controls and in bulbar ALS compared to spinal ALS. Bulbar ALS seems to be suffer from diffuse muscle atrophy than spinal ALS, as documented by muscle fiber morphometric analysis.

Conclusions. Muscle mass regulators are particularly down-expressed in bulbar ALS, suggesting a more rapid and diffuse atrophic process. We suppose that the biochemical and molecular indicators are involved in neuromuscular junction and reinnervation process.

HyperCKemia and safe anaesthesia table during surgery

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Reviews indicate that great majority of myopathy-related anaesthesia complications currently concerns subjects with clinically-unapparent and thus undiagnosed myopathy. To avoid these life-threatening untoward events we utilize during surgery the recently outlined "Safe Anaesthesia Table" (Trevisan CP, Accorsi A, Morandi LO, et al. *Undiagnosed myopathy before surgery and safe anaesthesia table*. Acta Myol 2013;32:100-5). It was applied without complications to 54 out of 2269 patients (2,4%) that in the last four years, at different Padua surgical institutions, were considered before surgery affected by possible undiagnosed myopathy. We present data focused on pre-surgical assessment of 769 consecutive cases, evaluated at ENT surgical Unit of Padua CMF. HyperCKemia was confirmed as the most significant marker of undiagnosed myopathy. Among children (357 aged 2-14) it was found in 7; while in adolescent and adult subjects (412 aged 15-72) it was apparent in 13. In their after-surgery clinical evaluation, in two children mild form of dystrophinopathy and calpainopathy were detected, while in three of the over-14 subjects dystrophinopathy, mitochondrial myopathy and proximal non-specific myopathy were the underlying pathologies. None was defined as Malignant Hyperthermia Susceptibility, although 14% of our ten-year series of HyperCKemia subjects were so diagnosed by caffeine-halothane contracture test.

Altogether, our data indicate: 1) Safe Anaesthesia Table allowed uncomplicated general anaesthesia in subjects with clinically unapparent myopathy; 2) HyperCKemia was the most frequent sign of undiagnosed myopathy; 3) HyperCKemia, as indicated by previous studies, may be expression of various silent myopathies, including dystrophinopathy and Malignant Hyperthermia Susceptibility.

The utility of myoimaging in the era of NGS. The example of a new mutation in *MYH7*

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Autosomal dominant (AD) eccentric core disease (ECD) is a congenital myopathy characterized byeccentric core disease (ECD) is a the presence of cores in the muscle fibers. Mutations in *RYR1* are observed in the large majority of eccentric core disease (ECD) is a AD-ECD families but recently a mutation in *MYH7*, encoding myosin heavy chain 7, has been eccentric core disease (ECD) is a detected in kindred with AD-ECD.

Herein, we present a family in which the proposita showed a late-onset distal muscle phenotype and eccentric core disease (ECD) is a subtle ECD in her muscle biopsy. Four relatives were also investigated. Traditional sequencing ofeccentric core disease (ECD) is a the *RYR1* gene revealed the heterozygous p.Glu294Lys variant in the proposita but not in four eccentric core disease (ECD) is a additional relatives who displayed a similar clinical phenotype.

Myoimaging showed in the proposita an almost complete involution of the anterior compartment eccentric core disease (ECD) is a leg muscles, particularly of the tibialis anterior. Similar MRI findings were detected in her relatives.

We used MotorPlex, a targeted gene array in next-generation sequencing, to screen in the proposita eccentric core disease (ECD) is a genes responsible for all known forms of inherited muscle disorders. Combination of myoimaging eccentric core disease (ECD) is a with results of MotorPlex allowed us to pinpoint the heterozygous p.Ser1435Pro in *MYH7* as the eccentric core disease (ECD) is a change with the highest probability of being a causative mutation. The heterozygous p.Ser1435Pro eccentric core disease (ECD) is a was also found in three living affected family members, but not in two healthy relatives.

In summary, combination of massive parallel gene analyses with recognition patterns at eccentric core disease (ECD) is a myoimaging might circumvent the difficulties created by the existence of multiple variants ofeccentric core disease (ECD) is a unknown significance emerging in diagnostic use of NGS methodologies.

Pre-operative training for scoliosis surgery in neuromuscular patients: the Genova experience

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Scoliosis surgery in neuromuscular (NM) patients may be associated with a high morbidity and even mortality. We report on our center' experience with a pre-operative training based on non-invasive ventilation (NIV) associated to cough assistance (CA) to improve respiratory outcome in neuromuscular patients undergoing surgical correction of scoliosis.

