

Established in 1982 as Cardiomyology

ACTA MYOLOGICA

(Myopathies, Cardiomyopathies and Neuromyopathies)

Vol. XXXIV - May 2015

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

Founders: Giovanni Nigro and Lucia Ines Comi

Four-monthly

EDITOR-IN-CHIEF

Giovanni Nigro

ASSISTANT EDITOR

Vincenzo Nigro

MANAGING EDITOR

Luisa Politano

CO-EDITORS

Valerie Askanas

Giuseppe Novelli

Lefkos Middleton

Reinhardt Rüdel

HISTORICAL STUDIES EDITOR

Georges Serratrice



PACINI
EDITORE
MEDICINA

Established in 1982 as *Cardiomyology*

ACTA MYOLOGICA

(Myopathies, Cardiomyopathies and Neuromyopathies)

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

Founders: Giovanni Nigro and Lucia Ines Comi

Four-monthly

EDITORIAL BOARD

Ekram Abdel-Salam, Cairo
Corrado Angelini, Padova
Enrico Bertini, Roma
Serge Braun, Paris
Kate Bushby, Newcastle upon Tyne
Kevin P. Campbell, Iowa City
Marinos Dalakas, Athens
Feza Deymeer, Istanbul
Salvatore Di Mauro, New York
Denis Duboc, Paris
Victor Dubowitz, London
King W. Engel, Los Angeles
Michel Fardeau, Paris
Irena Hausmanowa-Petrusewicz, Warszawa
Fayçal Hentati, Tunis
Byron A. Kakulas, Perth
Frank Lehmann-Horn, Ulm
Carlo Minetti, Genova

Clemens Müller, Würzburg
Francesco Muntoni, Londra
Carmen Navarro, Vigo
Gerardo Nigro, Napoli
Anders Oldfors, Göteborg
Eijiro Ozawa, Tokyo
Heinz Reichmann, Dresden
Serenella Servidei, Roma
Piraye Serdaroglu, Istanbul
Yeuda Shapira, Jerusalem
Osman I. Sinanovic, Tuzla
Michael Sinnreich, Montreal
Andoni J. Urtizberea, Hendaye
Gert-Jan van Ommen, Leiden
Lord John Walton of Detchant, Oxford
Steve Wilton, Perth
Klaus Zerres, Aachen
Janez Zidar, Ljubljana



© 1981 Gaetano Conte Academy. All rights reserved

This journal and the individual contributions contained in it are protected by the copyright of Gaetano Conte Academy and the following terms and conditions apply to their use:

Photocopying

Single photocopies of single articles may be made for personal use as allowed by national copyright laws. Permission of the publisher and payment of a fee is required for all other photocopying, including multiple or systematic copying, copying for advertising or promotional purposes, resale, and all forms of document delivery. Special rates are available for educational institutions that wish to make photocopies for non-profit educational classroom use.

EDITOR-IN-CHIEF

Giovanni Nigro, Napoli

ASSISTANT EDITOR

Vincenzo Nigro, Napoli

MANAGING EDITOR

Luisa Politano, Napoli

CO-EDITORS

Valerie Askanas, Los Angeles

Lefkos Middleton, Nicosia

Giuseppe Novelli, Roma

Reinhardt Rüdell, Ulm

HISTORICAL STUDIES EDITOR

Georges Serratrice, Marseille

BOARD OF THE MEDITERRANEAN SOCIETY OF MYOLOGY

G. Nigro, *President*

L.T. Middleton, G. Serratrice, *Vice-presidents*

Y. Shapira, *Secretary*

L. Politano, *Treasurer*

E. Abdel-Salam, M. Dalakas, F. Deymeer, F. Hentati, G. Meola, G. Siciliano, E. Tizzano, A. Toscano,

J. Zidar

Co-opted Members: V. Askanas, S. Di Mauro, K. Engel, I. Hausmanowa-Petrusewicz, R. Rüdell

Acta Myologica is cited in Index Medicus/MEDLINE, Medicine, Excerpta Medica Database (EMBASE), Index Copernicus and monitored for coverage in Chemical Abstracts Service. The Journal is available on PubMed Central (<http://www.ncbi.nlm.nih.gov/pmc/journals/1221/>).

Journal printed with total chlorine free paper and water varnishing.

Acta Myologica publishes 3 issues per year in May/July, September/October, December.

Acta Myologica will be sent free of charge to the members of the Gaetano Conte Academy and of the Mediterranean Society of Myology.

All correspondence should be addressed to Gaetano Conte Academy, Viale dei Pini 101 - 80131 Napoli - Italy.

Tel./Fax: +39-081-5874438

E-mail: giovanni.nigro@unina2.it

Editor in Chief: Giovanni Nigro

Tribunal Authorization, Napoli N. 3827, January 10, 1989

The editor remains at the complete disposal of those with rights whom it was impossible to contact, and for any omissions.

Photocopies, for personal use, are permitted within the limits of 15% of each publication by following payment to SIAE of the charge due, article 68, paragraphs 4 and 5 of the Law April 22, 1941, No 633.

Reproductions for professional or commercial use or for any other other purpose other than personal use can be made following a written request and specific authorization in writing from AIDRO, Corso di Porta Romana, 108, 20122 Milan, Italy, E-mail: segreteria@aidro.org and web site: www.aidro.org.

Publisher

Pacini
Editore

Via A. Gherardesca

56121 Pisa, Italy

Printed by Industrie Grafiche Pacini Editore S.p.A. - Pisa - May 2015

CONTENTS

EDITORIAL

Increasing awareness of Myology: it's time for its recognition as an independent discipline within the Medical Sciences

Giovanni Nigro, Luisa Politano. 1

ORIGINAL ARTICLES

Muscle exercise in limb girdle muscular dystrophies: pitfall and advantages

Gabriele Siciliano, Costanza Simoncini, Stefano Giannotti, Virna Zampa, Corrado Angelini and Giulia Ricci 3

Clinical features of patients with dystrophinopathy sharing the 45-55 exon deletion of DMD gene

Antonella Taglia, Roberta Petillo, Paola D'Ambrosio, Esther Picillo, Annalaura Torella, Chiara Orsini, Manuela Ergoli, Marianna Scutifero, Luigia Passamano, Alberto Palladino, Gerardo Nigro and Luisa Politano 9

LETTER TO THE EDITOR

Guenter Scheuerbrandt. 14

PROCEEDINGS OF THE XII CONGRESS OF MEDITERRANEAN SOCIETY OF MYOLOGY

Naples, Italy – May 18-20, 2015

Program (Summary) 17

Abstracts of invited lectures and oral communications 18

Invited MSM Congress closing lecture. 29

Abstracts of poster communications (in alphabetical order of the first Author) 31

Author Index 35

PROCEEDINGS OF THE XV CONGRESS OF THE ITALIAN SOCIETY OF MYOLOGY

Naples, Italy – May 20-23, 2015

Program (Summary) 39

Abstracts of invited lectures. 40

Abstracts of oral communications (in alphabetical order of the first Author) 50

Muscle Club Session. 62

Abstracts of poster communications (in alphabetical order of the first Author) 64

Author Index 86

NEWS FROM AROUND THE WORLD

MSM 90

GCA. 90

AIM 90

THE ITALIAN NETWORK FOR LAMINOPATHIES 90

LGMD-EuroNet 91

WMS 91

FORTHCOMING MEETINGS 92

EDITORIAL

Increasing awareness of Myology: it's time for its recognition as an independent discipline within the Medical Sciences

In 1891, Charcot and Bouchard – two eminent French scientists – published a famous textbook of Internal Medicine (1), which included large chapters dedicated to all branches of Medicine and Neurology. The medical specialties, as we know them today, are all derived from the Internal Medicine during the 19th century. J.M. Charcot, who first argued the need for a specialist to direct the Chair of Mental Disorders at the Salpêtrière Hospital in Paris, performed all necessary steps – scientific, institutional and political – to claim and obtain for himself the creation of the first Chair of Nervous and Mental Diseases of the Old Continent in 1882 (2, 3). The same fate befell during the 20th century in other disciplines, such as Cardiology, Endocrinology, Gastroenterology, Pulmonology etc. just to mention the most important, and, more recently, Microbiology, Hygiene, Genetics, ... and Myology.

Myology (from *latin myos* “muscle” and *logia*, “logy”) is the science that studies muscles, their physical structure, type of fibers, specific function, and the connections between different muscle groups. Interest of myology are also muscle disorders. For most of the 20th century, Myology was considered as a little part of Neurology, while currently it is recognized as an autonomous discipline both at the research and the medical level.

From a research point of view, we have witnessed the birth and flourishing of new scientific societies, such as the European Society for Muscle Research (1970), the Mediterranean Society of Myology (1993), the World Muscle Society (1995), the Institute of Myology (1996), the Italian Association of Myology (2000), the British Myology Society (2009), the French Society of Myology (2011), and numerous conferences concerning muscular disorders. In particular the annual meetings of the World Muscle Society and Italian Association of Myology have been this year at their 20th and 15th edition, respectively, while the biannual meeting of the Mediterranean Society of Myology will see its 12th edition in Naples. Similarly, the number of papers focusing on the different aspects of myology and of scientific journals specifically dedicated to myology is in a continuous and constant increase. A

search in PubMed, under the heading “muscle” reported, at the date of 13 April 2015, 912119 papers and under the heading “muscle disorders” 168,931 papers, in a similar fashion the first scientific journals *Muscle & Nerve*, edited by Bradley in the USA in 1964, *Acta Myologica* edited in Naples by Giovanni Nigro and Lucia Ines Comi in 1982, and *Neuromuscular Disorders*, edited by V. Dubowitz in London in 1990, were rapidly followed in the last few years by the creation of *Skeletal Muscles*, edited by K. Campbell in 2011, *European Journal of Translational Myology-BAM* edited in Padua by U. Carraro in 2014, and the *Journal of Neuromuscular Diseases*, edited by Bonnemant and Lochmuller both in 2014.

Furthermore the textbook “Myology” edited by Andrew Engel (that is) considered the “bible” for lovers of Myology, has reached its third edition.

In the meantime the attention to the care needs of people living with muscle diseases increased so that many Clinical Centers of Myology were created, achieving international fame. To be mentioned among others the Center of Cardiomyology and Medical Genetics, at the Second University of Naples, founded by Giovanni Nigro in 1976, the Institute of Myology initiated in Paris, in 1996, and the Dubowitz Neuromuscular Centre (DNC) in London. All these Centres provide clinical assessment, diagnostic services, advice on treatment and rehabilitation, and are involved in clinical trials and in basic research focused on understanding the cause of neuromuscular diseases, and identifying novel therapeutic intervention. They became a point of reference for patients, families and doctors, as sites where specialized consultations, fundamental and clinical research teams and teaching on muscle and muscle diseases are grouped together in public hospitals.

The major development reached by Myology in Italy, in Europe and in the States, has been efficiently described by C. Angelini in a paper published in *Acta Myologica* in 2011 (5).

In spite of this intense and fascinating process, Myology is still considered the “Cinderella” of the medical disciplines and the researchers who are involved in the

field as “isolated people”. Furthermore the scientific journals publishing on the topic are listed among journals of internal medicine and/or neurological sciences, at the bottom of the rankings.

We are convinced that it is time for Myology to be declared a separate discipline among the branches of Medicine and solicit the help of Myologists worldwide to make this dream possible.

Giovanni Nigro

President of Mediterranean Society of Myology

Luisa Politano

Cardiomyology and Medical Genetics

Second Naples University

References

1. Charcot JM, Bouchard C, Brissaud E, et al. Traité de Médecine. Paris: G. Masson Ed 1891.
2. Goetz G, Bonduelle M, Charcot GT. Constructing Neurology. New York: Oxford University Press 1995.
3. Signoret JL. La création de la chaire de Charcot. Revue Neurologique 1982;138:887-92.
4. Gelfand T. Comment Charcot a obtenu sa chaire. Histoire Des Sciences Médicales 1994;1: 307-12.
5. Angelini C. History of Myology in Italy and its international connections. Acta Myol 2011;30:151.

ORIGINAL ARTICLES

Muscle exercise in limb girdle muscular dystrophies: pitfall and advantages

GABRIELE SICILIANO¹, COSTANZA SIMONCINI¹, STEFANO GIANNOTTI², VIRNA ZAMPA³,
CORRADO ANGELINI⁴ AND GIULIA RICCI¹

¹ Department of Clinical and Experimental Medicine, Neurological Clinic, University of Pisa, Italy; ² Second Orthopaedic and Traumatologic Clinic, University of Pisa, Italy; ³ Department of Diagnostic and Interventional Radiology, University of Pisa, Italy;

⁴ Fondazione Ospedale S. Camillo, IRCCS, Lido Venice, Italy

Different genetic mutations underlying distinct pathogenic mechanisms have been identified as cause of muscle fibers degeneration and strength loss in limb girdle muscular dystrophies (LGMD). As a consequence, exercise tolerance is affected in patients with LGMD, either as a direct consequence of the loss of muscle fibers or secondary to the sedentary lifestyle due to the motor impairment. It has been debated for many years whether or not muscle exercise is beneficial or harmful for patients with myopathic disorders. In fact, muscular exercise would be considered in helping to hinder the loss of muscle tissue and strength. On the other hand, muscle structural defects in LGMD can result in instability of the sarcolemma, making it more likely to induce muscle damage as a consequence of intense muscle contraction, such as that performed during eccentric training. Several reports have suggested that supervised aerobic exercise training is safe and may be considered effective in improving oxidative capacity and muscle function in patients with LGMD, such as LGMD2I, LGMD2L, LGMD2A. More or less comfortable investigation methods applied to assess muscle function and structure can be useful to detect the beneficial effects of supervised training in LGMD. However, it is important to note that the available trials assessing muscle exercise in patients with LGMD have often involved a small number of patients, with a wide clinical heterogeneity and a different experimental design. Based on these considerations, resistance training can be considered part of the rehabilitation program for patients with a limb-girdle type of muscular dystrophy, but it should be strictly supervised to assess its effects and prevent possible development of muscle damage.

Key words: limb girdle muscle dystrophies, muscle fatigue, muscle exercise

Introduction

In limb girdle muscular dystrophies (LGMD), different genetic mutations, through distinct pathogenic mechanisms, determine a failure of muscle fibers in maintaining their physical structure during contraction, leading to sarcolemma breakdown, progressive muscle fibers degeneration and strength loss. Depending on that, exercise tolerance is affected in patients with LGMD, either as a direct consequence of loss of muscle fibers, but also secondary to the sedentary lifestyle due to the motor impairment. However, the cellular mechanisms that triggers skeletal muscle dysfunction and, ultimately, leads to muscle necrosis is still unclear, and available therapies are consequently inadequate.

Pathophysiology of fatigue and exercise intolerance in muscular dystrophies

Muscular dystrophies are genetic muscle diseases characterized by a progressive loss of motor unit constituents, due to different degeneration mechanisms, and by a wide range of phenotypes. Like dystrophinopathies (Duchenne and Becker muscular dystrophy, DMD, BMD), the majority of LGMD, that result from mutations in genes encoding specific structural protein, are prototypes of failure of the muscle fiber to maintain its physical structure during contraction, leading to sarcolemma breakdown, myofiber degeneration and necrosis.

As a consequence, more than 60% of dystrophic patients experiences severe fatigue as a common and precocious symptom of disease manifestation. Muscle fatigue

occurs when the intended physical activity can no longer be continued or is perceived as excessive effort and discomfort, depending on the interaction between the required force, the maximum force that the myofiber produces, as well as its endurance, also defined as fatigue resistance. At molecular level, although the loss of skeletal muscle mass accompanying the dystrophic process may be considered as a pathogenic factor in reduced muscle force generation in LGMD, several other and often interconnected mechanisms, as excitation-contraction coupling or energy breakdown, are involved in the genesis of muscle fatigue. However, the exact mechanism of the progressive muscle fibers necrosis in muscular dystrophies is still unknown, as well as the role of energy production in the cascade of events ending with muscle fiber degeneration. Related to that, evidence that the tricarboxylic acid cycle and some reactions in glycolysis are dependent on the integrity of cytoskeletal organization may suggest a role for defective energy metabolism in muscle fiber degeneration. In the clinical setting, a careful analysis of the muscle function is important to characterize the “phenomic” of muscle force impairment and fatigue in muscular dystrophies and to design outcome measures and optimal strategies to treat patients with decreased fatigue resistance (1).

A better knowledge of the muscle metabolic changes during exercise may be useful in understanding the role energy utilization plays in contractile insufficiency and pathogenic mechanisms. For these purposes, ³¹P-magnetic resonance spectroscopy (MRS) may be used to study skeletal muscle metabolism *in vivo*. The technique has become an important tool in the study of the pathophysiology of muscle diseases. ³¹P-MRS is used for providing information about the biochemical composition and metabolism of tissue without invasive sampling, and it has the unique ability to measure intracellular pH. Given that MRS is well tolerated and examinations are easily repeated, it may also be applied in longitudinal studies of disease progression or outcomes. Interestingly, studies performed by muscle ³¹P-MRS have shown significant differences in several metabolite ratios in dystrophic patients indicating a lower energy state (2). In particular, the reduced cytosolic acidification during exercise suggests a defective glycolytic activity in skeletal muscle of patients with Becker muscular dystrophy. A recent work performed by our group studied the exercise-related muscle metabolism in mildly affected BMD patients assessed by muscle ³¹P-MRS during an incremental workload. We observed that BMD patients, compared with normal controls, showed downregulation of resting pH and intramuscular membrane breakdown, an increased anaerobic metabolism during sustained submaximal contraction and the maintenance of oxidative function during recovery (3).

Lodi and coworkers in 1997 (4) used MR imaging and ³¹P-MRS to study skeletal muscle in seven patients with

LGMD with a variable deficiency of the alpha- (D), beta- (E), and gamma- (C) sarcoglycan but normal dystrophin expression on muscle biopsy. In LGMD patients, the authors observed that calf muscle phosphorylated compound content did not differ from controls, but the cytosolic pH was increased. Notably, the degree of calf muscle fat replacement inversely correlated with cytosolic pH and directly with phosphocreatine/adenosine triphosphate (PCr/ATP). Muscle oxidative metabolism was normal in LGMD2-C, -D, -E patients. The authors concluded that primary deficits of sarcoglycan complex lead to specific morphological and metabolic patterns of skeletal muscle involvement. In a recent study, we assessed exercise-related muscle metabolism by muscle ³¹P-MRS during an incremental workload in 10 mildly affected LGMD2A (6 males, 4 females, mean age 31.4 ± 9.5) patients and 3 LGMD2B patients, and 20 healthy controls (unpublished data). The incremental workload exercise test consisted of isometric intermittent plantar flexions of the dominant leg through an MR-compatible ergometer. Test normalization was obtained with reference to individual isometric maximal voluntary contraction (MVC), a valuable force indicator in contracting muscles. The in-magnet exercise protocol was made up of an incremental workload starting from 20% of the mean MVC (re-measured at rest 1 hour before the examination) and progressively increased by 10% MVC every 30 seconds until the subject's exhaustion (3). ³¹P MRS data were acquired from calf muscles. At rest, LGMD-2A and -2B subjects had a significantly higher cytosolic pH ($p < 0.03$), an increase in phosphodiester (PDE), as markers of membrane rupture, and adenosine diphosphate (ADP) ($p < 0.02$), while they showed a reduction of phosphocreatine (PCr) ($p < 0.01$) compared with controls. No significant differences were found in mean values of metabolic variables during exercise between LGMD subjects and controls, while, at the end of exercise, PCr recovery rate in LGMD2A was significantly reduced ($p < 0.02$) in comparison with LGMD2B subjects and healthy controls, suggesting an alteration of oxidative metabolism.

However, when considering metabolic pathway related to fatigue in muscular dystrophies, reports have shown that lactic acid does not have always a deleterious influence on muscle contraction and it does not cause muscle fatigue. In fact, although the reduced intracellular pH may alter muscle performance, given that the glycogen storage is more rapidly depleted when consumed anaerobically, producing lactic acid, it is also likely that its deleterious effects have been overestimated (5).

Alternatively, it has been proposed that the effects of ionic changes, i.e. the changes in the homeostasis of Ca^{2+} and reactive oxygen species (ROS) in the myoplasm, cause muscle fatigue.

Failure in calcium release is one of the major contributor to fatigue. Sarcoplasmic reticulum (SR) Ca^{2+}

stores decline during fatigue. It has been demonstrated that the increased inorganic phosphate (Pi) affect fatigue development by acting on SR Ca²⁺ handling, by reducing cross-bridge force and the Ca²⁺ sensitivity of the myofilaments, with secondary early drop in force (1). According to the “Ca²⁺ precipitation theory”, if Pi goes into the SR during fatigue, this can result in Ca-Pi precipitation and decrease of the Ca²⁺ available for release.