NM subjects who underwent scoliosis surgery between 2012 and 2014 were included. Mean follow-up was 2 years. All patients were trained to NIV and CA before scheduled operation and were started immediately after extubation. Age and type of surgery, training duration, intensive care unit (ICU) stay, complications and pulmonary function in medium term (6-12 months) and long term (> 12 months) were assessed.

Fourteen NM subjects (6 congenital myopathy, 4 Duchenne muscular dystrophy, 3 spinal muscular atrophy, 1 myotonic dystrophy type 1) were included: mean age 14.4 years (19.4-7.7), mean forced vital capacity (FVC) 45%pred (21-70%). Mean training duration was 5 days (3-7). Eleven underwent posterior arthrodesis, 3 correction by growing rods. Mean ICU stay was 18 hours (15-24 h). Main complications were surgical infections (2) and haemorrhagic gastritis (1). Mean improvement in medium-term postoperative FVC was 6.1% however long term follow up showed no significant differences.

Our data prove that this approach to neuromuscular scoliosis is effective in minimize time in ICU and maintain stable respiratory function.

Beneficial effect of ivabradine in dilated cardiomyopathy from Duchenne muscular dystrophy

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To date, no therapeutic consensus concerning progressive dilated cardiomyopathy or arrhythmia in dystrophinopathies currently exists. Beneficial effect of ivabradine (selective blockers of I(f) current in sinoatrial cell)has recently been reported in one patient affected by Becker muscular dystrophy (BMD)

We describe an 18-year-old male with Duchenne muscular dystrophy (DMD) due todeletion of exon 52 of dystrophin gene, who developed cardiologic symptoms effectively controlled by association of ivabradine.

The patient lost ambulation at 8 years of age, and, for echographic evidence of early sign of dilated cardiomyopathy associated with decrease in cardiac function, at age 9 years he started enalapril, and one year later carvedilol up to the dose of 25 mg/die. At 17 years, he developed acute respiratory failure and worsening of left ventricular function after spinal surgery. Furosemide (50 mg/die then down titrated to 25 mg/die) and spironolactone (25 mg/die) were started, and carvedilol was increased to 50 mg/die with benefit. Few weeks later the patient presented daily episodes of tachycardia (> 110 bpm) with dyspnea and he started non-invasive ventilation (NIV). At this time he started treatment with ivabradine at the dose of 5 mg/die associated with digoxin with complete resolution of the previous described symptoms. However, digoxin was suspended two weeks later for evidence of many ventricular extrasystoles (> 20000/die) at Holter recording. The suspension resulted in a reduction of the ventricular extrasystoles (4600/die at the last Holter recording) without onset of new clinical symptoms.

This is the first report showing that ivabradine can control heart symptoms in a DMD patient with dilated cardiomyopathy, by normalizing sinus tachycardia.

Bladder and gastrointestinal function involvement in late-onset Pompe disease

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Late-onset Pompe disease (LOPD) is a hereditary autosomal recessive lysosomal storage disease caused by acid alpha-glucosidase (GAA) deficiency. Striated skeletal tissue is not the only muscle tissue that accumulates glycogen; several autoptic studies have shown that even the smooth muscle tissue in many organs can accumulate this substrate.

Pompe patients followed at our Center were asked about symptoms in the upper and lower intestinal tract as well as urinary incontinence using the Gastrointestinal Symptoms Questionnaire (GSRS) and the International Consultation on Incontinence Questionnaire-Urinary Incontinence-Short Form (ICIQ-UI SF).

Eleven patients answered (6 females, 5 males), with a mean age of 51.8 yrs. All subjects were receiving enzyme replacement therapy (ERT). Most of them reported abdominal distension (72.7%) or flatulence (81.8%), whereas 36.3% complained of epigastric ache or abdominal pain. In some patients defecation episodes are increased (36.3%), in other patients are reduced with constipation (54.5%). Recurrent diarrhoea was reported by three patients (27.2%). Stool urgency was reported by one patient. Four women out of six reported mild urinary incontinence with urgency; a male patient suffered from a severe urinary incontinence but probably secondary to prostate surgical treatment.

Incontinence and gastrointestinal dysfunctions are rarely studied in myopathies but patients often complain of symptoms that may cause significant disability in social and professional life. The gastrointestinal and bladder function involvement is still poorly studied in adults with Pompe disease. These single-center data are preliminary and require a more extensive instrumental evaluation.