There is also a growing literature that indicates the “oxidative stress” as major source of signal pathway in the generation of muscle fatigue (6, 7). The superoxide anion (O₂•⁻) is one of the major reactive oxygen species (ROS). In addition, O₂•⁻ reacts with nitrogen oxide (NO), with the production of peroxynitrite (ONOO⁻), a reactive nitrogen species (RNS). Muscle contraction requires a large amount of ATP. The majority of ATP is produced by mitochondrial oxidative phosphorylation (OXPHOS); during exercise, mitochondria utilize an amount of O₂ which is up to 100-fold higher than the one used during resting. The high rate of O₂ consumption in skeletal muscles determines also an electron dispersion from the electron transfer chain during OXPHOS, with secondary generation of O₂•⁻ (8). ROS/RNS production in muscles causes oxidative stress that is dangerous for cellular DNA, proteins and lipids. ROS have been identified as endogenous mediators of muscle fatigue, highlighting the importance to develop antioxidants as therapeutic interventions for fatigue treatment (8).

The nitric oxide (NO) pathway has also been implicated in the genesis of muscle fatigue in muscular dystrophies. NO synthesized from L-arginine catalyzed by NO synthase is a widespread biological mediator with many functions, including cell signaling and protection from reactive oxygen intermediate superoxide. Loss of normal NO production in dystrophic muscle is expected to have a broad, disruptive effect on muscle function (9). Neuronal nitric oxide synthase (nNOS) is a key muscle enzyme in the production of NO, that is involved in the regulation of vasorelaxation and muscle blood supply. NOS is associated with dystrophin-associated protein complex at the sarcolemmal level, where it provides stability to the myofiber membrane during contraction. In the absence of

dystrophin, as observed in dystrophinopathies, the concentration of NOS at the cell membrane and in the cytoplasm decreases, and the concentration of NOS mRNA is also reduced (10). It has been hypothesized, both in mdx mice and boys with Duchenne muscular dystrophy (11-13), that the displacement and the secondary loss of nNOS and abnormalities in the levels of its expression in muscle significantly contribute to fatigue by inducing muscle ischemia during contraction. Interestingly, nNOS-null mice display a specific deficit in adapting to exercise and develop profound fatigue upon repeated muscle contraction (14). nNOS levels were also reduced in other genetic forms of muscle disease, including those resulting from defects in extracellular matrix proteins laminin α2 and collagen VI. Mutations in dysferlin are also shown to be characterized by reduced levels of nNOS. Loss of the sarcoglycan-sarcospan complex in muscle, as observed in sarcoglycanopathies, causes a reduction in the levels of nNOS expression at the membrane, even in the presence of normal dystrophin expression (15).

Effects of exercise training in muscular dystrophies

In healthy individuals physical exercise training is considered one of best intervention to improve muscle strength, endurance and cardiorespiratory function. Regular exercise can also prevent diseases such as diabetes mellitus, arteriosclerosis, some forms of cancer, bone fractures, overweight, and it may improve cognition and mood. Moreover, it can avoid the age-related loss of muscle, called sarcopenia. Physical fitness training is a planned and structured regimen of regular physical exercise. In particular, strength training is defined as a training performed primarily to improve muscle strength and endurance and it is typically carried out making repeated muscle contractions against resistance. Indeed, aerobic exercise training, or cardiorespiratory fitness training, is a training that consists of an activity or combination of activities that uses large muscle groups, that can be continuously maintained, and that is rhythmical and aerobic, for example walking, running, cycling, or swimming (Fig. 1).

PHYSICAL FITNESS TRAINING

a planned and structured regimen of regular physical exercise

Strength training is defined as a training performed to improve muscle strength and endurance. It is typically carried out making repeated muscle contractions against resistance

Aerobic exercise training consists of an activity or combination of activities that uses large muscle groups, that can be maintained continuously, and that is rhythmical and aerobic

Fig. 1.

Studies of exercise training in patients with different neuromuscular diseases seem to suggest a positive effect without susceptibility to muscle injury (16–18). In fact, strength training, which is performed to improve muscle strength and muscle endurance, or aerobic exercise programs, which involve training at moderate levels of intensity for extended periods of time (for example, distance cycling) might maximize muscle and cardiorespiratory function and prevent additional disuse atrophy (19). Previous studies demonstrated a beneficial effect of low- to moderate-intensity resistance and aerobic training in slowly progressive myopathies (20). An improvement in aerobic capacity may prevent type 2 diabetes, cardiovascular disease, and other lifestyle diseases. Patients with neuromuscular disorders are known to have a higher risk of developing disorders associated with obesity and a sedentary lifestyle, such as metabolic syndrome, when compared with the general population. Therefore, the positive effect of a training program on aerobic capacity in these patients is of substantial importance for their long-term health and quality of life (21, 22). However, clinicians are still afraid about muscle overuse during exercise in people with muscle disease and have a cautious approach to training. Limitations in patient number, design, and, most importantly, lack of supervision have often precluded any firm conclusion from previous studies (23). Importantly, as the pathophysiology of muscular dystrophies and myopathies differs, their reaction to training intervention might be different.

Muscle damage and exercise in LGMD

The damage of skeletal muscle during exercise may be caused by metabolic or mechanical mechanism. The metabolic damage is the result of ischemia or hypoxia during prolonged exercise, resulting in changes in ion concentration, accumulation of ROS and deficiency of ATP. Mechanical stimuli can determine muscle damage as direct consequence of overload of muscle fibers or inappropriate balance of exercise variables, with a secondary disruption of the sarcomeric Z lines (24) and plasma membrane with loss of muscle proteins, such as creatine kinase (CK), lactate dehydrogenase, aldolase, myoglobin, troponin.

Muscle contractions may determine mild and non-significant damage of muscle fibers during daily common activities (25). More severe injuries accompanied by myalgias are also possible, especially during predominantly lengthening (eccentric) contractions. There are three different types of contractions. If the force developed by the muscle is greater than the load on the muscle, a shortening (concentric) contraction occurs. When the force developed by the muscle and the load are equivalent, or the load is immovable, a fixed length, or isometric contraction, results.

The third type of contraction occurs when the load on the muscle is greater than the force developed by the muscle and the muscle is stretched, producing a lengthening (eccentric) contraction. Depending on the severity of the injury, complete recovery may require from 7 to 30 days. Training with protocols of lengthening (eccentric) contractions produces a hypertrophic and stronger muscle. The “trained” muscle is then able to perform the protocol of repeated lengthening contractions that previously caused injury without sustaining an injury. It may be hypothesized that tear and load due to exercise on a sick muscle could accelerate the disease process in muscular dystrophies, in which sick fibres of different sizes, with disorganised myofibrillar structure, often undergoing regeneration or necrosis, in association with fibrosis and adipose tissue, does not seem for contractile activity. Based on the above considerations, patients with neuromuscular disorders in the past have been advised to refrain from strenuous exercise. But, one of the consequences of this is that patients develop severe deconditioning, with potential acceleration of the primary muscle disease process.

However, in last decade, a growing number of studies has shown that exercise can be safe and beneficial for several muscle diseases, but, to date, it is still unknown what kind of exercise (aerobic versus strength training) should be recommended, and at what duration, frequency and intensities it should be performed. Starting from actual knowledge, there is the need to plan future studies aimed at addressing if motor training for muscle disease can play a therapeutic effect (26).

Endurance training in LGMD2I (Sveen et al., 2007) (27)

Sveen and coworkers (27) analyzed the effect of low-intensity aerobic training in 9 patients with limb-girdle muscular dystrophy type 2I (LGMD2I), caused by mutation of fukutin-related protein, that is a cytosolic protein that glycosylates alpha-dystroglycan. Alpha-dystroglycan and integrin alpha-7beta-1D are the two main laminin receptors in skeletal muscle, playing a major role for the integrity of the sarcolemma. Exercise could be deleterious in patients with LGMD2I, considering the importance of alpha-dystroglycan in linking the sarcolemma to the extracellular matrix. The AA also evaluated the effect of a training program consisted in fifty 30-minute training sessions on cycle ergometer for 12 weeks, at a heart rate corresponding to 65% of their maximal oxygen uptake (VO_{2max}). As a marker of exercise-inducing muscle damage, plasma CK was measured before and after a 12-week training period, 24 to 48 hours after the final training session. Plasma lactate and heart rate were used to validate the degree of exhaustion during cycle tests before and after training. Training improved VO_{2max} and maximal workload in patients with LGMD2I. Plasma

lactate levels and heart rate at rest and at $\text{VO}_{2\text{max}}$ did not differ significantly before and after training. Plasma CK levels tended to increase after training in patients, but also increased in nine matched healthy controls. Self-reported questionnaires showed that the majority of subjects with LGMD2I felt an improvement in physical endurance, leg muscle strength, and walking distance. No worsening of their condition or adverse events were reported.

The authors concluded that 12 weeks of low intensity aerobic training was effective and safe in increasing fitness in patients with LGMD2I. Training raised the patient's $\text{VO}_{2\text{max}}$ and workload capacity in watts by 21% and 27%, corresponding to the normal physiologic response to training in the healthy subjects. Increased work capacity was paralleled by self-reported improvements in endurance, leg muscle strength, and walking distance (27).

Resistance training in patients with LGMD2I and LGMD2A (Sveen et al., 2013) (28)

Sveen and coworkers presented the results of two pilot studies on the effect of resistance training in patients with LGMD2I, LGMD2A and Becker muscular dystrophy (BMD) (28). In particular, in one study they investigated the effect of low-intensity strength training (LOIT) in 2 patients with LGMD2A, in 4 patients with LGMD2I and 2 patients with BMD; in the other, they assessed the effect of high-intensity strength training (HIT) in 4 patients with LGMD2A, in 2 patients with LGMD2I and 1 patient with BMD. All recruited patients were ambulatory and considered to be mildly-moderately affected.

In LOIT study, the resistance-training program lasted 6 months. Two muscle groups were included in the training program: quadriceps (knee extension) and biceps brachii (elbow flexion). During the first 4 months of training the dominant side was trained, while both sides were trained for the final 2 months of training. The weight lifted during knee extension and elbow flexion started at 40% and was increased by 5% a week. Subjects were tested before and after 4 and 6 months of training for maximal strength and endurance (number of repetitions possible at 60% of maximal strength). They also completed a questionnaire, named Sickness Impact Profile (SIP), for assessment of their daily function and quality of life. As a marker of exercise-induced muscle damage, plasma CK was monthly measured during the training period. At the end of the training period, elbow flexion and knee extension showed a significant increase in muscle strength and endurance; there was no change in the results from the SIP questionnaire regarding daily activities.

In HIT study, the training program lasted for 3 months. Additional muscle groups were included for testing, including wrist flexion and extension and plantar flexion, and both the right and left extremities were trained from the beginning. Subjects followed a strength training

program with 3 sessions per week over the course of 12 weeks, with at least one day of rest between each training session. Supervision was provided during each session by a personal instructor. Patients were tested for maximum strength monthly. In order to measure endurance, patients performed as many repetitions as possible at 60% of their repeat maximum, which was found at the initial strength test. Each training session started with a 5-minute warm-up on a stationary cycle ergometer at low intensity, followed by three sets of each exercise performed with a 90-150-second interval between sets. In each set, the patient performed the maximum number of repetitions possible. Two patients with LGMD2A dropped out of the study because of training-induced CK elevations and myalgias. After 12 weeks of training, the strength of the patients improved in wrist flexion and extension, while the improvement in the other muscle groups was not significant. It was not observed a correlation between the initial strength level of the muscles and the percentage increase seen in the muscles. SIP questionnaire did not show changes in patient's self-reported daily status and quality of life.

In conclusion, the preliminary results of this work indicated that resistance training could be safe and effective in increasing muscle strength and endurance in muscular dystrophies with proximal weakness, such as LGMD. The authors highlighted, however, that, until more knowledge is convened, training should be carefully supervised in order to recognize potential adverse effects, particularly in high-intensity protocols (28).

Aerobic training in patients with LGMD2L (Vissing et al., 2014) (29)

LGMD2L is a recessively inherited dystrophy caused by mutations in ANO-5 gene, that encodes for the putative calcium-sensitive chloride channel anoctamin 5, which is thought to play a role in membrane repair. In this study, 6 ambulant patients with LGMD2L were selected in order to evaluate the effect of home-based, pulse-watch monitored, moderate-intensity exercise at home on a cycle ergometer for 30 minutes, 3 times weekly, for 10 weeks. Also in the present work, plasma CK was assessed as a marker of muscle damage. Training was performed at a heart rate interval corresponding to 70% of their $\text{VO}_{2\text{max}}$. Primary outcome measures were $\text{VO}_{2\text{max}}$ and time in the 5-repetitions-sit-to-stand test (FRSTST), requiring patients to rise and sit from a chair 5 times as rapidly as possible. The authors reported a significant improvement in oxidative capacity and muscle function (evaluated by $\text{VO}_{2\text{max}}$ and FRSTST time), with stable CK levels and no reports of adverse effects (29).

Conclusions

In LGMD, because muscle weakness is the main problem, muscular exercise can help to counteract the loss of muscle tissue and strength in LGMD. Although it is accepted that exercise has a positive role in many diseases, it is not possible to generalize this finding to muscular dystrophy, including LGMD, and there is the need to conduct a systematic search to point out the effects of muscular exercise in experimental settings. To date, there is no certain evidences about the type (endurance or strength), frequency and intensity of muscle exercise. However, a training with moderate (below 70% of predicted maximal aerobic capacity) aerobic exercise seems to play an useful and safe effect in muscular dystrophies (23, 26).

Acknowledgements

We are thankful to all patients and their associations, in particular the “Unione Italiana Lotta alla Distrofia Muscolare, UILDM” (www.uildm.org) and the “Beta-Sarcoglicanopatie” Italian association (beta-sarcoglicanopatie.it). We wish to acknowledge the Italian Association of Myology (AIM), and Telethon Italy for its support GUP10006.

References

- Allen DG. Skeletal muscle function: role of ionic changes in fatigue, damage and disease. *Clin Exp Pharmacol Physiol* 2004;31:485-93.
- Banerjee B, Sharma U, Balasubramanian K, et al. Effect of creatine monohydrate in improving cellular energetics and muscle strength in ambulatory Duchenne muscular dystrophy patients: a randomized, placebo-controlled 31P MRS study. *Magn Reson Imaging* 2010;28:698-707.
- Tosetti M, Linsalata S, Battini R, et al. Muscle metabolic alterations assessed by 31-phosphorus magnetic resonance spectroscopy in mild Becker muscular dystrophy. *Muscle Nerve* 2011;44:816-9.
- Lodi R, Muntoni F, Taylor J, et al. Correlative MR imaging and 31P-MR spectroscopy study in sarcoglycan deficient limb girdle muscular dystrophy. *Neuromuscul Disord* 1997;7:505-11.
- Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 2008;88:287-332.
- Reid MB. Free radicals and muscle fatigue: Of ROS, canaries, and the IOC. *Free Radic Biol Med* 2008;44:169-79.
- Ferreira LF, Reid MB. Muscle-derived ROS and thiol regulation in muscle fatigue. *J Appl Physiol* (1985) 2008;104:853-60.
- Kuwahara H, Horie T, Ishikawa S, et al. Oxidative stress in skeletal muscle causes severe disturbance of exercise activity without muscle atrophy. *Free Radic Biol Med* 2010;48:1252-62.
- Tidball JG, Wehling-Henricks M. Expression of a NOS transgene in dystrophin-deficient muscle reduces muscle membrane damage without increasing the expression of membrane-associated cytoskeletal proteins. *Mol Genet Metab* 2004;82:312-20.
- Wehling M1, Spencer MJ, Tidball JG. A nitric oxide synthase transgene ameliorates muscular dystrophy in mdx mice. *J Cell Biol* 2001;155:123-31.
- Heydemann A, McNally E. NO more muscle fatigue. *J Clin Invest* 2009;119:448-50.
- Güçüyener K, Ergenekon E, Erbas D, et al. The serum nitric oxide levels in patients with Duchenne muscular dystrophy. *Brain Dev* 2000;22:181-3.
- Tidball JG, Wehling-Henricks M. Nitric oxide synthase deficiency and the pathophysiology of muscular dystrophy. *J Physiol* 2014;592(Pt 21):4627-38.
- Kobayashi YM, Rader EP, Crawford RW, et al. Sarcolemma-localized nNOS is required to maintain activity after mild exercise. *Nature* 2008;456:511-5.
- Crosbie RH1, Barresi R, Campbell KP. Loss of sarcolemma nNOS in sarcoglycan-deficient muscle. *FASEB J* 2002;16:1786-91.
- Vignos PJ, Watkins MP. The effect of exercise in muscular dystrophy. *JAMA* 1966;197:843-8.
- McCartney N, Moroz D, Garner SH, et al. The effects of strength training in patients with selected neuromuscular disorders. *Med Sci Sports Exerc* 1988;20:362-8.
- Aitkens SG, McCrory MA, Kilmer DD, et al. Moderate resistance exercise program: Its effect in slowly progressive neuromuscular disease. *Arch Phys Med Rehabil* 1993;74:711-5.
- Voet NB, van der Kooi EL, Riphagen II, et al. Strength training and aerobic exercise training for muscle disease. *Cochrane Database Syst Rev* 2013;7:CD003907.
- Ansved T. Muscular dystrophies: influence of physical conditioning on the disease evolution. *Curr Opin Clin Nutr Metab Care* 2003;6:435-9.
- McCrory MA, Kim HR, Wright NC, et al. Energy expenditure, physical activity, and body composition of ambulatory adults with hereditary neuromuscular disease. *Am J Clin Nutr* 1998;67:1162-9.
- Aitkens S, Kilmer DD, Wright NC, et al. Metabolic syndrome in neuromuscular disease. *Arch Phys Med Rehabil* 2005;86:1030-6.
- Gianola S, Pecoraro V, Lambiase S, et al. Efficacy of muscle exercise in patients with muscular dystrophy: a systematic review showing a missed opportunity to improve outcomes. *PLoS One* 2013;8:e65414.
- da Luz CR, Nicastro H, Zanchi NE, et al. Potential therapeutic effects of branched-chain amino acids supplementation on resistance exercise-based muscle damage in humans. *J Int Soc Sports Nutr* 2011;8:23.
- Faulkner JA, Brooks SV, Opitck JA. Conditions of Occurrence and Prevention: Injury to Skeletal Muscle Fibers During Contractions. *Phys Ther* 1993;73:911-21.
- Vissing J, van Engelen BG. 160th ENMC International Workshop (First ENMC practical care workshop) Exercise training in patients with muscle diseases: 20-22 June 2008, Naarden, The Netherlands. *Neuromuscul Disord* 2013;23:182-7.
- Sveen ML, Jeppesen TD, Hauerslev S, et al. Endurance training: An effective and safe treatment for patients with LGMD2I. *Neurology* 2007;68:59-61.
- Sveen ML, Andersen SP, Ingelsrud LH, et al. Resistance training in patients with limb-girdle and becker muscular dystrophies. *Muscle Nerve* 2013;47:163-9.
- Vissing CR, Preisler N, Husu E, et al. Aerobic training in patients with anoctamin 5 myopathy and hyperckemia. *Muscle Nerve* 2014;50:119-23.

Clinical features of patients with dystrophinopathy sharing the 45-55 exon deletion of DMD gene

ANTONELLA TAGLIA^{1*}, ROBERTA PETILLO^{1*}, PAOLA D'AMBROSIO¹, ESTHER PICILLO¹, ANNALaura TORELLA², CHIARA ORSINI¹, MANUELA ERGOLI¹, MARIANNA SCUTIFERO¹, LUIGIA PASSAMANO¹, ALBERTO PALLADINO¹, GERARDO NIGRO³ AND LUISA POLITANO¹

¹ Cardiology and Medical Genetics, Department of Experimental Medicine; ² Laboratory of Medical Genetics, Department of Biochemistry, Biophysics and General Pathology; ³ Arrhythmologic Unit, Department of Cardio-Respiratory Sciences, Second University of Naples, Italy

Becker muscular dystrophy (BMD) was first described in 1953 by Emile Becker as a benign variant of Duchenne muscular Dystrophy (DMD). Compared with DMD, BMD is clinically more heterogeneous, with initial presentation in the teenage years and loss of ambulation beyond the age of 16 and a wide spectrum of clinical presentations, ranging from only myalgias and muscle cramps to exercise intolerance and myoglobinuria, asymptomatic elevation of serum creatin-kinase, or mild limb-girdle weakness and quadriceps myopathy. About 50% of patients become symptomatic by the age of 10 and the most part by the age of 20 years. However few patients can be free of symptoms till their fifties and cases of late-onset Becker Muscular Dystrophy have also been described.

In this report we describe the clinical features of patients with dystrophinopathy sharing a deletion of exons 45-55, occasionally or retrospectively diagnosed. These data are important for both the prognostic aspects of children presenting this dystrophin gene mutation, and for the genetic counseling in these families (reassuring them on the benign course of the disease), and last but not least to keep in mind a diagnosis of BMD in asymptomatic adults with mild hyperckemia.

Key words: Becker muscular dystrophy, dystrophin, asymptomatic BMD

Becker muscular dystrophy (OMIM 300376) is part of a spectrum of muscular disorders caused by pathogenic variants in DMD gene encoding for dystrophin protein; this ranges in severity from asymptomatic increased levels of CK, cramps and myoglobinuria to progressive

muscle diseases classified as Becker muscular dystrophy when skeletal muscle is primarily affected and as DMD gene-associated dilated cardiomyopathy (DCM) when the heart is primarily affected.

DMD gene (OMIM 300377), the largest found in nature measuring 2,5 Mb, was identified in 1987 through a positional cloning approach (1). It is composed by 79 exons and has 7 tissue specific promoters. Three promoters, localized upstream the first exon, control the transcription of the full length protein (dp427). They are named according to their main site of expression: (m - muscle), which induces transcription in skeletal and heart muscle but also in glial cells, (c - brain) specific for the brain and the retina as well, (p- Purkinje) which controls the expression in Purkinje cells and in muscle. The dystrophin gene also has at least 4 internal promoters, localized within introns, named according to the molecular weight of the produced protein: dp260 (retinal isoform, intron 29), dp140 (brain specific isoform, intron 44), dp116 (Schwann cells isoform, intron 44) and dp71 (general isoform, intron 55) (2). The shorter dystrophin proteins lack the actin-binding terminus but retain the cysteine-rich and carboxy-terminus domains that contain the binding sites for dystroglycan, distrobrevin and syntrophin. The genetic complexity is increased by the alternative splicing events; the spliced variants are formed both through the exclusion of some exons from the primary transcript (exon skipping) and

Address for correspondence: Luisa Politano, Cardiology and Medical Genetics, Dipartimento di Medicina Sperimentale, Seconda Università di Napoli. E-mail: luisa.politano@unina2.it

* These Authors contributed equally to this work.

by subversion of the reciprocal order of exons (exon scrambling) (2, 3). Taken together these mechanisms generate at least 18 transcripts (4).

Full-length dystrophin is a large rod-shaped protein with a molecular weight of 427kDa composed by 3685 amino acids organised in four structural domains: actin-binding domain, rod domain, cystein-rich domain and the COOH terminus region (4-6). These domains have different tri-dimensional structures and functional roles (7-11), as the protein is involved both in the interaction with integral membrane proteins (sarcoglycans, dystroglycans, syntrophin, and dystrobrevin complexes) assembled in the dystrophin-glycoprotein complex and in cellular communication and transmembrane signalling (12-14).

The most common mutational event is represented by intragenic deletions accounting for 65-70% of all mutations; duplications account for 10% of all mutations. Both might occur almost anywhere in the gene; however two deletion hot-spots are known – one located towards the central part of the gene, encoding for exons 45-55, and the other towards the 5', including exons 2-19.

There is no relation between size, region, domain of deletion and phenotype, which largely depends on whether or not the mutation disrupts the reading frame (15). Mutations which maintain the reading frame (in-frame mutations) generally result in abnormal but partially functional protein (BMD phenotype), while out of frame mutations cause a premature stop codon downstream, with the production of unstable mRNA that leads to a non-sense mediated decay and virtually undetectable levels of protein (DMD phenotype). However small amount of mRNA might escape this mechanism resulting in misfolded non functional proteins which exert heavy dominant negative effects (16).

The central and the distal rod-domains are likely to be functionally dispensable, as deletions in these domains have been associated with isolated hyperCKemia, myalgia and cramps, but not with weakness. This is the case of deletions in exons 32-44, 48-51 and 48-53, who had normal or near normal dystrophin concentrations (17-20). As a general rule deletions of large portions of *rod* domain result in BMD phenotype as long as they maintain the C and N-termini.

The reading frame hypothesis holds for over 90%; exceptions exist and involve both patients with BMD who carry out of frame deletions and DMD patients with in frame mutations, generally involving exons 3-7. The two mechanisms which may explain BMD phenotype in patients with out of frame deletions are the *exon skipping* event, occurring via an alternative splicing, and the presence of an additional translation start site located within exon 8 (21, 22). On the other hand, in-frame deletions disrupting the 5' actin binding domain may result in DMD phenotype (23).

In about 20-35% of dystrophinopathic patients sharing nonsense point mutations, small frameshifting deletions/insertions and splice site mutations have been identified (24); given the size of the gene, the identification of these mutations remain difficult.

BMD displays a high phenotypic variability ranging in severity from asymptomatic hyperCKemia, cramps and myoglobinuria to mild-moderate muscular involvement, characterized by a progressive symmetric muscle weakness and atrophy (proximal greater than distal) sparing calf muscles often hypertrophic; to be stressed that weakness of quadriceps femoris may be the only sign for a long time.

The clinical distinction between DMD and BMD is conventionally based on the age of wheelchair dependency: before age 13 years in DMD and after age 16 years in BMD; however BMD patients may remain ambulant until the late 40s and over.

Despite the milder skeletal muscle involvement, intractable heart failure from dilated cardiomyopathy is a common cause of morbidity and the most common cause of death; it may be the main clinical feature in patients affected by subclinical and mild BMD (25). Mean age at cardiomyopathy diagnosis is 14.6 years, similar to that in DMD (14.4 years) with the mean age of death the mid-40s (26, 27). In one study involving 28 individuals with subclinical and benign BMD between ages 6 and 48 years (28), 19 (68%) had myocardial involvement, although only two were symptomatic. Saito et al. (29) also demonstrated that of 21 individuals ranging from age 3 to 63 years (mean age 40 years), 33% had cardiac failure despite relatively mild skeletal muscle findings.

A significant role in the clinical variability of BMD patients likely relies on the structure of the internally truncated dystrophin produced by in-frame deletions in the central Rod domain. Indeed deletions leading to hybrid repeats should lead to more favourable phenotypes than deletions leading fractional repeats, although other factors may influence the clinical outcome, such as the presence/absence of binding factors, or other factors (SNPs and microRNAs) which could modulate the expression or the function of the protein (30). In particular, the deletion of exons 45-55 is considered to have a favourable prognosis, as the related phenotypes so far described, although limited to a small number of patients, range from asymptomatic patients to patients with only myalgia or exercise intolerance, mild BMD; however some cases of dilated cardiomyopathy have been also reported (31-35).

In this paper we wide the number of patients sharing this deletion, describing the clinical features of 9 new patients diagnosed at Cardiomyology and Medical Genetics of Second Naples University.

Patients and methods

Among the 249 BMD patients, regularly followed at our Clinical Service, Centre of Reference for muscular dystrophies for Campania and other Regions of Southern Italy, we retrospectively evaluated clinical data from nine patients in which a deletion of exons 45-55 in DMD gene has been found.

Clinical data included: family history, age at diagnosis and at last control, type of diagnosis (pre-clinical or clinical) and presence of muscle, cardiac and respiratory symptoms. The age of onset of myocardial and respiratory involvement was also noted.

Muscular involvement has been assessed through dynamic tests (Gower's time, time to get up from the floor and to climb 4 standard steps, in seconds) and since 2010 through North Star and 6MWT. Muscle strength was evaluated by manual MRC scale. Serum CK levels were also evaluated.

Myocardial involvement was assessed by evaluating ECG and Ecocardiography records, with a particular focus on ejection fraction (EF) and left ventricular volumes (VTD) adjusted for m2 (VTD/m2). We indicated as the onset of dilated cardiomyopathy an EF value < 50% and a VTD/m2 > 70.

Respiratory involvement was assessed through spirometric tests (FVC, absolute values and percentages). The onset of restrictive syndrome was considered for FVC values < 70%.

Results

The results are reported in Table I.

The average age of patients was 31,06 years (sd 24,10) at diagnosis, and 33,07 (sd 22,03) at the last control. For all of them but one the diagnosis was pre-symptomatic; however just one (D.G.) complained fatigue at the time of diagnosis but not at last control (19 years). Five patients were diagnosed after routine lab tests that showed high serum CK levels, while for four of them (T.A., T.R., M.C. and M.M.) the diagnosis was obtained retrospectively (T.R. maternal uncle of V.F.; M.C. T.A. and M.M. respectively maternal grandfather, mother's cousin and mother's paternal uncle of S.A.). All patients have normal muscular strength (evaluated according MRC scale); the typical quadriceps hypotrophy and calf hypertrophy were observed in all. Serum CK levels ranged from 3,8 to 35,2x.

An initial ventricle dilation was observed at the last control in two patients, who presented a VTD/m2 > 70 :

Table I. Clinical features of patients with dystrophinopathy sharing the 45-55 exon deletion of DMD gene.

I.D.	Age at diagnosis	Age at last control	Symptoms at diagnosis	Family history	Diagnosis	Muscular involvement	Myocardial involvement	Respiratory involvement	CK
D.G.	11y 4m	19y 1m	Fatigue	Negative	Occasional	Calf hypertrophy	VTD/m2 70,6 EF 61%	No	5.5x
D.F.	14y 4m	17y 6m	No	Negative	Occasional	Quadriceps hypotrophy and calf hypertrophy	VTD/m2 65,4 EF 60,2%	No	24.4x
P.F.	49y 3m	49y3m	No	Negative	Occasional	Quadriceps hypotrophy and calf hypertrophy	VTD/m2 71 EF 63,3%	No	7.9x
S.A.	5y	8y	No	Negative	Occasional	Calf and quadriceps hypertrophy	EF 68%	No	23.4x
T.A.	39y 6m	39y6m	No	Negative	A posteriori	Quadriceps hypotrophy	VTD/m2 61,7 EF 66%	Obstructive	5.2x
M.C.	62y3m	62y3m	No	Positive	A posteriori	Calf hypertrophy	VTD/m2 62 EF 64%		3.5x
M.M.	66a	66a	No	Positive	A posteriori	Calf hypertrophy	VTD/m2 64,5 EF 63%		3x
V.F.	4y 9m	7y 2m	No	Positive	Occasional	Calf hypertrophy	EF 66,9%	No	35.2x
T.R.	34y 3m	36y 7m	No	Positive	A posteriori	Quadriceps hypotrophy and calf hypertrophy	VTD/m2 93,6 EF 53,5%	No	3.8x

Legenda

A posteriori: in these patients, maternal grandfathers of young children with BMD (< 10 years) the diagnosis was made after the occasional observation of hyperCKemia.

P.F. had a VTD/m2 of 71 at the age of 49 years, but his EF was 63,3%, and T.R. had a VTD/m2 of 93,6% at the age of 36 years, with EF of 53,5%.

Respiratory involvement (FVC was below 70%) was absent; only one patient had an obstructive syndrome related to cigarette smoke.

Discussion

Becker muscular dystrophy is a clinically heterogeneous disorder which may vary from asymptomatic forms to more relevant muscular and cardiac involvement (18, 31, 36). Genotype-phenotype correlation has been characterized in a previous study: deletions in the N-terminal region and in the rod-domain proximal to exon 45 have been associated with earlier onset of symptoms than mutations in the distal region (37). In particular patients sharing deletion of exons 45-55 seem to have a less severe muscular involvement, with only a few cases of dilated cardiomyopathy so far described (31-35).

Myocardial involvement, in particular, has been investigated in different studies so far; our group demonstrated that a real, dilated cardiomyopathy is the most frequent type of myocardial involvement after the age of 20 and that the severity of cardiac involvement can be unrelated to that of skeletal muscle damage, confirming that cardiac dysfunction is a primary feature of Becker muscular dystrophy (38-41). Even in the absence of overt cardiomyopathy, there is an increased susceptibility to ventricular arrhythmias, whose severity appears closely related to the degree of left ventricular systolic dysfunction (42, 43). In addition we demonstrated that BMD patients with deletions of exons 48-49 have a more severe cardiac involvement than others with different deletions (44). In another study Kaspar et al. focused on the correlation between the genotype and the age of onset of dilated cardiomyopathy in 3 different groups of BMD patients. They concluded that patients with deletions involving N-terminal domain (Group 1- deletion exons 2-9) and those causing a disruption of phase in rod domain (Group 2 – deletion exons 45-49) had an earlier onset of cardiomyopathy (early and mid 20's respectively) than that observed in case of in-phase deletions of rod-domain (Group 2) or disrupting hinge 3 domain (Group 3 – deletion exons 45-55) (mid 30's and 40's) (45). The not constant presence of cardiomyopathy seen in patients sharing deletion of exon 45-55 may be related to the different breakpoint locations eventually involving cardio-specific *cis* or *trans* regulatory elements (33, 34).

The clinical data reported herein are in keeping with previous observations, as none of our patients exhibited symptomatic muscle. However all patients showed as a constant feature quadriceps hypotrophy and calf hyper-

trophy. Furthermore only two patients showed at the last control (age 49 and 36, respectively) an increase in VTD/m2 values, suggesting an initial dilated cardiomyopathy to be monitored over time.

The definition of the clinical phenotype in patients carrying a deletion of exons 45-55 is important because its impact on several aspects of the disease: a) prognosis, often extremely benign, allowing less frequent follow-up and less aggressive therapies than in patients with more deleterious mutations; b) genetic counselling in these families, reassured about future pregnancies and prenatal diagnoses. The case of patients indirectly diagnosed in their sixties thanks to a positive family history (mother's father and uncle of S.A.) highlights the importance to extend the clinical assessment and eventually the molecular diagnosis on the paternal side of mothers of new very young diagnosed BMD patients.

Acknowledgements

We are grateful to patients and families for their cooperation. Naples Human Mutation Gene Biobank (NHMG), member of the Telethon Network of Genetic Biobanks (Project N. GTB12001H) founded by Telethon Italy, and Eurobiobank Network provided us with the specimens.

References

1. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol* 2003;2:731-40.
2. Sadoulet-Puccio HM, Kunkel LM. Dystrophin and its isoforms. *Brain Pathol* 1996;6:25-35.
3. Surono A, Takeshima Y, Wibawa T, et al. Circular dystrophin RNAs consisting of exons that were skipped by alternative splicing. *Hum Mol Genet* 1999;8:493-500.
4. Blake DJ, Weir A, Newey SE, et al. Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol Rev* 2002;82:291-329.
5. Koenig M, Monaco AP, Kunkel LM. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell* 1988;53:219-28.
6. Le Rumeur E, Winder SJ, Hubert JF. Dystrophin: more than just the sum of its parts. *Biochim Biophys Acta* 2010;1804:1713-22.
7. Norwood FL, Sutherland-Smith AJ, Keep NH, et al. The structure of the N-terminal actin-binding domain of human dystrophin and how mutations in this domain may cause Duchenne or Becker muscular dystrophy. *Structure* 2000;8:481-91.
8. Winder SJ, Gibson TJ, Kendrick-Jones J. Dystrophin and utrophin: the missing links. *FEBS Lett.* 1995;369:27-33.
9. Jung D, Yang B, Meyer J, Chamberlain JS, et al. Identification and characterization of the dystrophin anchoring site on betadystroglycan. *J Biol Chem* 1995;270:27305-10.
10. Yang B, Jung D, Rafael JA, et al. Identification of alpha-syntrophin binding to syntrophin triplet, dystrophin, and utrophin. *J Biol Chem* 1995;270:4975-8.
11. Ahn AH, Kunkel LM. Syntrophin binds to an alternatively spliced exon of dystrophin. *J Cell Biol* 1995;128:363-71.

12. Rando TA. The dystrophin-glycoprotein complex, cellular signaling, and the regulation of cell survival in the muscular dystrophies. *Muscle Nerve* 2001;24:1575-94.
13. Campbell KP, Kahl SD. Association of dystrophin and an integral membrane glycoprotein. *Nature* 1989;338:259-62.
14. Petrof BJ. Molecular pathophysiology of myofiber injury in deficiencies of the dystrophin-glycoprotein complex. *Am J Phys Med Rehabil* 2002;81:S162-74.
15. Monaco AP, Bertelson CJ, Liechti-Gallati S, et al. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. *Genomics* 1988;2:90-5.
16. Mühlemann O, Eberle AB, Stalder L, et al. Recognition and elimination of nonsense mRNA. *Biochim Biophys Acta* 2008;1779:538-49.
17. Angelini C, Fanin M, Freda MP, et al. Prognostic factors in mild dystrophinopathies. *J Neurol Sci* 1996;142:70-8.
18. Beggs AH, Hoffman EP, Snyder JR, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. *Am J Hum Genet* 1991;49:54-67.
19. Gospe SM Jr, Lazaro RP, Lava NS, et al. Familial X-linked myalgia and cramps: a non progressive myopathy associated with a deletion in the dystrophin gene. *Neurology* 1989;39:1277-80.
20. Melis MA, Cau M, Muntoni F, et al. Elevation of serum creatine kinase as the only manifestation of an intragenic deletion of the dystrophin gene in three unrelated families. *Eur J Paediatr Neurol* 1998;2:255-61.
21. Arahata K, Beggs AH, Honda H, et al. Preservation of the C-terminus of dystrophin molecule in the skeletal muscle from Becker muscular dystrophy. *J Neurol Sci* 1991;101:148-56.
22. Winnard AV, Mendell JR, Prior TW, et al. Frameshift deletions of exons 3-7 and revertant fibers in Duchenne muscular dystrophy: mechanisms of dystrophin production. *Am J Hum Genet* 1995;56:158-66.
23. Nevo Y, Muntoni F, Sewry C, et al. Large in-frame deletions of the rod-shaped domain of the dystrophin gene resulting in severe phenotype. *Isr Med Assoc J* 2003;5:94-7.
24. Roberts RG, Gardner RJ, Bobrow M. Searching for the 1 in 2,400,000: a review of dystrophin gene point mutations. *Hum Mutat* 1994;4:1-11.
25. Cox GF, Kunkel LM. Dystrophies and heart disease. *Curr Opin Cardiol* 1997;12:329-43.
26. Connuck DM, Sleeper LA, Colan SD, et al. Pediatric Cardiomyopathy Registry Study Group; Characteristics and outcomes of cardiomyopathy in children with Duchenne or Becker muscular dystrophy: a comparative study from the Pediatric Cardiomyopathy Registry. *Am Heart J* 2008;155:998-1005.
27. Bushby KM. The limb-girdle muscular dystrophies-multiple genes, multiple mechanisms. *Hum Mol Genet* 1999;8:1875-82.
28. Melacini P, Fanin M, Danieli GA, et al. Myocardial involvement is very frequent among patients affected with subclinical Becker's muscular dystrophy. *Circulation* 1996;94:3168-75.
29. Saito M, Kawai H, Akaike M, et al. Cardiac dysfunction with Becker muscular dystrophy. *Am Heart J* 1996;132:642-7.
30. Nicolas A, Raguénès-Nicol C, Ben Yaou R, et al. French Network of Clinical Reference Centres for Neuromuscular Diseases (COR-NEMUS). Becker muscular dystrophy severity is linked to the structure of dystrophin. *Hum Mol Genet* 2015;24:1267-79.
31. Beroud C, Tuffery-Giraud S, Matsuo M, et al. Multiexon skipping leading to an artificial DMD protein lacking amino acids from exons 45 through 55 could rescue up to 63% of patients with Duchenne muscular dystrophy. *Hum Mutat* 2007;28:196-202.
32. Ferreiro V, Giliberto F, Muniz GM, et al. Asymptomatic Becker muscular dystrophy in a family with a multiexon deletion. *Muscle Nerve* 2009;39:239-43.
33. Miyazaki D, Yoshida K, Fukushima K, et al. Characterization of deletion breakpoints in patients with dystrophinopathy carrying a deletion of exons 45-55 of the Duchenne muscular dystrophy (DMD) gene. *J Hum Genet* 2009;54:127-30.
34. Nakamura A, Yoshida K, Fukushima K, et al. Follow-up of three patients with a large in-frame deletion of exons 45-55 in the Duchenne muscular dystrophy (DMD) gene. *J Clin Neurosci* 2008;15:757-63.
35. Anthony K, Cirak S, Torelli S, et al. Dystrophin quantification and clinical correlations in Becker muscular dystrophy: implications for clinical trials. *Brain* 2011;134(Pt 12):3547-59.
36. Comi GP, Prelle A, Bresolin N, et al. Clinical variability in Becker muscular dystrophy. Genetic, biochemical and immunohistochemical correlates. *Brain* 1994;117:1-14.
37. Magri F, Govoni A, D'Angelo MG, et al. Genotype and phenotype characterization in a large dystrophinopathic cohort with extended follow-up. *J Neurol* 2011;258:1610-23.
38. Nigro G, Comi L, Politano L, et al. Evaluation of the cardiomyopathy in Becker muscular dystrophy. *Muscle Nerve* 1995;18:283-91.
39. Mansi L, Pace L, Politano L, et al. Left ventricular function and perfusion in Becker's muscular dystrophy. *J Nucl Med* 1997;38:563-7.
40. Agretto A, Politano L, Bossone E, et al. Pulsed Doppler tissue imaging in dystrophinopathic cardiomyopathy. *J Am Soc Echocardiogr* 2002;15:891-9.
41. Cittadini A, Ines Comi L, Longobardi S, et al. A preliminary randomized study of growth hormone administration in Becker and Duchenne muscular dystrophies. *Eur Heart J* 2003;24:664-72.
42. Ducceschi V, Nigro G, Sarubbi B, et al. Autonomic nervous system imbalance and left ventricular systolic dysfunction as potential candidates for arrhythmogenesis in Becker muscular dystrophy. *Int J Cardiol* 1997;59:275-9.
43. Nigro G, Nigro G, Politano L, et al. Is the value of QT dispersion a valid method to foresee the risk of sudden death? A study in Becker patients. *Heart* 2002;87:156-7.
44. Nigro G, Politano L, Nigro V, et al. Mutation of dystrophin gene and cardiomyopathy. *Neuromuscul Disord* 1994;4:371-9.
45. Kaspar RW, Allen HD, Ray WC, et al. Analysis of dystrophin deletion mutations predicts age of cardiomyopathy onset in becker muscular dystrophy. *Circ Cardiovasc Genet* 2009;2:544-51.