Immune-mediated, statin-related myopathy due to anti 3-hydroxy-3-methylglutaryl-coenzyme. A reductase antibodies: a case report

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A 67 years-old woman presented an increase in CK value dating back to 2011 after she started to take statins (376 UI/l). Despite statin therapy suspension in 2012, CK levels progressively increased over two years up to a value of 6733 IU/l. In April 2014 she started to complain of progressive proximal muscle weakness and fatigability and to present episodes of (paroxysmal) atrial fibrillation without any respiratory or swallowing dysfunction. EMG study showed a mixed pattern. Muscle biopsy showed a primitive necrotizing myopathy without inflammatory infiltrates. Screening for malignancy did not reveal any abnormalities and thyroid function was normal.

Both clinical history and diagnostic test results led to a hypothesis of immune-mediated myopathy induced by statins, for this reason an anti 3-hydroxy-3-methylglutaryl-coenzyme A reductase antibody assay was performed and found positive.

The patient was given high-dose steroid therapy with partial benefit consisting in moderate CK decrease (up to 1000 IU/l) and mild improvement of muscle strength. Azathioprine was administrated for about one month, then stopped for adverse hepatic reaction.

We therefore decided to administer iv IgG and we obtained a progressive, generalized normalization of muscular strength and a further reduction, though not complete normalization, of CK levels.

We are now going to start methotrexate in combination with iv IgG. Statin-induced immune-mediated myopathy is a still underestimated and underdiagnosed disease that should, however, be considered given the large use of statin therapy in our society.

Teriparatide (rhPTH) treatment in Duchenne Muscular Dystrophy, 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 fractures may occur leading to severe pain and worsening of respiratory function. Daily

injection of 20 mcg teriparatide (rhPTH) was previously proven to enhance bone formation and bone mineral density (BMD) and to reduce vertebral fracture rate in postmenopausal and glucocorticoid-induced osteoporosis, but no data are available in DMD.

We describe the case of a 20-year old GC treated DMD subject, suffering from multiple symptomatic, severe vertebral fractures, treated with rhPTH. BMD was measured by DXA at lumbar spine (L1-L4) and vertebral fractures were defined by morphometric examination, in accordance to Genant's classification, at baseline and after 12 months. Bone resorption (CTX) and bone formation (BGP) markers were assessed in addition to other laboratory and clinical data every 6 months for 12 months.

Three wedge fractures of grade 3 were recognized at baseline and a Z-score of -6.7SD. Calcifediol supplementation, previously given to maintaine adequate 25(OH)D levels, was administered in addition to rhPTH. After 12 months, Z-score value increased significantly (+10%) and no further clinical and/or morphometric vertebral fractures were detected. We observed an early increase (after 6 months) of bone turn-over markers, expecially for BGP, which maintained after 12 months, highlighting the anabolic window of treatment. Moreover, rhPTH significantly reduced back pain intensity after 3 months, with disappearance at 6 months.

These encouraging data suggest to extend treatment to a larger group of DMD patients with severe osteoporosis to test its efficacy.

Beta-sarcoglycanopathy: What's new? The Family Group of Beta-sarcoglycanopathy ONLUS was founded in 2013

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LGMD2E is a recessive autosomal disease caused by mutation in the gene, located on chromosome 4q12, encoding the beta-sarcoglycan, a major component of the DPC. Age of onset is between 2 years and the mid-teenage years. The clinical presentation includes progressive limb weakness (mainly of proximal muscles). Cardiac involvement occurs in 20% of the cases. For LGMD2E there is a substantial absence of dedicated scientific research up to now; no specific treatments are known and patients only receive some physical therapy to prevent worsening of muscle contractures.

Of interest, sarcoglycanopathies should be cured by gene therapy since the sarcoglycans genes are relatively short and with few exons, making them suitable for adenovirus-based therapy. Actually, the phase II – clinical trial for gene therapy of alpha-sarcoglycanopathy – is ongoing in USA. Since 2012, the families of the GFB ONLUS have been funding a project of gene therapy for LGMD2E led by prof. J. Mendell in Columbus Ohio USA with important two objectives:

1. Determination of pre-clinical efficacy of the transfer of human b-sarcoglycan gene, using recombinant adeno-associated virus to act as delivery vehicle, in b-sarcoglycan deficient mice.

2. Regulatory preparation for a "recombinant adeno-associated virus human b-sarcoglycan" gene transfer intramuscular clinical trial, including formal toxicology/biodistribution study and clinical vector production. The project approaches completion.

In 2013 it was established the volunteer organization named Family Group of Beta-sarcoglycanopathy Onlus (GFB Onlus www.lgmd2e.org) for stimulating both basic and clinical research**.**

In 2014 there was a second conference in Venice, where LGMDEuroNET was founded.