Letter to the Editor

Dear Editor,

This letter goes to you and about 1,400 other Duchenne families and scientists because you had received, often for many years, my reports about research for finding therapies for boys with Duchenne muscular dystrophy. One year ago (January 2014), I sent you a “good bye” letter, because I thought that – at my age of then 83 years – it was time to find someone who could continue my work in a similar way as I had done it. Unfortunately, this does not happen as I hoped for and even my internet pages containing many of my older reports and interviews (www.duchenne-information.eu) have been taken away and I do not know how they can be used by me again.

Just at this time, when exon skipping, the most advanced Duchenne research approach, has encountered serious difficulties, it is unfortunate that my reports are not available anymore which I had written since the year 2000 especially for Duchenne families. I therefore thought a few months ago that I should try to find out whether you and many others who are receiving this letter would like me to continue my reports, but perhaps concentrating first on exon skipping and later on other techniques also, which are not too far from becoming approved as effective treatments.

Thus, I have written this short report only about the work done by the Dutch company Prosensa on exon skipping during the last two years after the failure of the phase-III clinical trial with drisapersen to skip exon 51 of the dystrophin gene. You know from my last report, written at the beginning of 2013, that this trial was the “pivotal”, the decisive one, because it was designed to definitely prove that exon skipping really is able to slow down the degeneration of Duchenne muscles. We all had hoped that this trial would really show the results that would soon lead to the effective and approved skipping drugs we are waiting for. Unfortunately, on 20 September 2013, it became publicly known that the results of this trial were not significant. Thus, in the last two years, Prosensa, which was left alone by its large partner GlaxoSmithKline, started to find by itself the reasons of this failure and it still continues that, but now as part of the American company BioMarin near San Francisco which has much experience with developing therapies for rare diseases.

But after this short updated exon skipping report you might be interested to follow for yourself on the internet the new advances of important Duchenne research. So you may ask the following selected companies and patient organizations to send you regularly their press releases or newsletters.

Two companies are working on exon skipping, Prosensa-BioMarin in Leiden, the Netherlands, (www.prosensa.eu), and Sarepta in Cambridge in the United States (www.sarepta.com). The PTC company in New Jersey in the USA (www.ptcbio.com) has now received permission to market their drug Translarna (earlier called PTC124 and Ataluren), for reading through premature stop codons, which, however, can be used only by patients, who have these stop codons produced by some special point mutations. There are also two other quite advanced research approaches for developing Duchenne drugs that are independent of the patient's mutation. The company Summit near Oxford (www.summitplc.com) develops the potential drug SMT C 1100 for the upregulation of utrophin, and Santhera near Basel in Switzerland (www.santhera.com) works on Catena/Raxone for slowing the degradation of respiratory function in older patients. The parent association Parent Project Muscular Dystrophy in the USA (www.parentprojectmd.org) publishes regularly summaries about practically all research work on treating Duchenne muscular dystrophy. And also our European center for neuromuscular diseases in Newcastle upon Tyne in England (www.treat-nmd.eu) publishes newsletters regularly. All the information on these websites is in English. And there are many other associations and companies working on Duchenne research which I cannot mention here.

I am hoping that many of you who are receiving this letter will let me know what you think of my starting again my reports for as long as I am not too old for doing that. With kind regards,

Guenther Scheuerbrandt, PhD.
gscheuerbrandt@t-online.de

Im Talgrund 2,
79874 Breitnau, Germany
14 April 2015

**PROCEEDINGS of the XII CONGRESS
OF MEDITERRANEAN SOCIETY OF MYOLOGY**

Naples, Italy

May 18-20, 2015



XII CONGRESS OF MEDITERRANEAN SOCIETY OF MYOLOGY

Naples, Italy - May 18-20, 2015

Program (Summary)

MONDAY MAY 18, 2015

h. 13.00-19.00 Registration of delegates

- Setting up of posters

h. 14.00-14.30 Opening Ceremony

h. 14.30-16.30 Session 1. Spinal Muscular Atrophies

- J. Melki: History of the gene and advances in genetic and translational research
- E. Tizzano-Ferrari: Developmental and evolutive aspects of the disease.
- E. Mercuri: Update on therapeutic trials in SMA

Oral communications on the topic

h. 16.30-17.00 Coffee break

h. 17.00-19.00 Session 2. Inflammatory Myopathies

- M. Dalakas: Advances in the classification, diagnosis and immunopathogenesis of inflammatory myopathies
- M. Mirabella: Inflammatory myopathies: novel diagnostic approach
- B. De Paepe: Treatment of idiopathic inflammatory myopathies: targets inside the cytokine network

Oral communications on the topic

h. 19.30-21.00 Welcome Reception

TUESDAY MAY 19, 2015

h. 9.00-11.00 Session 3. Next Generation Sequencing and Neuromuscular Disorders

- M. Bartoli: Next Generation Sequencing in Neuromuscular Disorders
- V. Nigro: Next Generation Sequencing in Limb-Girdle-Muscular Dystrophies
- F.M. Santorelli: Pro&Cons on the clinical use of massive parallel sequencing methodologies.

Oral communications on the topic

h. 11.00-11.30 Coffee break

h. 11.30-13.30 Session 4. Heart involvement in Neuromuscular Disorders

- D. Duboc: Cardiovascular morbidity and mortality in Laminopathies. The crucial role of Right ventricle involvement.
- G. Nigro: Heart involvement in Myotonic Dystrophy type 1
- A. Florian: Cardiovascular Magnetic Resonance in Muscular Dystrophies
- A. Amodeo: New therapeutic options in the end stage heart failure in Duchenne Muscular Dystrophy

Oral communications on the topic

h. 13.30-15.00 Lunch

Meeting of the Editorial Board of Acta Myologica

h. 15.00-17.00 Session 5. Laminopathies

- G. Bonne: The broad spectrum of laminopathies
- V. Andr s: Cardiovascular disease in premature ageing syndromes LMNA-related
- G. Lattanzi: Role of cytokine signaling in LMNA-linked muscular dystrophies: new findings and perspectives

Oral communications on the topic

h. 17.00-17.30 Coffee break

h. 17.30-19.30 Poster Discussion Session

h. 18.30-19.30 MSM Members General Assembly – Election of the new Board of MSM

h. 19.30-20.00 MSM Board Meeting

h. 21.00-23.00 Social dinner and Award Ceremony for G. Conte Winners Prize

WEDNESDAY MAY 20, 2015

h. 8.30-9.30 Session 6. Mucopolysaccharidosis: a multidisciplinary disease requiring a multifaceted approach

- G. Andria: Clinical aspects of Mucopolysaccharidosis
- R. Manara: Neuroradiological aspects of Mucopolysaccharidosis
- R. Parini: Results of ERT in Mucopolysaccharidosis

h. 09.30-11.00 Invited Lectures by the “Gaetano Conte Prize” Winners

h. 11.00-11.30 Coffee break

h. 11.30-13.30 Session 7. New therapeutic approach in Neuromuscular Disorders

- S. Messina: Novel translational approach in Duchenne Muscular Dystrophy treatment
- G. Meola: New therapeutic approach on Myotonic Dystrophies
- V. Saccone: Histone deacetylase inhibitors: a potential approach for the treatment of DMD
- P. Bettica: Givinostat: a new therapeutic approach for DMD

Oral communications on the topic

h. 13.30-14.00 Invited Congress Closing Lecture

- V. Dubowitz: The progress of Myology in the last 30 years

h. 14.00 Congress Closure

ABSTRACTS OF INVITED LECTURES and ORAL COMMUNICATIONS**Session 1. Spinal Muscular Atrophies****INVITED LECTURES****1.1 History of the gene and advances in genetic and translational research in spinal muscular atrophy**

J. Melki

UMR-1169, Inserm and Paris Sud University. Le Kremlin-Bicêtre, France

Spinal muscular atrophy (SMA) is a frequent autosomal recessive lower motor neuron disease caused by mutations of the survival motor neuron (SMN1) gene. A highly homologous gene, SMN2, remains present in patients. The only functional difference between SMN1 and SMN2 is a synonymous transition leading to alternative splicing of SMN2 exon 7 and lower full length SMN protein. SMA disease is caused by reduced level of SMN protein. SMA severity depends on the amount of residual SMN protein encoded by SMN2 and therefore SMN2 gene copy number.

Biomedical research of this condition allowed a better understanding of the function of SMN protein and the SMA pathogenesis through the development of cellular and animal models of SMA. SMN belongs to a complex involved in the assembly of small nuclear ribonucleoproteins, which are keys for pre-mRNA splicing. However, the link between SMA and RNA metabolism remains unclear: tissue-specific altered spliced mRNAs or tissue more sensitive to reduced SMN protein level? A better knowledge of SMA pathogenesis will likely accelerate the development of new therapeutic targets.

To date, there are no effective treatments in SMA. However, the SMN2 gene is an attractive target aiming at enhancing SMN2 transcription, reducing SMN2 exon 7 exclusion or stabilizing SMN Δ 7 protein. Alternative strategies are mainly aimed at preserving motor neurons through neuroprotection or delivering SMN1 gene by viral vectors. When and where SMN is critical for the neuromuscular system should allow optimal therapeutic design in SMA.

1.2 Developmental and evolutive aspects of spinal muscular atrophy

E. Tizzano

Head Clinical and Molecular Genetics and Rare Diseases Unit, Hospital Valle Hebrón, Barcelona Spain

Spinal muscular atrophy (SMA) is an autosomal recessive disorder that affects motor neurons. It is caused by mutations in the survival motor neuron gene 1 (SMN1).

SMA patients can be classified into three main types (type I non sitters with onset before 6 months; type II, sitters with onset before 18 months; and type III, walkers with onset after 18 months). This classification is useful to help doctors communicate with each other internationally to develop strategies for clinical trials. The SMN2 gene, which is the highly homologous SMN1 copy that is present in all patients, is unable to prevent the disease. Severe patients have one or two SMN2 copies whereas chronic type II have 3 copies and type III have 3 or 4 copies. This correlation is not absolute and prognosis is difficult to establish based only in SMN2 copy number.

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, investigation is ongoing to ascertain whether muscle, neuromuscular junctions, or motor neurons alone are the critical target tissue in SMA. The neuropathologic description of SMA comes largely from postnatal necropsy samples, which describe the end-stage of the disease. The human developmental period appears to play an essential role in SMA pathogenesis. With the exception of severe congenital SMA (type 0), varying age at onset in the four SMA types provides evidence of a latency period without clear manifestations in most SMA patients. This presymptomatic period may be considered as a therapeutic window to establish better therapeutic strategies and to prevent or delay the onset of clinical symptoms.

Given that studies of patients' preclinical status are lacking, the study of SMA during development helps to gain insight into the mechanism of disease in the prenatal and presymptomatic stage. Several findings in fetal studies confirm that SMA pathology is already present in the first trimester. Thus increased cell death, failure in the maintenance of the neuromuscular junction and arrest of muscle development are already present around 12-15 weeks of fetal life. In contrast, fetal movements investigated by ultrasound are indistinguishable from the control fetuses. Thus, the apparent compensation in affected fetuses at early stages warrants further investigation.

Developmental findings in SMA spinal cord, neuromuscular junction and muscle suggest that pathogenic responses to lower levels of SMN protein differ in these key tissues and their dynamic interaction is disrupted early in SMA disease, lending support to the view of SMA as a developmental disorder. As therapeutic advances emerge, the possible benefits of early presymptomatic intervention in SMA should be demonstrated.

Supported by SMA EUROPE and Fundación Mutua Madrileña

1.3 Update on therapeutical trials in SMA

E. Mercuri

Pediatric Neurology, Catholic University, Rome

Multiple therapy approaches have been developed targeting two main mechanisms: replacement of the mutated gene SMN1 and upregulation of the full-length transcription of SMN2. Replacement of SMN1 is currently performed as part of phase 1-2a for infants with SMA type 1 through i.v. delivery of AAV9 capable of crossing the blood-brain barrier and delivering the SMN1 gene to α -motoneurons. The other main approach regards the upregulation of a full-length SMN2 transcript through antisense molecules. One of them, by ISIS Pharmaceuticals is in phase 3 trials for SMA types 1, 2 and 3. An alternative approach using a small molecule also capable of producing a full length SMN protein by including exon 7 into the SMN2 transcript, led by Roche, is currently in phase 1-2a for SMA types 1, 2 and 3. A different approach involves neuroprotective drug capable of slowing down disease progression in SMA. One of them, olesoxime, has successfully been tested in a phase 3 trial in SMA 2 and 3.

Session 2. Inflammatory myopathies

INVITED LECTURES

2.1 Idiopathic inflammatory myopathies: novel diagnostic approach

M. Mirabella, M. Lucchini, C. De Fino, R. Morosetti, A. Broccolini

Institute of Neurology, Catholic University, Roma, Italy

Idiopathic inflammatory myopathies (IIM) are a heterogeneous group of acquired systemic diseases resulting in muscle weakness and disability. Although classification of these myopathies is still debated, the four most common subtypes include dermatomyositis (DM), polymyositis (PM), necrotizing myopathy (NM) and inclusion body myositis (IBM). They are characterised by distinct clinical presentations, histology and immunopathology and response to treatment. They all share muscle weakness progressive over a time variable from weeks to years, elevated muscle enzymes and lymphomonocytic inflammation in muscle. Pathological examination on muscle biopsy is the key diagnostic tool to establish diagnosis. The gold standard to characterize a myositis includes morphological, immunohistochemical and immunopathological analysis of muscle biopsy. Main goal of IIM differential diagnosis is to accurately identify myositis type, ruling out mimics with secondary inflammatory changes (muscular dystrophies, metabolic, infectious, toxic or drug-induced myopathies) as well other systemic and neurological diseases. In sera of many patients, myositis-specific antibodies can be detected, some

of which are associated with specific phenotypes and have a prognostic value. Muscle MRI, besides being used to select a target muscle for biopsy, is increasingly employed in the diagnostic workup of myopathies providing useful information regarding pattern of muscle involvement and degree of inflammatory activity. There is a lack of evidence-based treatment guidelines for myositis due to the rarity of the disease. Precise characterization of immunopathology and morphological features related to disease-specific pathomechanisms is crucial to recognize uncommon phenotypes, identify specific subgroup of patients and help to design more selective and individualized therapeutic approach.

2.2 Advances in the classification, diagnosis and immunopathogenesis of inflammatory myopathies

M. Dalakas

Not arrived

2.3 Treatment of idiopathic inflammatory myopathies: targets inside the cytokine network

B. De Paepe

Department of Neurology, Myopathology & Neuromuscular Reference Centre, Ghent University Hospital, Belgium

The idiopathic inflammatory myopathies (IM) constitute a heterogeneous group of chronic disorders which include dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (IBM) and necrotizing autoimmune myopathy (NAM). They represent distinct pathological entities but, most often, share predominant inflammation in muscle tissue. Many of the immunopathogenic processes behind the IM remain poorly understood until today, but the crucial role played by cytokines as essential regulators of leukocyte activation and migration, has long been recognized. The IM are characterized by strong expression of subsets of Tumor Necrosis Factors (TNF α , LT β , BAFF), Interferons (IFN $\alpha/\beta/\gamma$), Interleukins (IL-1/6/12/15/18/23) and Chemokines (CXCL9/10/11/13, CCL2/3/4/8/19/21) in the muscle tissue. Exploration of neutralizing agents as a therapeutic approach for IM patients is ongoing, in an attempt to find alternative treatments for patients that do not respond to conventional immune-suppressants. Reported effects of anti-TNF α treatment in IM are conflicting and new onset DM/PM has been described after administration of anti-TNF α agents to treat other diseases. Treatment with anti-IFN α monoclonal antibody has been shown to suppress the IFN type 1 gene signature in DM/PM patients and improved muscle strength. Beneficial effects of anti-IL-1 and anti-IL-6 therapy have also been reported. These results show promise for the development of future therapies. In addition, they have pinpointed cytokine profiling as an amenable approach to predict treatment outcome and to guide future therapeutic decisions through the subtyping of patients.

ORAL COMMUNICATIONS

2.O.1 Novel, rapid and sensitive diagnostic test for sporadic Inclusion-body myositis (s-IBM) employing NBR1 ELISA

C. D'Agostino, W.K. Engel, V. Askanas

USC Neuromuscular Center, University of Southern California Keck school of Medicine, Good Samaritan Hospital, Los Angeles, CA, USA

s-IBM is the most common progressive myopathy of older persons, leading to severe disability and there is no effective treatment. Characteristic are intra-myofiber vacuoles and multiprotein aggregates, and endomysial mononuclear-cell inflammation. Defective protein degradation, involving both 26S proteasome and autophagic-lysosomal pathways, importantly contribute to the pathogenesis. NBR1 and p62/SQSTM1 are proteasome-lysosome shuttle-proteins participating in degradation of ubiquitinated proteins. We previously showed both NBR1 and p62 accumulated within s-IBM myofibers, and reported that p62 immunohistochemistry is a diagnostic test for s-IBM, which is now widely used. However, immunohistochemistry is laborious, and immuno-positive fibers, if few, can be overlooked. We have now developed a better diagnostic test by adapting a newly-available NBR1 ELISA kit (Enzo-Life Science) as a rapid (3 hours), sensitive, quantitative method to measure NBR1 – it requires only 10µg of muscle biopsy-homogenate protein (3 10µm frozen sections). ELISA was performed on: 15 s-IBM, 10 non-neuromuscular controls, 8 ALS, 5 PM, 4 DM and 6 PN patients. In s-IBM, NBR1 values ranged 90-209 pg/ml, mean 122pg/ml. All controls had significantly lower values (~70%, $p < 0.005$), ranging 17-65 pg/ml, mean 39pg/ml. s-IBM is still under-diagnosed or misdiagnosed. This diagnostic test for s-IBM should facilitate early detection of s-IBM and development of treatments.

2.O.2 Inflammatory myopathy in horses chronically affected by *Babesia caballi* and *Theileria equi*

T.B. Pagano, M.P. Pasolini, A. Costagliola, D. De Biase, P. Santoro, L. Auletta, M. Greco, S. Papparella, O. Paciello
Laboratory of Neuromuscular comparative pathology, Department of Veterinary Medicine, University of Naples Federico II, Napoli, Italy

Equine piroplasmosis is a common protozoal disease of equids, but its chronic effects on skeletal muscle have been poorly investigated. The aim of this study was to assess histological and hematobiochemical findings of inflammatory myopathy occurring in horses natu-

rally affected by chronic piroplasmosis. Sixteen horses referring clinical signs of myopathy and serologically positive to one or both agents of equine piroplasmosis in absence of acute clinical signs of the disease were selected for histopathology, hematobiochemical analysis and molecular detection of inflammatory cytokines. Myopathic features were found in all biopsies from affected animals, mainly consisting of increased variability in fiber size, fiber degeneration, and mild to severe inflammatory changes varying from a predominantly lymphoplasmacytic infiltrate to mixed neutro-granulocytic forms mainly arranged in a perivascular pattern. 93% of biopsies were strongly immunohistochemically positive to Major Histocompatibility Complex Class I (MHC I) and II (MHC II); the predominant leukocyte population was CD3+, CD8+ and CD4+ in an equal proportion, with lesser number of CD79α+. RT-PCR on muscle samples revealed a significant increase of IFNγ, IL12 and TGFα compared to normal controls. Increased CPK serum activity was found in 62,5% of cases. Our study is the first report of inflammatory myopathy associated with equine piroplasmosis; it may be possibly related both to an immune-mediated mechanism, and to the chronic systemic release of muscle-impairing inflammatory cytokines.

2.O.3 Activation of the Nuclear Factor of Activated T-cells 5 pathway is characteristic of the perifascicular muscle fiber atrophy observed in dermatomyositis

B. De Paepe¹, C. Jimenez-Mallebrera², E. Iglesias³, C. Jou⁴, J.-J. Martin⁵, J. Weis⁶, Sandrine Herbelet¹, J. De Bleecker¹

¹Neuromuscular Reference Center, Laboratory for Neuropathology, Ghent University Hospital, Belgium; ²Neuromuscular Unit, Neurology Department; ³Pediatrics Department; ⁴Pathology Department, Hospital Sant Joan de Deu Barcelona, Spain; ⁵Department of Ultrastructural Neuropathology, Born-Bunge Institute, Antwerp University Hospital, Belgium; ⁶ Institute for Neuropathology, RWTH Aachen University Hospital, Germany

Nuclear factor of activated T-cells 5 (NFAT5), the central regulator of cellular response to osmotic stress, has also been recognized as an inducer of pro-inflammatory pathways. Therefore, we studied the expression of NFAT5 and a selection of its molecular targets in juvenile (n = 4) and adult (n = 15) dermatomyositis (DM) patients, comparing with healthy tissues (n = 20), using fluorescent immunolocalisation and western blotting. In normal skeletal muscle, constitutive NFAT5 staining was present in myonuclei. In DM muscle additional strong sarcoplasmic NFAT5 staining was present in the perifascicular atrophic fibers, corresponding to the activated form phosphoryl-

ated on its serine 1197 residue. Normal skeletal muscle contained constitutive levels of the osmoprotective factor aldose reductase (AR), while Taurine Transporter (TauT) staining was absent. AR was increased in the atrophic fibers, TauT showed patchy staining in perifascicular areas representing mostly CD56+ regenerating muscle fibers. Quantitative western blotting detected AR in all muscle samples but

could not show changes in AR protein levels in patients (0.45 ± 0.01 in DM versus 0.52 ± 0.10 in controls); TauT could be detected in 2 of 4 DM samples, but not in healthy muscle. Additionally, several other molecular NFAT5-targets were either increased (Heat Shock Protein 70 family) or induced (α -chemokine monocyte chemoattractant protein -1 (CCL2) and inducible NO synthase (iNOS)) in perifascicular atrophic muscle fibers. Our results show that the NFAT5 pathway is activated in the perifascicular atrophic fibers, indicating that NFAT5 may represent an important regulatory mechanism and implicating osmolyte accumulation in this hallmark myopathological feature associated with DM.

Session 3. Next Generation Sequencing and Neuromuscular Disorders

INVITED LECTURES

3.1 Next generation sequencing in Neuromuscular Disorders

M. Bartoli, S. Gorokhova, M. Krahn

Aix Marseille University, INSERM UMR_S 910, Myologie Translationnelle, Marseille, France

Next Generation Sequencing, is rapidly gaining wide use in clinical practice due to possibility of simultaneous exploration of multiple genomic regions. More than 300 genes have been implicated in neuromuscular disorders, meaning that many genes need to be considered in a differential diagnosis for a patient affected with myopathy. By providing sequencing information for numerous genes at the same time, NGS greatly accelerates the diagnostic processes of myopathies compared to the classical “gene-after-gene” approach by Sanger sequencing. In this presentation, we will describe multiple advantages of this powerful sequencing method for applications in myopathy diagnosis. We will also outline recent studies that used this approach to discover new myopathy-causing genes and to diagnose cohorts of patients with muscular disorders. Finally, we will highlight the key aspects and limitations of NGS that a neurologist considering this test needs to know in order to interpret the results and to deal with other issues concerning the test.

3.2 Next generation sequencing in limb girdle muscular dystrophies and other myopathies

M. Savarese^{1,2}, G. Di Fruscio^{1,2}, A. Torella^{1,2}, M. Mutarelli², C. Bruno³, C. Fiorillo³, M. Fanin⁴, L. Ruggiero⁵, A. Garofalo^{1,2}, T. Giugliano^{1,2}, M. Dionisi², O. De Concilio^{1,2}, F. Del Vecchio Blanco^{1,2}, G. Piluso^{1,2}, O. Musumeci⁶, G. Siciliano⁷, M. Mora⁸, L. Morandi⁸, G. Tasca⁹, A. D’Amico¹⁰, E. Bertini¹⁰, C. Minetti³, G. Ricci⁷, F.M. Santorelli¹¹, S. Sacconi¹², S. Janssens¹³, K. Claes¹³, T. Mongini¹⁴, C. Angelini¹⁵, G.P. Comi¹⁶, R.G. Tupler¹⁷, L. Politano¹⁸, V. Nigro^{1,2}

¹Dip. di Biochimica, Biofisica e Patologia Generale, Seconda Università di Napoli, Napoli, Italy; ²Telethon Institute of Genetics and Medicine, Pozzuoli, Italy; ³U.O.C. Neurologia Pediatrica e Malattie Muscolari, IRCCS Istituto Giannina Gaslini, Genova, Italy; ⁴Dip. di Neuroscienze, Università di Padova, Padova, Italy; ⁵Dip. di Neuroscienze e Scienze riproduttive ed odont., Università di Napoli “Federico II”, Napoli, Italy; ⁶Dip. di Neuroscienze, Università Messina, Messina, Italy; ⁷Dip. di Medicina Clinica e Sperimentale, Università degli Studi di Pisa, Pisa, Italy; ⁸RCCS Istituto Carlo Besta, Milano, Italy; ⁹Fondazione Don Carlo Gnocchi, Italy; ¹⁰Dip. di Neuroscienze, IRCCS Ospedale Pediatrico Bambino Gesù, Roma, Italy; ¹¹Medicina Molecolare, IRCCS Fondazione Stella Maris, Pisa, Italy; ¹²Centre de Référence des Maladies Neuromusculaires, Nice Hospital and UMR CNRS6543, Nice University, Nice, France; ¹³Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; ¹⁴Dip. di Neuroscienze, Università di Torino, Torino, Italy; ¹⁵IRCCS San Camillo, Lido Venice, Italy; ¹⁶Dip. di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università di Milano, Milano, Italy; ¹⁷Dip. di Scienze della Vita, Università di Modena e Reggio Emilia, Modena, Italy; ¹⁸Cardiomyology and Medical Genetics, Department of Experimental Medicine, Second University of Naples, Naples

In the last few years, a vast number of studies have demonstrated that Next Generation Sequencing (NGS) is the best method to discover small variations in genetically heterogeneous conditions, in which different genes can be involved with overlapping phenotypes. In the pre-NGS era, the large variability of human DNA sequences and the occurrence of several pathogenic variants in the same individual were overlooked. We have developed and applied NGS-based platforms, named MotorPlex, to test 89-93 disease genes associated with a genetic myopathy. More than 500 individuals have been sequenced so far. These were clinically classified as limb girdle muscular dystrophies, congenital myopathies or other myopathies, but no conclusion was previously reached on the basis of the traditional gene-by-gene testing. In some cases, the disease classification was also uncertain. We concluded the genetic diagnosis of a specific and expected Mendelian condition in 43% of cases, while in the remainder of cases further studies are required. Trio analysis was always necessary to improve the interpretation of results and to facilitate validation steps. To cover the gaps of NGS, we are using additional tools, such as Motorchip (ArrayCGH), RNA-Seq or Whole Exome Sequencing.

Our study represents one of the first screening of myopathic patients and demonstrates the importance of NGS in the diagnostic flowchart. Considering the decreasing cost of NGS and the rapid evolution of targeting methods, these methods are the best upcoming tests for initial approach of complex patients.

3.3 Pros & Cons on the clinical use of massive parallel sequencing methodologies

F.M. Santorelli, R. Trovato, I. Ricca, A. Rubegni
Molecular Medicine, Neurogenetics, & Neuromuscular Diseases, IRCCS Stella Maris, Pisa, Italy

Inherited neuromuscular disorders (NMD) are chronic genetic diseases posing a significant burden on patients and the health care system. Despite tremendous research and clinical efforts, the molecular causes remain undefined for about half of the patients, due to genetic heterogeneity and conventional molecular diagnosis based on the limitations of the “one gene at a time” strategy.

Recent improvements in sequencing technology have yielded the advent of massively parallel DNA sequencing systems, which produce short read lengths at very high densities. The clinical application of the “next-gen” technology has already returned many potential benefits in performing genetic analyses, especially for large-scale projects in several fields including NMD. With appropriate adaptations and set up, this strategy could be implemented into a routine genetic diagnosis set-up as a first screening approach to detect most kind of mutations, potentially before the need of more invasive and specific clinical investigations such as a muscle biopsy. Yet, limitations still exist in technical and cost/benefit terms. The take-back messages from a large set of data remain laborious and the yield disappointing for clinicians while deciphering genetic etiologies in NMD.

We will present a personal view on advantages and limitations of next-gen analyses in NMD and profile new skills to be met in clinical adaptation to the new era of medical genomics.

ORAL COMMUNICATIONS

3.O.1 Limb-Girdle Muscular Dystrophy in Egypt

N.A. Fahmy
Neuromuscular Unit and Muscle and Nerve Research laboratory, Neuropsychiatry Department, Faculty of Medicine, Ain Shams University, Egypt

Limb-girdle muscular dystrophy (LGMD) is a purely descriptive term, generally reserved for childhood- or adult-onset muscular dystrophies that are distinct from the much more common X-linked dystrophinopathies. To study the clinical, pathological, MRI imaging and genetic characteristics of LGMD patients in Egypt. Patients were selected

from those with progressive muscular dystrophy referred to Muscle and Nerve Research Laboratory, Cairo, Egypt. We studied 77 patients with dystrophic muscle biopsy. Patients had neurological assessment, family pedigree study, Serum Creatine Kinase, ECHO cardiography, electromyography, Dystrophin and Dysferlin gene testing. A battery of histochemical tests, immunohistochemistry using both fluorescent and automated methods against a panel of antibodies were done. Mini-multiplex Western Blotting was also done. We found 40 patients with limb-girdle muscular dystrophy, 12 with dysferlinopathy, 6 with calpainopathy, 6 with sarcoglycanopathy and 16 with non-specified limb-girdle muscular dystrophy. Dysferlinopathy patients showed 3 patients with proximal type, 3 patients with Miyoshi myopathy and 6 patients with anterior compartment myopathy. The Immunohistochemistry, western blotting study and genetic findings would be presented. LGMD is a common condition in Egypt. Many clinical, pathological and genetic characteristics are found and help in its diagnosis.

3.O.2 Huge variability in a huge gene: TTN variants identified in a large NGS-resequencing project

M. Savarese^{1,2}, A. Evilä³, G. Di Fruscio^{1,2}, A. Vihola³, A. Torella^{1,2}, G. Tasca⁴, F. Magri⁵, Sandra Janssens⁶, L. Ruggiero⁷, K. Claes⁶, M. Mora⁸, O. Vanakker⁶, L. Morandi⁸, L. Santoro⁷, S. Sacconi⁹, E. Mercuri¹⁰, J. De Bleecker¹¹, L. Politano¹², G.P. Comi⁵, P. Hackman³, B. Udd^{3,13,14}, V. Nigro^{1,2}
¹Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Naples, Italy; ²Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Naples, Italy; ³Folkhälsan Institute of Genetics, University of Helsinki, Helsinki, Finland; ⁴Unit of Neuromuscular Disorders, Laboratory of Molecular Medicine, Bambino Gesù Children's Research Hospital, Rome, Italy; ⁵Dino Ferrari Center, IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuroscience Section, Dipartimento di Fisiopatologia medicochirurgica e dei trapianti, Università di Milano, Milano, Italy; ⁶Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; ⁷Dipartimento di Neuroscienze e Scienze riproduttive ed odontostomatologiche, Università degli Studi di Napoli “Federico II”, Napoli, Italy; ⁸Muscle Cell Biology Lab, Neuromuscular Diseases and Neuroimmunology Unit, Fondazione Istituto Neurologico C. Besta, Milano, Italy; ⁹Centre de Référence Maladies Neuromusculaires-SLA, Hôpital Archet 1, CHU de Nice, Nice, France; ¹⁰Pediatric Neurology Unit, Catholic University School of Medicine, Rome, Italy; ¹¹Department of Neurology, Ghent University Hospital, Ghent, Belgium; ¹²Cardiomyology and Medical Genetics, Department of Experimental Medicine, Second University of Naples, Naples, Italy; ¹³Neuromuscular Research Center, University of Tampere and Tampere University Hospital, Tampere, Finland; ¹⁴Department of Neurology, Vaasa Central Hospital, Finland.

Titin is the largest and, probably, the most complex human protein. It is a scaffold protein aiding in myofibrillar assembly during myogenesis and it acts as a molecular spring determining the passive elasticity of the muscle,

besides many regulatory roles. Titin is encoded by the 363-exon titin gene (TTN). Mutations in this gene have been associated with different genetic diseases, including hypertrophic and dilated cardiomyopathy and different forms of myopathy: an early-onset severe myopathy, a proximal form with early respiratory muscle involvement, a dominant distal myopathy and a recessive limb girdle muscular dystrophy. Until the recent development of the Next Generation Sequencing and its use in routine diagnosis, the molecular analysis of TTN gene had been hampered by its huge genomic size. Recently, several different studies, based on NGS approaches, have evidenced a high number of novel truncating or missense variants associated with human diseases. Here we report the TTN variants identified in a large project of targeted resequencing in which all the TTN exons of about 500 patients affected by unclassified forms of LGMD or myopathy have been analyzed. In particular, we compare the results obtained with an external control cohort, composed of all the variants listed in the NHLBI-ESP and representing the general population, and with an internal control cohort of 62 unaffected individuals analyzed by NGS. The huge variability that we observe and describe in the TTN gene suggests the need for a careful approach in the interpretation of data generated, which has to include a comprehensive segregation analysis and mandatory functional assays.

Session 4. Heart involvement in Neuromuscular Disorders

INVITED LECTURES

4.1 Cardiovascular morbidity and mortality in Laminopathies. The crucial role of right ventricle involvement

D. Duboc, G. Durand Viel, H.-M. Becane, B. Eymard, P. Laforêt, A. Béhin, T. Stovskovic, K. Wahbi
Department of Cardiology, Hop Cochin and Myology Institute Hop Salpêtrière, Paris, France

Patients carrying Lamin A/C gene mutations are frequently exposed to cardiovascular complications. Severe dysrhythmias (AF, AV Block, Ventricular Arrhythmias), Left ventricular dysfunction, right ventricular dysfunction, peripheral emboli associated to atrial fibrillation are the main complications frequently observed and often life threatening.

In a retrospective analysis concerning 136 patients we have noticed that 52 patients were indicated for an Implantable Cardiac Defibrillator. In this subgroup 13 patients received one or more appropriate choc for episode of life threatening ventricular arrhythmia.

Thirteen patients experienced one or more episode of peripheral emboli mainly cerebral and 12 patients were

heart transplanted for severe intractable heart failure.

Ten patients presented a predominant right ventricle involvement. 4 of these patients died from right heart failure and 3 has to be transplanted for this reason.

In conclusion, Lamin A/C gene mutations associated disease is associated to a high risk of cardiovascular morbidity and mortality. Right ventricular involvement is frequent and associated with a poor prognosis

4.2 Heart involvement in Myotonic Dystrophy type 1

G. Nigro
Chair of Cardiology Second University of Naples, Naples, Italy

Myotonic dystrophy (Dystrophia Myotonica, DM) is the most frequently inherited neuromuscular disease of adult life. It is a multisystemic disease with major cardiac involvement. Core features of myotonic dystrophy are myotonia, muscle weakness, cataract, respiratory failure and cardiac conduction abnormalities. Classical DM, first described by Steinert and called Steinert's disease or DM1 (Dystrophia Myotonica type 1) has been identified as an autosomal dominant disorder associated with the presence of an abnormal expansion of a CTG trinucleotide repeat in the 3' untranslated region of DMPK gene on chromosome 19. Conduction system abnormalities, atrial or ventricular arrhythmias and, less commonly, myocardial dysfunction are observed in patients with DM1 and may occasionally represent the initial manifestation of the disease, even in the absence of overt neuromuscular involvement. Thus, cardiologists should be aware of this diagnosis. Conversely, in all patients presenting with DM1, a careful clinical and diagnostic evaluation needs to be performed for the identification of patients at risk of major cardiac events.

An attitude of a low threshold for invasive procedures is suggested, considering the unclear rate of cardiac disease progression and the risk of sudden death in some subsets of patients. However several questions are still unanswered to improve the stratification of DM1 patients at high risk of SCD and/or heart failure.

4.3 Cardiovascular Magnetic Resonance in Muscular Dystrophies

A. Florian
Department of Cardiology and Angiology, University Hospital Muenster, Muenster, Germany

In several forms of muscular dystrophies (MD), concomitant cardiomyopathy is described, which in some cases may be the predominant manifestation of the underlying genetic disease.

In the last years, cardiovascular magnetic resonance (CMR) has been increasingly used for the diagnosis as

well as for follow-up of cardiac involvement in MD patients. CMR offers an accurate and reproducible tool for LV systolic function assessment together with the possibility of myocardial tissue characterization and fibrosis detection based on late gadolinium enhancement (LGE) imaging. Early detection of cardiomyopathy and timely initiation of proper therapy is thus facilitated.

In Duchenne and Becker MD (DMD/BMD) cardiac involvement as depicted by CMR occurs in approximately 70% of patients. A typical LGE pattern – in the sub-epicardium of the left ventricular (LV) lateral wall – that precedes LV systolic dysfunction is described. Moreover, presence of transmural LGE showed independent value additive to LV systolic dysfunction in the risk stratification of BMD/DMD patients. Cardiac involvement is also a frequent finding in female carriers of DMD, but rarely observed in BMD carriers. Carriers with cardiac involvement demonstrate the same myocardial fibrosis pattern as their male counterparts with overt disease.

Myotonic dystrophy type 1 and 2 patients may also present myocardial fibrosis (up to 40% of cases) as depicted by LGE CMR, but more heterogeneous in pattern and usually with preserved LV function.

Lastly, in the highly heterogeneous group of Limb-Girdle MD a midmyocardial LGE pattern in the septum that precedes the onset of ventricular dilatation and systolic dysfunction is described.

4.4 New therapeutic options in the end stage heart failure in Duchenne Muscular Dystrophy

A. Amodeo

Department of Pediatric Cardiology and Cardiac Surgery, Bambino Gesù Children Hospital, Rome, Italy

End-stage dilated cardiomyopathy (DCM) is one of the most challenging complication in patients with Duchenne Muscular Dystrophy. We report our experience with use of left ventricular assist device (LVAD) as destination therapy (DT) for the management of this new subgroup of children

From February 2011 to February 2015 five children with Duchenne syndrome and DCM were assisted with a LVAD. The median age at surgery was 15,6 years (range 14,2-17,4). Preoperatively, all patients underwent a multidisciplinary assessment. Four children received VAD after long-term medical inotropic support while one underwent implantation after 12 days of VA-ECMO.

All children survived to hospital discharge. All patients after early extubation required non-invasive positive pressure ventilation and cough machine cycles. The early post-operative course was characterized in one patient by mediastinal re-exploration. The second child, for iatrogenic spleen lesion, required several abdominal surgery. The last three patients had uneventful post op. At mean follow-

up time of 21.2 months (range 1-44.8), we have two late death, both not related to cardiac causes. One child died at 44,8 months from implantation for sepsis secondary to pulmonary infection while the second died in a peripheral hospital for massive bleeding due to a otorhinolaryngology maneuver 28,6 months after surgery.

Our experience showed the possibility to use VAD as DT in Duchenne with end stage DCM. Given the increasing pediatric and adult population of Duchenne DCM, our results represent a significant step forward for the treatment of these patients with otherwise no therapeutic option.

ORAL COMMUNICATIONS

4.O.1 Severe and early onset cardiomyopathy in females with Danon disease

C. Hedberg Oldfors^a, G. Máthé^a, K. Thomson^b, M.

Tulinus^c, K. Karason^d, I. Östman-Smith^e, A. Oldfors^a

Departments of Pathology^a, Paediatrics^{c,e}, Molecular and Clinical Medicine^d, University of Gothenburg, Gothenburg, Sweden, Regional Molecular Genetics Laboratory^b, Churchill Hospital, Oxford, UK

Danon disease is caused by mutations in the lysosome-associated membrane protein-2 gene, LAMP2, located on the X chromosome. Female carriers with LAMP2 mutations most often present with late onset cardiomyopathy and slow disease progress. We investigated the explanted heart and skeletal muscle biopsies in two girls, aged ten and thirteen years, who underwent cardiac transplantation because of hypertrophic cardiomyopathy secondary to LAMP2 mutations and a 41-year old female with late-onset familial LAMP2 cardiomyopathy with more typical clinical phenotype. The two girls in contrast had clinical features that mimicked severe primary hypertrophic cardiomyopathy caused by mutations in genes encoding sarcomeric proteins. Immunohistochemistry in cardiac muscles showed a remarkable pattern with lack of LAMP2 protein in large regions including thousands of cardiomyocytes that also showed myocyte hypertrophy, lysosomal enlargement and disarray.

In other equally large regions there was preserved LAMP2 expression and nearly normal histology. The skeletal muscle biopsy revealed no pathological changes. An uneven distribution of LAMP2 protein may cause deleterious effects depending on which regions of the myocardium that are lacking LAMP2 protein in spite of an overall moderate reduction of LAMP2 protein. This may be a more common mechanism behind early aggressive disease in females than an overall skewed X-chromosome inactivation in the tissue.

In conclusion, female carriers of LAMP2 gene mutations with early onset of disease may display a clinical picture that mimics sarcomeric hypertrophic cardiomy-

opathy. The resemblance may cause diagnostic delay; not least since LAMP2 associated cardiomyopathy in females occurs without any skeletal muscle involvement.