Botulin Toxin A affects muscle cells, but not muscle-derived fibroblasts

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Botulinum toxin (BTX) is widely used for treating muscle spasticity. Experimental results on BTX treatment in vivo are limited because spasticity has been difficult to reproduce in animal models. Reports are mainly focused on the effects of BTX at neuromuscular junction, while relatively little is known about the toxin effect on the muscle cell itself. We investigated cellular mechanisms and key mediators targeted by BTX using an in vitro approach in muscle cell cultures from normal controls.

On these cell lines we separated, by immunomagnetic selection, myoblast- and fibroblastenriched populations and differentiated myoblasts to myotubes. Myoblast, myotube and fibroblast cultures in basal conditions or treated with incobotulinumtoxinA, a purified type A BTX (BTX-A), underwent microarray investigation. Bioinformatics analysis revealed a major action of BTX-A on the transcriptome of muscle cells while the transcriptome of muscle-derived fibroblasts appeared very little affected. In myoblasts, mainly genes involved in cell cycle were downregulated; in myotubes, genes involved in inflammation were upregulated and genes involved in muscle contraction were downregulated.

Validation at transcript level, by RT-PCR, and at protein level, by immunocytochemistry and western blot, confirmed microarray results.

We demonstrate that BTX-A treatment in vitro affects intrinsic properties of the muscle cell.

The BTX effect on skeletal muscle in vivo could involve different aspects: by modulating cell cycle, BTX-A could reduce fiber regeneration, by increasing inflammation it could act on myonecrosis, and by downregulating contractile proteins it could reduce muscle stiffness. A future evaluation in patient spastic muscle may help ameliorating management and treatment of spasticity with BTX.

Pilot study of serial casting of ankles in muscular dystrophy patients

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Contractures of Achilles tendons (TAs) deteriorate the performance in daily living activities of neuromuscular patients. Nocturnal use of ankle-foot orthoses (AFOs) helps to prevent the progression of deformities. In clinical practice ankle serial casting is used to reduce TA contractures and to allow an improving in AFOs fitting, however only scanty reports focused on these aspects.

The aim of this work was to assess the effect of TAs' serial casting on: 1) joint physical examination (range of Motion (ROM)) 2) functional performances (six minute walking test (6MWT) and North Star Ambulatory Assessment (NSAA)) and 3) patient's perspective (using a self reported questionnaire), in ambulant patients affected by Duchenne muscular dystrophy (DMD) and limb gird muscular dystrophy (LGMD) 2A.

The protocol included three casting five days apart with the TA on a stretch and was proposed to patients with contractures <35°.

We included 17 patients (15 DMD, age range: 4-14yrs, 2 LGMD-2A). Our results showed in all patients a significant improvement of ROM of ankles. Only the younger patients had an improvement at 6MWT but no significant changes has been observed in NSAA scale. Thirteen out of seventeen patients reported an improvement of mobility and autonomy (self-reported questionnaire).

The procedure has been well tolerated by all patients, no adverse events have been reported. All patients received indication of daily stretching of TA and use of AFOs after treatment. Although further studies in a larger cohort will be required, our results suggest that serial casting may be a valid alternative to surgery.

Cutaneous features of myotonic dystrophy type 1 and type 2

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Vitamin D deficiency in myotonic dystrophy type 1 and 2 (DM1 and DM2) has been recently demonstrated by us and others. We also showed that cutaneous synthesis of vitamin D, rather than malabsorption and liver dysfunction, may be responsible for vitamin D deficiency found in DM. Skin changes, such as baldness and epithelial tumors, have been described in DM1. The aim of this study was to explore in detail the cutaneous features of DM1 and DM2 patients. Clinical skin examination was performed in 60 DM1 (mean age 44.6 years) and 15 DM2 patients (mean age 51,4) by means of dermoscopy. The number of nevi and other skin alterations were correlated to CTG expansion size and vitamin D levels. Compared to the general population, in DM1 and DM2 patients, a higher frequency of junctional nevi (52% and 50%, respectively), dysplastic nevi (30% and 17%), xerosis (33% for both forms), seborrheic keratosis (18% and 25%) and seborrheic dermatitis (28% and 25%) were found. Dermatofibromas were present in 9 DM1 and 1 DM2 patients, only 2 DM1 patients showed a pilomatrixoma, in 1 DM1 patient a basalioma was removed. In all, 22 nevi were excised and none showed melanoma features. In DM1 patients, the total number of nevi significantly correlated with CTG expansion size, whereas the number of junctional nevi and xerosis lesions inversely correlated with vitamin D levels. In conclusion, DM1 and DM2 patients display a high frequency of skin abnormalities, some of which correlate with genotype severity and vitamin D levels. Conversely, pilomatrixomas are not frequent in our population. Skin examination is highly informative, and should be performed in all DM1 and DM2 patients.

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