4.0.2 Glycogen storage cardiomyopathy associated with GYG1 and RBCK1 deficiency

A. Oldfors

Department of Pathology, The Sahlgrenska Academy at University of Gothenburg, Sweden

Two novel glycogen storage diseases are due to defects of the E3 ubiquitin ligase RANBP-type and C3HC4-type Zinc Finger-Containing 1 (RBCK1) and the autoglucosylating protein glycogenin-1 (GYG1) that acts as a primer for glycogen synthesis.

These two diseases share characteristic features in skeletal muscle with accumulation of abnormal glycogen partly with features of polyglucosan. The storage material is ubiquitinated and labeled with sequestosome / p62 in both conditions. In spite of similar light and electron microscopical features the storage material is in general less amylase resistant in GYG1 deficiency than in RBCK1 deficiency.

The cardiac involvement is variable and in general more severe in RBCK1 deficiency than in GYG1 deficiency, but may lead to severe congestive heart failure and require cardiac transplantation in both conditions. As in skeletal muscle the cardiac storage material is less amylase resistant in GYG1 deficiency than in RBCK1 deficiency. Unlike GYG1 deficiency some patients with RBCK1 deficiency develop an autoinflammatory disease in childhood.

The pathogenesis in GYG1 deficiency involves reduced amount of the glycogen priming protein and/or loss of its ability to autoglucosylate. Total lack of functional GYG1 is compatible with formation of apparently normal glycogen in addition to the abnormal storage. The substitute for GYG1 in glycogen priming remains enigmatic as well as the pathogenesis of the abnormal glycogen. The pathogenesis of glycogen storage disease with polyglucosan in RBCK1 deficiency remains to be explored.

Session 5. Laminopathies

INVITED LECTURES

5.1 The broad spectrum of laminopathies

G. Bonne

Sorbonne Universités, UPMC Univ Paris 06, INSERM UMRS_974, CNRS FRE3617, Center of Research in Myology, F-75013, Paris, France

Laminopathies are due to mutations in the LMNA gene encoding Lamin A and C and comprise highly heterogeneous human disorders including cardiac and muscular dystrophies, lipodystrophies and progeria. Lamins A/C are

constituents of the nuclear lamina, a meshwork of proteins underneath the nuclear envelope. Since the discovery of the first LMNA mutation in the Emery-Dreifuss muscular dystrophy (EDMD), more than 450 different LMNA mutations were reported (www.umd.be/LMNA/). In order to dissect the pathomechanisms of LMNA mutations in striated muscle, we created knock-in mouse models that reproduced LMNA mutation identified in patients presenting with cardiac and muscular dystrophies. We demonstrated an aberrant increase in MAP kinases in hearts from Lmna H222P knock-in mice hearts, a mouse model of EDMD. These results provide proof of principle for MAP kinase inhibition as a therapeutic option to prevent or delay the onset of cardiomyopathy in EDMD. These preliminary findings were the foundation of our preclinical work. Pharmacological or genetic blockade of signaling in the MAP kinase cascade in Lmna H222P knock-in mice improves left ventricular dilatation and deterioration in cardiac contractility. Treatment also decreases cardiac fibrosis, an end-stage and irreversible feature of cardiomyopathy in EDMD. While it remains unclear how alterations in A-type lamins lead to activation of MAP kinase signaling in the heart, these studies clearly show that the abnormal activation is involved in the pathophysiology of EDMD. Inhibitors of MAP kinase signaling are currently in human clinical trials for other indications and could potentially be tested in human subjects with EDMD.

5.2 Cardiovascular disease in LMNA-related premature ageing syndromes

V. Andrés

Vascular Biology Program, Centro Nacional de Investigaciones Cardiovasculares Carlos III, Melchor Fernández Almagro 3, 28029 Madrid, Spain

The world population is experiencing progressive aging, the main cardiovascular disease (CVD) risk factor. Nuclear lamins A and C (A-type lamins, LMNA gene) are filamentous proteins with play architectural and functional roles. More than 400 mutations throughout the LMNA gene have been linked to human diseases termed laminopathies, which include tissue-specific disorders and premature aging syndromes. Hutchinson-Gilford progeria syndrome (HGPS) is a very rare disorder caused by the lamin A mutant called progerin. HGPS patients exhibit excessive atherosclerosis, vascular calcification and death at an average age of 13 years, predominantly from myocardial infarction or stroke. Evidence is accumulating that progerin is also expressed in aged tissues of control individuals, suggesting its role during physiological aging. To gain insight into the molecular and cellular mechanisms underlying CVD in progeria, we use mouse and human cells and genetically-engineered mouse models in which expression of progerin is manipulated ei-

ther globally or in a tissue-specific manner. Our studies aim at uncovering new and possibly cell-type-specific mechanisms governing premature aging, and related atherosclerosis, vascular calcification and heart disease in the setting of HGPS. We also seek to identify gender- and age-related changes in protein abundance and oxidation in organs affected in progeria and normal aging or only in normal aging with the ultimate goal of identifying molecular mechanisms common to both processes as well as specific to each.

5.3 Role of cytokine signaling in LMNA-linked muscular dystrophies: new findings and perspectives

G. Lattanzi^{1,2}

¹CNR Institute for Molecular Genetics, Unit of Bologna, Bologna, Italy; ²Rizzoli Orthopedic Institute, Bologna, Italy

An increasing number of studies in human and mouse models of laminopathies highlight dysregulation of cytokine levels downstream of lamin mutations. Moreover, involvement of cytokines has been suggested in inflammatory and non-inflammatory muscle diseases. These considerations prompted us to set up a wide screening of cytokine regulation in Italian patients affected by diverse laminopathies. In sera collected from a large cohort of individuals affected by Emery-Dreifuss Muscular Dystrophy, Limb-Girdle Muscular Dystrophy 1B, Dilated Cardiomyopathy with conduction system disease, type 2 Familial Partial Lipodystrophy, Mandibuloacral Dysplasia, Atypical Progeria Syndrome, we found an interesting effect of lamin mutations on a few cytokines including IL17, IL6, VEGF and TGFbetas. Some of these results were consistent with data obtained in laminopathic primary cell cultures. The biological effects of TGFbeta 2 were tested in cellular models of bone, muscle and adipocyte differentiation to unravel potential pathogenetic mechanisms. Moreover, the signaling effectors of lamin-dependent TGFbeta2 pathways were identified. The results pave the way to further investigation in animal models and suggest biomarkers and therapeutic targets for laminopathies.

ORAL COMMUNICATIONS

5.0.1 Novel mutations in LMNA gene and associated phenotypes

A. Taglia, P. D'Ambrosio, A. Torella¹, E. Picillo, R. Petillo, G. Piluso¹, V. Nigro¹, L. Politano

Cardiology and Medical Genetics, Department of Experimental Medicine; ¹ Lab. of Medical Genetics, Department of Biochemistry, Biophysics and General Pathology, Second University of Naples

Mutations in the lamin A/C gene (LMNA) have been associated with several phenotypes ranging from systemic to prevalent of muscle, heart, skin, nerve etc. More re-

cently they have been associated with dilated cardiomyopathy (DCM) and severe forms of arrhythmogenic right ventricular cardiomyopathy (ARVC).

We report five novel mutations - 4 missense and 1 deletion - in 5 unrelated patients showing different phenotypes, ranging from the early onset congenital form of laminopathy to severe classical LGMD phenotype. All these newly identified variants were not found in 250 ethnically-matched control subjects.

The variant c.103-105del CTG in LMNA gene was described post-mortem in a young patient, with a merosin positive congenital muscular dystrophy, who presented at the age of 9 a first degree A-V block and died from a fatal supraventricular paroxysmic tachycardia. Two patients who presented as onset symptom, lower limbs muscle weakness, developed heart conduction defects requiring pace-maker implantation at the age of 38 and 26 years respectively. The first, carried the mutation c.1339G>C, and died at the age of 40y4m by intractable heart failure; the latter carrying the mutation c.265C>T is still alive, at the age of 30. In the fourth patient who showed a classical LGMD phenotype without heart involvement, and died aged 68 years of respiratory insufficiency, the missense mutation c.1579C>T was observed.

Finally the mutation c. 111A>G was found in an asymptomatic young girl presenting isolated hyperCKemia. This study further expands the role of the LMNA gene in the pathogenesis of cardiac laminopathies, suggesting that LMNA should be included in mutation screening of all patients with suspected arrhythmogenic cardiomyopathy, particularly when they have ECG evidence for conduction defects.

5.0.2 Analysis of X chromosome inactivation in carriers of X-linked Emery Dreifuss Muscular Dystrophy

E. Viggiano¹, N. Carboni², A. Madej-Pilarczyk³, E. Picillo¹, S. Del Gaudio⁴, A. Palladino¹, G. Nigro⁵, I. Hausmanowa-Petrusewicz³, L. Politano¹

¹Cardiology and Medical Genetics, Department of Experimental Medicine, Second University of Naples; ²Divisione di Neurologia, Ospedale San Francesco di Nuoro, Nuoro; ³Mossakowski Medical Research Centre, Warsaw, Poland; ⁴Section of Biotechnology and Molecular Biology "A. Cascino", Department of Experimental Medicine, Second University of Naples; ⁵Arrhythmologic Unit, Department of Cardiothoracic Sciences, Second University of Naples, Italy

Emery-Dreifuss muscular dystrophy (EDMD), is a skeletal myopathy characterized by progressive muscular weakness, joint contractures, and cardiac disease. LMNA gene, encoding lamins A and C, is responsible for the autosomal forms, usually with a dominant transmission, while EMD gene, encoding emerin, causes the X-linked form of EDMD.

Female carriers of X-linked EDMD are usually asymptomatic, but they may show cardiac involvement, such as arrhythmias, often fatal.

Aim of the present work was to evaluate whether the X chromosome inactivation (XCI) can play a role in the pathogenesis of cardiomyopathy in carriers of X-linked EDMD, as we noted in Duchenne Muscular Dystrophy carriers.

To this aim in 31 EDMD carriers from 15 families, followed at the Cardiomyology and Medical Genetics of Second University of Naples, Nuoro San Francesco Hospital, and the Mossakowski Medical Research Centre of Warsaw, the pattern of X chromosome inactivation was determined in the lymphocytes, using the AR methylation-based assay.

The carriers were divided into two groups, symptomatic (6) and not symptomatic (25); furthermore the last group was subdivided in a) less or b) more 35 years, as arrhythmic events usually occurs in EDMD carriers after the age of 35.

The results showed that, unlike previously observed in DMD carriers, all females analyzed, both symptomatic and asymptomatic, presented a random XCI, suggesting that the onset and presence of symptoms in EDMD carriers should not be related with a skewed XCI in leucocytes.

However such a correlation cannot be completely ruled out, as XCI pattern in the leucocytes does not reflect the pattern observed in conduction tissue cardiac cells.

Session 6. Mucopolysaccharidosis: a multidisciplinary disease requiring a multifaced approach

INVITED LECTURES

6.1 Clinical aspects of mucopolysaccharidosis

G. Andria

Department of Translational Medicines, Section of Pediatrics, Federico II University, Naples

Mucopolysaccharidoses (MPS) are lysosomal storage disorders that share many clinical features. The spectrum of manifestations in the severe forms include facial dysmorphism, bone dysplasia, hepatosplenomegaly, neurological abnormalities, developmental regression and a reduced life expectancy, while patients affected by the attenuated form, may have almost normal phenotype and good quality of life. MPS have an autosomal recessive mode of inheritance, except the MPS II (Hunter syndrome), which is X-linked. The diagnosis initially is carried out by detecting increased urinary excretion of mucopolysaccharides (GAG), specific enzymatic deficiency in serum, fibroblasts, leukocytes, and gene mutations by molecular testing. Treatments are palliative,

but there have been important developments in the use of specific strategies such as enzyme replacement therapy for some MPS (type I, II, IV, VI, VII). Moreover, transplantation of hematopoietic stem cells (HSCT) can improve the prognosis in some selected patients with MPS I, but this procedure is associated with significant risks. Other approaches, still under development, are based on the use of drugs (for example, genistein) that reduce the synthesis of the accumulated substrate on gene therapy. The transfer of the normal gene to correct the genetic defect, using vectors, has been successfully used in animal models and is being translated to patients through clinical trials already underway.

6.2 Neuroradiological aspects in Mucopolysaccharidosis

R. Manara

Dipartimento di Medicina e Chirurgia, Università di Salerno, Salerno, Italy

Head and spine involvement is common in mucopolysaccharidosis (MPS) and includes a variety of bone and parenchymal abnormalities. Changes are not type-specific, their prevalence may differ across subtypes and might include skull changes (macrocephaly, craniosynostosis, hyperostosis, craniofacial anomalies, venous outflow abnormalities, "J-" or "omega"-shaped pituitary sella, closed meningo(encephalo)cele, abnormalities of the ossicular chain, teeth and temporomandibular joint), brain changes (perivascular spaces enlargement, communicating hydrocephalus and atrophy, CSF space enlargement around the optic nerve, optic nerve atrophy, arachnoid cysts, Chiari I malformation, megacisterna magna, megacerebellum), atlo-occipital junction changes (atlanto-axial instability, spinal canal stenosis), spinal cord changes (myelopathy, syringomyelia) and vertebral changes (platyspondylia, scoliosis, kifosis, spinal canal stenosis, dural ectasia). These changes might represent peculiar but clinically non-significant MPS-related features or entail a high potential risk thus requiring prompt diagnosis and, in selected cases, close follow-up or surgical correction.

6.3 Results of enzyme replacement therapy in mucopolysaccharidosis

R. Parini¹, S. Gasperini¹, M. Rigoldi²

¹ *Rare Metabolic Diseases Unit, Pediatric Clinic, Fondazione MBBM, San Gerardo Hospital and* ² *Rare Adult Diseases Unit, Internal Medicine, San Gerardo Hospital, Monza, Italy*

Until recently only palliative care was available for the management of patients affected by Mucopolysaccharidoses (MPS). Enzymatic replacement therapy (ERT) was approved in the early 2000s for MPS I, MPS II and

MPS VI. The ERT for MPS IV A has recently been approved (2014). ERT consists in infusing intravenously the recombinant enzymes which are internalized in the cells through M6P receptors to reach the lysosomes in order to replace the defective enzymes. The phase 3 or 2/3 trials performed for each of these treatments showed that ERT was effective in significantly reducing urinary glycosaminoglycan excretion and organomegaly and substantially improving many other signs/symptoms like pulmonary function, joint mobility and endurance. Heart valves and bones, however appear to be resistant to ERT. Furthermore, ERT is unable to cross the blood-brain barrier (BBB), precluding the possibility to modify CNS pathology. The long-term follow-up of the patients included in clinical trials and of the other patients treated after registration of these drugs showed that improvement is sustained over the time with the result of a better quality of life. Adverse events were rare and, in general, mild and easily treatable. To address the CNS manifestations, studies are ongoing to explore the efficacy of intrathecal administration. As MPSs are progressive diseases it is crucial to start treatment early, before severe tissue damage is established. This is demonstrated by the clinical evolution of a small cohort of patients who started treatment at few months of life. Neonatal screening for MPS needs to be discussed.

Session 7. New therapeutic approach in Neuromuscular Disorders

INVITED LECTURES

7.1 Novel translational approach in Duchenne Muscular Dystrophy treatment

S. Messina

Department of Neurosciences and Centro Clinico Nemo Sud, University of Messina, Messina, Italy

Most mutations that truncate the reading frame of the DMD gene cause loss of dystrophin expression and lead to Duchenne muscular dystrophy. However several cases have been identified that did not follow this reading frame rule. Although these patients had nonsense mutations, which are predicted to result in no protein translation, their muscle biopsy revealed significant amounts of dystrophin expression and the clinical phenotype was a very mild dystrophinopathy.

Amelioration of disease severity can result from alternate translation initiation beginning in DMD exon 6 that leads to expression of a highly functional N-truncated dystrophin. This novel isoform results from usage of an internal ribosome entry site (IRES) within exon 5 that

is glucocorticoid-inducible. IRES activity is confirmed in patient muscle by both peptide sequencing and ribosome profiling. Generation of a truncated reading frame upstream of the IRES by exon skipping leads to synthesis of a functional N-truncated isoform in both patient-derived cell lines and in a new DMD mouse model, where expression protects muscle from contraction-induced injury and corrects muscle force to the same level as control mice. These findings support a novel therapeutic approach for patients with mutations within the 5' exons, which account for around 6% of DMD patients.

7.2 New therapeutic approach on Myotonic dystrophies

G. Meola^{1,2}

¹Department of Biomedical Sciences for Health, University of Milan, IRCCS Policlinico San Donato, Milan, Italy; ²Laboratory of Muscle Histopathology and Molecular Biology, IRCCS Policlinico San Donato, Milan, Italy

Myotonic dystrophy type 1 (DM1) and 2 (DM2) are autosomal dominant multisystemic disorders characterized by myotonia, muscle weakness and wasting, cardiac conduction defects and neuropsychological manifestations. The DM pathogenesis is based on the dominant gain-of-function of the DMPK or CNBP transcripts containing expanded CUG/CCUG repeats that accumulate in cell nuclei as foci. Mutant RNAs alter the activity of RNA-binding proteins leading to embryonic patterns of alternative splicing in adult tissues. The remarkable progress in understanding the disease pathobiology resulted in the design of molecular therapies, which have been successfully tested in animal models. Phenotype reversal can be obtained by the modulation of altered levels of RNA-binding proteins (MBNL1, CUGBP1, p68...) or targeting the expanded transcripts. To date, two main experimental therapeutic strategies of targeting expanded repeat RNA were described: (i) antisense oligomer-induced (ASO) degradation of toxic CUGexp-containing

RNA and (ii) inhibition of pathogenic interaction of CUGexp RNA with nuclear proteins. In cellular or animal models of DM, the efficient degradation of CUGexp transcripts was induced by several mechanisms like RNA interference, ribozymes or chemically modified ASOs which activate nuclear RNase H. For an in vivo blocking of CUGexp/protein interaction either ASOs or small compounds that bind to CUG repeat hairpin were tested. Only CAG-25 morpholino and pentamidine were described so far as efficient tools to inhibit MBNL1 binding by toxic RNA. It is noteworthy that recently Isis-Biogen has started a phase 1/2 study in patients with DM1 using an antisense oligonucleotide specifically designed to reduce toxic DMPK RNA.

7.3 Histone deacetylase inhibitors: a potential epigenetic treatment for Duchenne muscular dystrophy

V. Saccone¹, S.A. Consalvi¹, M. Sandona¹, L. Tucciarone¹, P.L. Puri^{1,2}

¹IRCCS Fondazione Santa Lucia, Via del Fosso di Fiorano 64, 00143, Roma, Italy; ²Sanford-Burnham Medical Research Institute, Sanford Children's Health Research Center, La Jolla, California, United States of America

The most severe muscular dystrophy is the Duchenne Muscular Dystrophy (DMD). We identified a muscle-Interstitial population -fibro-adipocyte progenitor (FAP)- that can promote either muscle regeneration or fibroadipogenesis in dystrophic muscles contributing to pathology. This bivalence can be solved by HDAC inhibitors activating in FAPs a BAF60C-myomiR network and promoting the regenerative activity at expense of FAP fibro-adipogenic potential increasing muscle genes and myo-miRs expression. HDACi beneficial effects are restricted to young mdx mice and are lost in aged mdx mice, whose muscles have exhausted the regeneration potential and are replaced with fat and fibrotic scars. Perturbing the components of the network we aimed to find strategies towards restoring the regeneration potential in dystrophic muscles at advanced stages of disease.

Our research, promises to shift the direction of the current research in regenerative medicine toward a "qualitative" modulation of adult tissue-stem cell niche and will open new perspectives in the pharmacological treatment of neuromuscular diseases challenging the current dogma that limits to satellite cells the target of interventions toward stimulating endogenous regeneration of diseased muscles. Based on our pre-clinical studies in mdx mice, the HDACi Givinostat has moved into a phase II clinical trial with DMD boys. We are identifying and characterizing the activity of human FAPs from muscle biopsies of DMD patients in order to identify molecular biomarkers that can predict the disease progression and the response to pharmacological interventions.

7.4 Givinostat: a new therapeutic approach for the treatment of Duchenne Muscular Dystrophy

P. Bettica

Not arrived

ORAL COMMUNICATIONS

7.0.1 Pompe disease: pathophysiology and novel approaches to therapy

J.-A. Lim, N. Raben

Laboratory of Muscle Stem Cells and Gene Regulation, NIAMS, NIH, Bethesda, MD

The clinical spectrum of Pompe disease, a deficiency of lysosomal acid alpha-glucosidase (GAA), ranges from

fatal cardiomyopathy and skeletal muscle myopathy in infants to attenuated late-onset myopathy in adults. The only available treatment, designed to provide the missing enzyme, proved to be successful in reversing cardiac but not skeletal muscle abnormalities. The failure of the therapy to fully deliver on its promise has been due not only to the inefficient drug supply to muscle but also to the inadequate and entrenched view of the disease pathogenesis: enlargement of glycogen-filled lysosomes and lysosomal rupture leading to muscle destruction. We have shown that the pathological cascade in muscle involves dysfunctional autophagy and inhibition of the autophagic flux. Another abnormality is the accelerated production of large lipofuscin deposits - a sign of mitochondrial dysfunction. Indeed, damaged mitochondria with reduced $\Delta\Psi_m$ and altered calcium buffering capacity, a decreased oxygen consumption and ATP production, and defective mitophagy were detected in Pompe muscle. The disease has the characteristics of autophagic myopathy, premature muscle ageing/lipofuscinosis. Several new therapeutic approaches have been successfully tested in vitro and in GAA-KO: suppression of autophagy, modulation of calcium levels by Ca²⁺ channel blockers, and restoration of autophagic flux by the overexpression of TFEB and TFE3, the two transcriptional regulators of lysosomal/autophagosomal biogenesis. Mapping the genome-wide TFE3 binding sites (ChIP-seq) showed a significantly lower number of peaks in GAA-deficient cells compared to controls, raising a possibility that a deficiency of a lysosomal enzyme may also be associated with epigenetic abnormalities

INVITED MSM CONGRESS CLOSING LECTURE

I.C.C.L The progress of Myology in the last 30 years

V. Dubowitz

Emeritus Professor of Paediatrics, Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London WC1N 1EH, UK

We live in exciting times. Progress has been remarkable; like coming out of the wilderness into the promised land. On the Genetic front Worton's location of the Duchenne gene at Xp21 and Kunkel's cloning and characterization of the gene was followed by a stream of new gene discoveries. In parallel came remarkable advances in technology from positional cloning to whole exome sequencing. An important milestone was establishment of ENMC in early 1990's promoting collaboration in Europe through workshops and leading the field in genetic advances.

Muscle imaging started in early 1980s with Heckmatt's pioneering real-time ultrasound imaging, showing selective involvement of muscles, which proved a

valuable screening tool in the clinic and eclipsed the need for electromyography in children. This was followed by CT scanning and MRI, which has established characteristic patterns of muscle involvement in individual diseases.

On the therapeutic front a major development in the 1980s was the introduction of non-invasive, positive-pressure, nasal mask ventilation in Duchenne boys in their late teens, going into respiratory failure from diaphragmatic weakness, with a prognosis of months. They started surviving into the 20's and 30's. Efforts are also in progress to combat the late cardiac failure.

Corticosteroids introduced in the late 1980s and universal acceptance by the late 1990s has had a major impact on the clinical course of Duchenne.

Advances on the genetic front have opened the way for potential gene therapy starting with Duchenne dystrophy, which seems on the brink of coming to fruition.

The discovery of animals with Duchenne mutations has given a major boost to laboratory studies and potential therapy. The mouse is enigmatic in lacking dystrophin, but showing no significant pathology, except diaphragm, and fairly normal activity and lifespan. In contrast the dystrophic Golden Retriever dog has severe clinical problems comparable to Duchenne and is a good therapeutic model for Duchenne.

Recognition of cases of Duchenne with out of frame mutations and absent dystrophin but a mild clinical course and also exceptional dogs with the Golden Retriever mutation and absent dystrophin, suggest a new avenue for potential therapeutic mechanisms.

Finally a word of appreciation to our President, Giovanni Nigro, who founded this active and prestigious Society some years ahead of the World Muscle Society, in which he was also a founding father, and helped to put myology on the world stage.

ABSTRACTS OF POSTER COMMUNICATIONS

(in alphabetical order of the first Author)

Hydrotherapy program in Duchenne Muscular Dystrophy: motor functional evaluation and body self perception

K. Gorni, C. Grandi, G. Giuliano, V. Morettini, V. Baiardi, V. Sansone
NeMo Clinic, Milan, Italy

Hydrotherapy has been implemented in rehabilitation programs to reduce spasticity and pain and to improve recovery of muscle damage after traumatic injury but there is still limited data on its use and efficacy in Duchenne Muscular Dystrophy DMD.

To verify the efficacy of an aquatic rehabilitation program in a DMD population. The study will consider both motor function and body self-perception

10 DMD patients, both ambulant and wheel-chair bound, aged from 3 to 17 years were subjected to weekly hydrotherapy for 45 min for a 6 weeks period. MFM, North Star, PUL, 6MWT assessments and hip, knee and ankle angle measurement were performed at the beginning and the end of the study. Body perception was evaluated using pictures drawn by kids, representing their human figure and the Goodenough-Harris Draw-a-Person Test of body self-perception and body scheme knowledge.

Preliminary results suggest that water physical therapy maintains motor function and improves passive range of motion. Initial analysis of drawings indicate that body self-awareness is also positively affected by this rehabilitation program.

Our preliminary results suggest that hydrotherapy may improve motor function and body self-perception in DMD. Confirmatory data in larger number of patients will create the rationale to include hydrotherapy in the standard treatment program for DMD.

Exome sequencing identifies mutations in two genes encoding the LIM-proteins N-RAP and FHL1 in a BAG3 myofibrillar myopathy

M. Meregalli², F.C. D'Avila^{1,5}, S. Lupoli^{1,5}, M. Barcella^{1,5}, A. Orro³, F. De Santis², C. Sitzia², A. Farini², P. D'Ursi³, S. Erratico⁴, L. Milanesi³, D. Braga^{1,5}, D. Cusi^{1,5}, C. Barlassina^{1,5}, Y. Torrente²

¹ Department of Health Sciences, Università degli Studi di Milano, Milan; ² Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Fondazione IRCCS Cà Granda Ospedale; Maggiore Policlinico, Centro Dino Ferrari, Milan; ³ Institute for Biomedical Technologies, CNR, Segrate, (Milan); ⁴ YStem s.r.l., Milan; ⁵ Filarete Foundation, Milan

Myofibrillar myopathies (MFMs) are genetically heterogeneous dystrophies characterized by disintegration of

Z-disks and myofibrils. The characteristic degradation of myofibrils is followed by ectopic accumulation of multiple proteins.

MFMs have been associated with mutations in genes encoding Z-disk or Z-disk-related proteins. Recently, BAG3 mutation has been described as causative of MFM. At now, the genetic basis of MFM with BAG 3 mutation is not fully traced. In this work we studied by exome sequencing a MFM female patient carrying the c.626 C>T (P209L) mutation in BAG3 gene. We found that this BAG3 variant is associated to mutations of N-RAP and FHL1 genes that encode muscle specific LIM domain containing proteins resulting in a decreased expression of NRAP and FLH1 accumulation in aggregates in affected skeletal muscle tissue. Molecular dynamic analysis of mutated FHL1 domain suggests a modification of its surface charge, which could explain its accumulation in muscle fibers.

To our knowledge this is the first study reporting the simultaneous presence of genetic variants in three genes possibly causative of MFM: BAG3 and FHL1, already independently associated to MFMs, and NRAP linked for the first time to MFM.

DMPK and SIX-5 gene expression in lens of patients with Myotonic Dystrophy type 1

E. Picillo, M. Lanza¹, M. Scutifero, M. De Bernardo², N. Rosa², L. Politano
Cardiomyology and Medical Genetics, Dept. of Experimental Medicine, Second University of Naples; ¹ Eye Department, Second University of Naples, Naples, Italy; ² Department of Medicine and Surgery, University of Salerno, Salerno, Italy

Myotonic dystrophy (DM1) is the most common muscle disease in adults, affecting 1:8000 individuals. It is caused by an expanded (CTG)_n repeat on chromosome 19q35, that lies in the 3' UTR of DMPK gene and in the promoter region of immediately downstream SIX-5 gene.

DM1 is a multisystemic disorder affecting muscle, heart, respiratory and endocrine apparatus and eye. Cataract is often the first sign of the disease in asymptomatic patients. In order to explore whether CTG expansion could be the cause of senile cataract in humans, we analyzed CTG expansion in lenses of DM1 patients and normal individuals undergoing senile cataract surgery (SCS group), and studied the expression of DMPK and SIX-5 genes in both populations. The study was carried out on 34 lens specimens [9 from DM1, mean age 45,8 ± 6,18

(range 39-53 years) and 25 from SCS group, mean age 74.3 ± 9.43 (range 57-85 years)].

An expanded CTG repeat was found in the lens of DM1 group, but not in SCS group. A slight reduction of DMPK expression (2.77 ± 0.30 vs 2.94 ± 0.05 ; $p = 0.007$) and an increased expression of SIX-5 (3.62 ± 0.13 vs 3.12 ± 0.07 ; $p < 0.002$), was observed in the lens of DM1 group compared with SCS group, confirming data reported by other Authors.

However when we compared these results with those observed in clear lenses - surgically excised because a lens trauma - from 2 controls, aged 41 and 39 years respectively, we observed a higher expression of DMPK and a virtual absence of SIX-5 expression in the clear lenses compared with SCS and DM1 groups. These data - which require further confirmation - suggest that the pathogenesis of human cataract should be related to a reduced expression of DMPK and an overexpression of SIX-5 genes in the lens.

Fasciitis frequently accompanies myopathy in acute critical illness muscle wasting: evidence from qualitative ultrasound and muscle biopsy analysis

Z.A. Puthuchery¹, R. Phadke², J. Rawal¹, M.J.W. McPhail³, P.S. Sidhu⁴, A. Rowleron⁵, J. Moxham⁵, S. Harridge⁵, N. Hart⁵, H.E. Montgomery¹

¹ Institute of Health and Human Performance, UCL, London, UK, ² UCL Institute of Neurology, National Hospital, UCLH, London, UK, ³ St. Mary's Hospital, Imperial College, London, UK, ⁴ King's College Hospital, London, UK, ⁵ King's College London, London, UK

A rapid and early loss of skeletal muscle mass underlies the physical disability that is common amongst survivors of critical illness (CI) with possible adverse implications for muscle function. Our main objectives were to characterise changes in muscle echogenicity, pennation angle and fascial characteristics that occur early in CI, and relate these to histologically defined myonecrosis and fascial pathology.

Subjects comprised a subgroup of patients recruited to the Musculoskeletal Ultrasound in CI: Longitudinal Evaluation (MUSCLE) study. Comparisons were made between sequential Vastus Lateralis (VL) biopsies and ultrasound assessment of Rectus Femoris (RF) echogenicity. Change in RF pennation angle was measured.

In 30 patients, change in muscle echogenicity was greater in patients who developed myonecrosis than in those who did not (8.2%, (95%CI -5.3- 21.7), versus -15.0%, (95%CI -28.9- -1.09), $p = 0.016$). The AUROC for prediction of myonecrosis was 0.74 (95%CI 0.565-0.919, $p = 0.024$) increasing to 0.85 (95%CI 0.703-0.995,

$p = 0.003$) after excluding those with potential iatrogenic muscle damage. Fasciitis was observed in 18 out of 30 biopsies (60%), was dominated by macrophages by day 7/10 and paralleled myonecrosis in severity.

Mean pennation angle decreased from $7.6^\circ \pm 4.0$ to $5.5^\circ \pm 2.1$ ($p = 0.01$) over the first 10 days of CI. Myonecrosis and fascial inflammation can be detected non-invasively using ultrasound in CI. Fasciitis precedes and frequently accompanies myonecrosis and is dominated by macrophages in the sub-acute phase. Rapid decreases in pennation angle are seen. These findings may have functional implications for survivors of critical illness.

A new missense mutation in LMNA gene: case report description.

G. Ricci¹, E. Schirizzi¹, L. Chico¹, N. Botto², A. Barison³, G. Siciliano¹

¹ Department of Clinical and Experimental Medicine, Neurological Unit, University of Pisa, Pisa, Italy; ² CNR, Institute of Clinical Physiology, G. Pasquinucci Hospital, Massa, Italy; ³ Fondazione Toscana Gabriele Monasterio, Pisa, Italy

Mutations in the LMNA gene are responsible for more than ten different disorders called laminopathies which affect various tissues in an isolated (striated muscle, adipose tissue or peripheral nerve) or systemic (premature aging syndromes) condition. Overlapping phenotypes are also observed. There is a large genetic heterogeneity associated with the wide clinical variability. We describe the case of a 43-year-first time at the age of 42 for a two-year story of diffuse body pain, especially localized to the extremities, myalgias and asthenia, in association with skin rash of trunk and limb (diagnosed as "flagellatum erythema"). In the same period, he was subjected to pace maker implantation for the diagnosis of sinus node disease; in his pathologic anamnesis he also reported a previous episode of ectopic atrial tachycardia treated by radiofrequency ablation.

There was no family history suggestive of neuromuscular or cardiologic diseases. The neurological examination showed a severe motor impairment due to the complained diffuse pain. The creatine kinase level was slightly increased (350 U/L), as well as the serum lactate level after forearm ischemic muscle testing. Needle electromyography revealed myopathic signs in the four limbs; conductivity of peripheral nerves was normal. The enzyme assay to measure the level of alpha-LMNA gene, required for the concomitant cardiologic involvement, revealed the presence of a new missense mutation c.290A>C in exon 1 (p.LYS97THR).

Russian Neuromuscular Children's Centre experience of Emery-Dreifuss muscular dystrophy

D.V. Vlodavets¹, D.O. Kazakov¹, E.D. Belousova¹, P.G. Carlier²

¹ Pirogov Russian National Research Medical University, Moscow, Russian Federation; ² Institute of Myology, Pitié-Salpêtrière University Hospital, Paris, France.

Emery-Dreifuss muscular dystrophy (EDMD) is a degenerative myopathy characterized by weakness and atrophy of muscle without involvement of the nervous system. Most of the patients have very recognizable phenotype: myopathy, flexion deformities of the ankles and elbows dating from early childhood, mild pectus excavatum, signs of cardiac involvement, mostly arrhythmia, involvement of the forearm muscles and absence of muscle pseudohypertrophy. The disease caused by several genes EMD, LMNA, SYNE1, SYNE2, FHL1, TMEM43, and it could be inherited as autosomal recessive, or autosomal dominant, or X-linked forms. In our clinic we observe 25 patients with EDMD. Most of them have mutation in Lamin A/C gene – 11 patients; 2 patients have mutations in Emerin, 1 patient has mutation in FHL1 gene and 1 patient has mutation in SYNE1. So in our population 10 patients have no genetic confirmation but clear EDMD phenotype. CK level in normal or slightly elevated in some patients up to 500-600 U/l. Muscle MRI is very useful and revealed very specific pattern of fat infiltration on posterior calf – involvement of medial head of m. Gastrocnemius, with lateral head spared; m. Soleus spared in most patients; on thigh: involvement of mm. Vastus intermedius and lateralis. Most patients showed involvement of the extensor muscles of the spine and m. Sternocleidomastoideus. It is very interesting that most of the patients developed cardiomyopathy and arrhythmia only since the age of 8-10 y.o. and need the care of experienced cardiologist. Before this age no signs of cardiologic involvement were found.

Open access cryobank of primary cell cultures from Duchenne muscular dystrophy patients

D. Vlodavets¹, D. Reshetov²

¹ Pirogov Russian National Research Medical University, Moscow, Russian Federation; ² Marlin Biotech LLC, Moscow, Russian Federation

Duchenne muscular dystrophy (DMD) and the milder Becker muscular dystrophy (BMD) both are the result of mutations in the gene that encodes dystrophin protein. DMD is the most common neuromuscular disorder in the world with the incidence 1 in 5000 newborn boys.

About 50% of DMD boys could be treated by exon-skipping technologies aimed to restore reading frame of the gene affected by mutations. Skipping of the exon 51 could be relevant for 13% of DMD boys, exon 45 and 53 for 8% each, 44 - 6%, 52 and 50 - 4% each, 55 and 8 - about 2% each.

There is a clear need for cell cultures from different DMD patients to develop and test new methodologies for exons skipping. In our clinic we observe boys with DMD and BMD. We started a Natural History clinical trial with gathering DNA from blood and fibroblasts from the skin to develop a cryobank of primary cell cultures from Duchenne muscular dystrophy patients. To the date we have collected fibroblasts from 41 DMD patients covering a large spectrum of DMD gene mutations.

Fibroblast primary cell cultures are available upon request. Please contact us via email: info@marlinbiotech.com

Case report of vaccine-associated paralytic poliomyelitis in a patient from Russia.

D.V. Vlodavets¹, A.K. Shakaryan^{1,2}, D.O. Kazakov¹, E.D. Belousova¹, P.G. Carlier³, I.V. Shulyakova¹

¹ Pirogov Russian National Research Medical University, Moscow, Russian Federation; ² Chumakov Institute of poliomyelitis and viral encephalitis, RAMS, Moscow, Russian Federation; ³ Institute of Myology, Pitié-Salpêtrière University Hospital, Paris, France

Poliomyelitis, often called polio or infantile paralysis, is an infectious disease caused by the poliovirus. Nearly 5-10 % of poliovirus infection cases can be clinically recognized as unspecific infection disease (fever, vomiting, diarrhea, headache etc.) with full recover in 1-2 weeks. In 0,5% of these cases patients can develop neuroinfection with inflammatory damage of motoneurons of spinal cord's anterior horn or pons cerebri. Clinically it manifests as acute flaccid paralyses of limbs with residual weakness, hypotrophy, and skeletal deformity. There is no specific treatment, but polio is vaccine-preventable disease. According to Russian National Immunization Plan, polio-vaccine is to be administered at 3-4,5-6 months of age, with revaccination at 18 and 20 months of age, and at the age of 14 years. We present a clinical case of neuromuscular disorder in a patient with vaccine-associated poliomyelitis (VAPP). According to medical documentation, he received first dose of oral polio vaccine (OPV) at the age of 4 months. In 3 days, the boy showed symptoms of infection (fever, diarrhea, vomiting, weakness), and in few days he developed acute flaccid paralysis of the right leg. Subsequently asymmetry progressed and hypotrophy and shortening of the right leg manifested. He began to sit at age of 9 months and could walk independently only at 18 months. Since the age of 7 years the patient has devel-

oped an equinus feet. When we first examined the patient, he was 10 and presented with marked motor disturbances: gait disturbances, stairs ascent problems, proximal hypotrophy and hypotonia of the right leg, equinus feet. DTR was absent from the right knee-jerk, it was normal on the left side; ankle DTR were well presented. CK level was 547 U/l. EMG showed signs of motor neuron damages with MUAP(s) more than 5600 mV. Muscle MRI showed marked asymmetric damages of thigh and lower leg muscles: the most affected was the right quadriceps as opposed to the posterior compartment of the left thigh that was almost intact; in the lower leg, Tibialis anterior and posterior muscles were the most involved on the left side and the Gastrocnemius on the right side. Such degree of asymmetry in skeletal muscle degenerative changes are encountered in 4 conditions: anoctamin5-related dystrophy, fascio-scapulo-humeral dystrophy, inclusion-body myositis and poliomyelitis. We described here a rare case of in VAPP, a condition that has to be included in the differential diagnosis of very asymmetric degenerative changes in neuro-muscular patients.

Efficacy and safety of a novel oral anticoagulant drug in prevention of stroke in patients with Myotonic Dystrophy type 1

V. Russo¹, A. Rago¹, A.A. Papa¹, F. di Meo¹, A. Palladino², P. D'Ambrosio², L. Politano², G. Nigro¹

¹ Department of Cardiology, Second University of Naples;

² Cardiomyology and Medical Genetics, Department of Experimental Medicine, Second University of Naples

Myotonic dystrophy type 1 (DM1) is a clinically and genetically heterogeneous disorder. Cardiac involvement, that often precedes the skeletal muscle one, occurs in 80% of DM1 patients and represents the second most common cause of death, together with respiratory causes. Paroxys-

mal supraventricular tachyarrhythmias (atrial fibrillation, atrial flutter, atrial tachycardia) are a common finding on 12 lead ECG or 24 hour Holter monitoring, occurring in up to 30 % in DM1 patients. Atrial tachycardias are observed in up to 7.3% of patients, both as un-sustained and sustained forms. Atrial fibrillation and flutter may be the first clinical manifestation of muscular dystrophy in young patients and seem to increase mortality in this population. Paroxysmal atrial tachy-arrhythmias are also easily inducible at EPS, even in the absence of previously documented spontaneous episodes. However clinical implications of these findings remain uncertain. Several studies have associated electrocardiographic baseline abnormalities with an increased risk of sudden death, often leading to pacemaker (PMK) or cardioverter defibrillator (ICD) in 4.1% to 11% and 1.1% to 5.3%, respectively. Cognitive impairment, mental retardation and attention disorders may be part of DM1 disease and may be associated with less effective VKA oral anticoagulation, that requires more frequent coagulation monitoring and dose adjustments, to ensure an adequate level of anticoagulation. Recently, the US Food and Drug Administration (FDA) approved 3 oral anticoagulants - dabigatran, rivaroxaban, and apixaban - in less than 4 years. Dabigatran, a direct thrombin inhibitor, only inhibits factor IIa, while rivaroxaban and apixaban directly inhibit factor Xa and indirectly factor IIa. Notable advantages exist in the use of these new agents, although some disadvantages should be considered, as well. However, an appropriate patient selection, guided by a thorough understanding of benefits and risks of NOACs, plays a key role. Compared with warfarin, dabigatran is the only oral anticoagulant showing a lower rate of both hemorrhagic and ischemic stroke. In this report we evaluated the efficacy and safety of dabigatran for stroke prevention in DM1 patients presenting paroxysmal atrial fibrillation.

AUTHOR INDEX

- Amodeo A., 24
 Andrés V., 25
 Andria G., 27
 Angelini C., 21
 Askanas V., 20
 Auletta L., 20
 Baiardi V., 31
 Barcella M., 31
 Barison A., 32
 Barlassina C., 31
 Bartoli M., 21
 Becane H.-M., 23
 Béhin A., 23
 Belousova E.D., 33
 Bertini E., 21
 Bettica P., 29
 Bonne G., 25
 Botto N., 32
 Braga D., 31
 Broccolini A., 19
 Bruno C., 21
 Carboni N., 26
 Carlier P.G., 33
 Chico L., 32
 Claes K., 21, 22
 Comi G.P., 21, 22
 Consalvi S.A., 29
 Costagliola A., 20
 Cusi D., 31
 D'Agostino C., 20
 Dalakas M., 19
 D'Ambrosio P., 26, 34
 D'Amico A., 21
 D'Avila F.C., 31
 De Bernardo M., 31
 De Biase D., 20
 De Bleecker J., 20, 22
 De Concilio O., 21
 De Fino C., 19
 Del Gaudio S., 26
 Del Vecchio Blanco F., 21
 De Paepe B., 19, 20
 De Santis F., 31
 Di Fruscio G., 22
 di Meo F., 34
 Dionisi M., 21
 Duboc D., 23
 Dubowitz V., 29
 Durand Viel G., 23
 D'Ursi P., 31
 Engel W.K., 20
 Erratico S., 31
 Evilä A., 22
 Eymard B., 23
 Fahmy N.A., 22
 Fanin M., 21
 Farini A., 31
 Fiorillo C., 21
 Florian A., 23
 Garofalo A., 21
 Gasperini S., 27
 Di Fruscio G., 21, 22
 Giugliano T., 21
 Giuliano G., 31
 Gorni K., 31
 Gorokhova S., 21
 Grandi C., 31
 Greco M., 20
 Hackman P., 22
 Harridge S., 32
 Hart N., 32
 Hausmanowa-Petrusewicz I., 26
 Hedberg Oldfors C., 24
 Herbelet S., 20
 Iglesias E., 20
 Janssens S., 21, 22
 Jimenez-Mallebrera C., 20
 Jou C., 20
 Karason K., 24
 Kazakov D.O., 33
 Krahn M., 21
 Laforêt P., 23
 Lanza M., 31
 Lattanzi G., 26
 Lim J.-A., 29
 Lucchini M., 19
 Lupoli S., 31
 Madej-Pilarczyk A., 26
 Magri F., 22
 Manara R., 27
 Martin J.-J., 20
 Máthé G., 24
 McPhail M.J.W., 32
 Melki J., 18
 Meola G., 28
 Mercuri E., 19, 22
 Meregalli M., 31
 Messina S., 28
 Milanesi L., 31
 Minetti C., 21

- Mirabella M., 19
 Mongini T., 21
 Montgomery H.E., 32
 Mora M., 21, 22
 Morandi L., 21, 22
 Morettini V., 31
 Morosetti R., 19
 Moxham J., 32
 Musumeci O., 21
 Mutarelli M., 21
 Nigro G., 23, 26, 34
 Nigro V., 21, 22, 26
 Oldfors A., 25
 Oldfors A., 24
 Orro A., 31
 Östman-Smithe I., 24
 Paciello O., 20
 Pagano T.B., 20
 Palladino A., 26, 34
 Papa A.A., 34
 Papparella S., 20
 Parini R., 27
 Pasolini M.P., 20
 Petillo R., 26
 Phadke R., 32
 Picillo E., 26, 31
 Piluso G., 21, 26
 Politano L., 21, 22, 26, 31, 34
 Puri P.L., 29
 Puthucheary Z.A., 32
 Raben N., 29
 Rago A., 34
 Rawal J., 32
 Reshetov D., 33
 Ricca I., 22
 Ricci G., 21 32
 Rigoldi M., 27
 Rosa N., 31
 Rowlerson A., 32
 Rubegni A., 22
 Ruggiero L., 21, 22
 Russo V., 34
 Saccone V., 29
 Sacconi S., 21, 22
 Sandona M., 29
 Sansone V., 31
 Santorelli F.M., 21, 22
 Santoro L., 22
 Santoro P., 20
 Savarese M., 21, 22
 Schirinzi E., 32
 Scutifero M., 31
 Shakaryan A.K., 33
 Shulyakova I.V., 33
 Siciliano G., 21, 32
 Sidhu P.S., 32
 Sitzia C., 31
 Stovskovic T., 23
 Taglia A., 26
 Tasca G., 21, 22
 Thomson K., 24
 Tizzano E., 18
 Torella A., 21, 22, 26
 Torrente Y., 31
 Trovato R., 22
 Tucciarone L., 29
 Tulinius M., 24
 Tupler R.G., 21
 Udd B., 22
 Vanakker O., 22
 Viggiano E., 26
 Vihola A., 22
 Vlodayets D., 33
 Vlodayets D.V., 33
 Wahbi K., 23
 Weis J., 20

**PROCEEDINGS OF THE XV CONGRESS
OF THE ITALIAN SOCIETY OF MYOLOGY**

Naples, Italy

May 20-23, 2015



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. Politano¹, M. Scutifero¹, A. Zaccaro¹, U. Balottin², A.L. Berardinelli², M. Camia², M.C. Motta², G. Vita³, S. Messina³, M. Sframeli³, G. Vita³, M. Pane⁵, M.E. Lombardo⁴, R. Scalise⁴, A. D'Amico⁵, M. Catteruccia⁵, G. Colia⁵, C. Angelini⁶, A. Gaiani⁶, C. Semplicini, R. Battini⁷, G. Astrea⁷, M.G. D'Angelo⁸, E. Brighina⁸, F. Civati⁸, M. Patalano⁹, A. Sagliocchi⁹, L. Magliano⁹

¹ Cardiology and Medical Genetics, Experimental Medicine Dept., Second University of Naples (SUN), Italy;

² Child Neuropsychiatry Dept., IRCCS "Mondino" Pavia, Italy; ³ Neurosciences, Psychiatry and Anaesthesiology Dept., Messina University, Italy; ⁴ Paediatric Neurology Dept., Catholic University, Rome, Italy; ⁵ Neuromuscular and Neurodegenerative Disorders Unit, "Bambino Gesù" Children Hospital, Rome, Italy; ⁶ Neurosciences Dept., Padova University, Italy; ⁷ Neurology Dept., Pisa University, Italy; ⁸ NeuroMuscular Unit-NeuroRehabilitation Dept., IRCCS "E. Medea", Bosisio Parini, Lecco, Italy; ⁹ Psychology Dept., SUN, Naples, Italy

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 responsibility 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 from 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 etiology 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

L. Riccio, G. Riccardi, G. Iolascon, F. Gimigliano,

L. Politano, R. Gimigliano, V. Riccio

Dip. Multidisciplinare di Specialità Medico-Chirurgiche 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 PaCO₂ and CO₂ 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 CO₂ 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 conditions 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. Bernasconi¹, C. Evangelisti², P. Cavalcante¹, C. Cappelletti¹, N. Carboni³, L. Politano⁴, G. Boriani⁵, G. Ricci⁶, L. Vercelli⁷, L. Ruggiero⁸, A. Gambineri⁹, A. D'Amico¹⁰, C. Rodolico¹¹, L. Maggi¹, L. Morandi¹, R. Mantegazza¹, G. Lattanzi²

¹ Unità Neurologia IV, Fondazione Istituto Neurologico "Carlo Besta", Milan, Italy; ² Istituto di Genetica Molecolare, Istituto Ortopedico Rizzoli e CNR, Bologna, Italy; ³ Divisione Neurologia, Ospedale "San Francesco", Nuoro, Italy;

⁴ Cardiomiologia e Genetica Medica, Seconda Università di Napoli, Italy; ⁵ Divisione di Cardiologia, Policlinico "S. Orsola-Malpighi", Bologna, Italy; ⁶ Dipartimento di Neuroscienze, Università di Pisa, Italy; ⁷ Dipartimento di Neuroscienze "Rita Levi Montalcini", Università di Torino, Italy; ⁸ Dipartimento di Medicina Sperimentale, Seconda Università di Napoli, Italy; ⁹ Unità Operativa di Endocrinologia, Policlinico "S. Orsola-Malpighi", Bologna, Italy; ¹⁰ Neurologia Pediatrica, Ospedale "Bambino Gesù", Roma, Italy; ¹¹ UOC Neurologia e Malattie Neuromuscolari, Policlinico "G. Martino", Messina, Italy. On behalf of the Italian Network for Laminopathies

Emerging evidences suggest that nuclear lamina defects dysregulate the NF- κ B 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- β 2 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- β 2 pathway. To identify the signalling pathways triggered by TGF- β 2 in laminopathic cells, we used an experimental model expressing various LMNA mutants: wild-type lamin A was able to modulate TGF- β 2 expression, whereas pathogenetic LMNA mutants failed to negatively regulate TGF- β 2 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- β 2. 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. Angelini¹, M. Fanin², G. Cenacchi³, E. Pinzan¹, V. Pegoraro¹, E. Tasca¹, V. Nigro⁴

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 hypotrophy 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 atrophy 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 hypothesize 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 import-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

A. Taglia, M. Savarese¹, P. D'Ambrosio, R. Petillo, C. Orsini, M. Ergoli, E. Picillo, V. Nigro¹, L. Politano
Cardiology and Medical Genetics, Dept. of Experimental Medicine and ¹ Laboratory of Medical Genetics, Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Italy

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

G. Parenti

Department of Translational Medical Sciences, "Federico II" University, Naples, and Telethon Institute of Genetics and Medicine, Pozzuoli, Italy

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

M. Moggio, on behalf of the Italian group of GSDII Neuromuscular Unit, Fondazione IRCCS “Ca’ Granda”, Ospedale Maggiore Policlinico, Milano, “Dino Ferrari” Centre, University of Milan, Italy; Neurological Units, Universities of Verona, Brescia, San Raffaele Milan, Padova, Torino and Istituto Besta, Milano

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)

A. Toscano, F. Montagnese, O. Musumeci
Department of Neurosciences, University of Messina, Italy

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 α -glucosidase, 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

E. Bertini

Unit of Neuromuscular and Neurodegenerative Disorders,
Laboratory of Molecular Medicine, Rome, Italy

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

G.P. Comi

Centro Dino Ferrari, Fondazione Ca' Granda, Ospedale Maggiore Policlinico, Università di Milano, Italy

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 phosphordiamidate 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 drisa-

persen 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

C. Rodolico

Department of Neurosciences, University of Messina, Italy

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 choline acetyltransferase (CHAT) has been associated with presynaptic CMS, while synaptic basal-laminin-associated defects are due to mutations in the collagen tail subunit of the acetylcholinesterase (COLQ) or in laminin $\beta 2$ 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 post-synaptic 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

A. Evoli, P.E. Alboini, V. Damato, R. Iorio¹
Institute of Neurology, Catholic University, Roma, Italy; ¹ Don Carlo Gnocchi, ONLUS Foundation, Milan, Italy

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

R. Mantegazza, F. Baggi
Neurological Institute "Carlo Besta", Milan, Italy

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 MyopathiesM. Aguenouz, O. Musumeci, M. Beccaria, A. Toscano
Department of Neurosciences, University of Messina, Italy

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 CIC-1 chloride channel mutationsC. Altamura, G.M. Camerino, P. Imbrici, S. Portaro¹, O. Musumeci¹, A. Toscano¹, J.-F. Desaphy, D. Conte Camerino
Department of Pharmacy, Drug Sciences, University of Bari "Aldo Moro", Italy; ¹ Department of Neurosciences, University of Messina, Italy

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 CIC-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 hCIC-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 CIC-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 $\beta 1$ 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 screeningS. Assereto, S. Baratto, M. Massacesi, L. Galletta, F. Zara, C. Bruno, C. Minetti, E. Gazzerro
G. Gaslini Institute, Genova, Italy

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 $\approx 4,500$ 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 networksS. Baldanzi¹, P. Cecchi², C. Simoncini¹, G. Ricci¹, L. Volpi¹, S. Fabbri², G. Migaletto², R. Lorio⁴, F. Bevilacqua⁴, A. Petrucci⁵, C. Angelini⁴, M. Cosottini³, G. Siciliano¹¹ Department of Clinical and Experimental Medicine,² Neuroradiology Unit, AOUP, ³ Neuroradiology Unit, Department

of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Italy; ⁴IRCCS San Camillo, Lido Venice, Italy; ⁵“San Camillo Forlanini” Hospital, Rome, Italy.

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

L. Bello¹, P. Campadello¹, A. Barp¹, C. Semplicini¹, G. Sorar¹, C. Angelini^{1,2}, E. Pegoraro¹

¹ Dipartimento di Neuroscienze, Università di Padova, Italy; ² IRCCS Ospedale “S. Camillo”, Venezia, Italy

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

E. Bertini¹, A. Sferra¹, G. Tasca¹, T. Rizza¹, A. D'Amico¹, G. Zanni¹, S. Baresi¹, D. Diodato¹, E. Piermarini¹, D. Martinelli², C. Dionisi-Vici², M. Niceta³, M. Tartaglia⁴, B. Dallapiccola³, C. Compagnucci¹

¹ Unit of Neuromuscular Disorders, Laboratory of Molecular Medicine, “Bambino Gesù” Children's Research Hospital, Rome, Italy; ² Unit of Metabolism, “Bambino Gesù” Children's Research Hospital, Rome, Italy; ³ Scientific Direction, “Bambino Gesù” Children's Research Hospital, Rome, Italy; ⁴ ISS, Rome, Italy

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 dysmorphic 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 motoneurons 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)

C. Capanni^{1,2}, M. Loi^{1,2}, V. Cenni^{1,2}, S. Duchi^{3,4}, S. Squarzone^{1,2}, C. Lopez-Otin⁵, R. Foisner⁶, G. Lattanzi^{1,2}

¹ CNR-National Research Council of Italy, Institute of Molecular Genetics, Unit of Bologna, Italy; ² Laboratory of Musculoskeletal Cell Biology, IOR, Bologna, Italy; ³ Osteoarticular Regeneration Laboratory, "Rizzoli" Orthopaedic Institute, Bologna, Italy; ⁴ Department of Biomedical and Neuromotor Sciences (DIBINEM), Alma Mater Studiorum University of Bologna, Italy; ⁵ Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Oviedo, Oviedo, Spain; ⁶ "Max F. Perutz Laboratories, Medical University of Vienna, Austria

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

G. Civiletto¹, T. Varanità², R. Cerutti¹, T. Gorletta³, S. Barbaro³, S. Marchet³, C. Lamperti³, C. Viscomi¹, L. Scorrano², M. Zeviani¹

¹ Medical Research Council, Mitochondrial Biology Unit, Cambridge, UK; ² Department of Biology, University of Padova, Italy; ³ Foundation "Carlo Besta" Institute of Neurology, IRCCS, Unit of Molecular Neurogenetics, Milano, Italy

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

I. Colombo¹, S. Pagliarini², S. Testolin¹, C.M. Cinnante³, G. Fagioli¹, P. Ciscato¹, A. Bordoni², F. Fortunato², F. Magri², S.C. Previtali⁴, D. Velardo⁴, M. Sciacco¹, G.P. Comi², M. Moggi¹

¹ Neuromuscular and Rare Disease Unit, Department of Neuroscience, Foundation IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, University of Milan, Italy; ² "Dino Ferrari" Centre, Department of Pathophysiology and Transplantation Neuroscience Section (DEPT), Neurology Unit, Foundation IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, University of Milan, Italy; ³ Unit of Neuroradiology, Department of Neuroscience, Foundation IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, University of Milan, Italy; ⁴ Institute of Experimental Neurology (INSPE), Division of Neuroscience and Department of Neurology, IRCCS "San Raffaele Scientific Institute", Milan, Italy

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 (Glycogenin-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

A. D'Amico¹, J. Martone², S. Previtali³, G. Baranello⁴, G. D'Angelo⁵, A. Berardinelli⁶, S. Messina⁷, M. Pane⁸, L. Morandi⁴, E. Mercuri², M. Catteruccia¹, E. Bertini¹, I. Bozzoni².

¹ Unit of neuromuscular and neurodegenerative Disorders, "Bambino Gesù" Children's Hospital, Rome, Italy; ² Department of Biology and Biotechnology "Charles Darwin", Sapienza Università di Roma, Italy; ³ Institute of Experimental Neurology (INSPE), Division of Neuroscience, IRCCS "San Raffaele" Scientific Institute, Milan, Italy; ⁴ Developmental Neurology and Neuromuscular Disease and Immunology Units Fondazione IRCCS Istituto Neurologico "C. Besta", Milan, Italy; ⁵ IRCCS "Eugenio Medea", Bosisio Parini, Lecco, Italy; ⁶ Child Neurology and Psychiatry Unit, "Casimiro Mondino" Foundation, Pavia, Italy; ⁷ Centro "Nemo Sud", University of Messina; Department of paediatrics and neurology, Catholic university, Rome, Italy

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

G. Di Fruscio^{1,2}, M. Savarese^{1,2}, R. De Cegli², M. Mutarelli², V. Singhmarwah², D. Di Bernardo², S. Banfi^{1,2}, A. Ballabio², V. Nigro^{1,2}

¹ Dipartimento di Biochimica, Biofisica e Patologia Generale, Seconda Università di Napoli, Italy; ² Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA), Italy

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

M. Filosto¹, M. Aureli², F. Rinaldi¹, D. Schiumarini², M. Valsecchi², B. Castellotti³, A. Todeschini¹, S. Rota¹, E. Pari¹, M. Filocamo⁴, C. Gellera³, A. Padovani¹

¹ Clinical Neurology, Section for Neuromuscular Diseases and Neuropathies, University Hospital "Spedali Civili", Brescia, Italy; ² Department of Medical Biotechnology and Translational Medicine, University of Milan, Italy; ³ Unit of Genetics of Neurodegenerative and Metabolic Diseases, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy; ⁴ Center for Genetic and Biochemical Diagnostics of Metabolic Diseases, "Giannina Gaslini" Institute, Genoa, Italy

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

C. Fiorillo¹, M. Savarese², G. Astrea³, D. Cassandrini³, G. Brisca¹, L. Ruggiero⁴, M. Fanin⁵, L. Vercelli⁶, A. D'Amico⁷, M. Pane⁸, G. Tasca⁷, S. Gibertini⁹, R. Trovato³, M. Morandi⁹, M. Mora⁹, C. Angelini⁵, E. Pegoraro⁵, T. Mongini⁶, E. Bertini⁷, E. Mercuri⁸, L. Santoro⁴, F.M. Santorelli³, C. Minetti¹, V. Nigro², C. Bruno¹

¹ Department of Pediatric Neurology and Muscular Disorders, IRCCS Istituto "Giannina Gaslini", Genoa, Italy; ² Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Naples, Italy; ³ Neuromuscular and Molecular Medicine Unit, IRCCS "Stella Maris" Foundation, Pisa, Italy; ⁴ Department of Neurological Science, University "Federico II", Naples, Italy; ⁵ Department of Neurosciences, University of Padova, Italy; ⁶ Malattie Neuromuscolari Dipartimento di Neuroscienze Ospedale "Molinette", Torino, Italy; ⁷ UOC Malattie Neuromuscolari e Neurodegenerative, Ospedale Pediatrico "Bambino Gesù", Roma, Italy; ⁸ Neuropsichiatria infantile Policlinico Gemelli, Università Cattolica del "Sacro Cuore", Roma, Italy; ⁹ Department of Diagnostic and Experimental Research, Istituto Nazionale Neurologico "C. Besta", Milan, Italy

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

S. Gibertini, S. Zanotti, M. Curcio, F. Salerno, F. Cornelio, R. Mantegazza, M. Mora

Neuromuscular Diseases and Neuroimmunology Unit, Foundation IRCCS Neurological Institute "C. Besta", Milano, Italy

Excessive extracellular matrix deposition progressively replacing muscle fibres is the endpoint of most severe mus-

cle 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-I gene

F. Invernizzi¹, L. Morandi², M. Mora², F. Salerno², F. Blaservitch², R. Mantegazza², S. Marchetti¹, B. Garavaglia¹, C. Lamperti¹

¹ UO Neurogenetica Molecolare, ² UO Neurologia IV, Fondazione IRCCS Istituto Neurologico "C. Besta", Milano, Italy

The 3 main enzymes involved in biosynthesis of glycogen are glycogenin-I, glycogen synthase and branching enzyme. Glycogen synthase and branching enzyme allow further elongation and branching of the glucose polymer primer while Glycogenin-I is a glycosyltransferase 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 cramps and muscle pain and PAS-positive materials at the muscle biopsy.

We identified mutations in Glycogenin I 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 I 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 Ophthalmoplegia

C. Lamperti¹, A. Reyes², L. Melchionda¹, F. Carrara¹, M. Moggio³, G. Fagioli³, L. Morandi⁴, G. Comi⁵, S. Bonato⁵, N. Bresolin⁵, M. Zeviani^{1,2}, D. Ghezzi¹

¹ *UO Neurogenetica Molecolare, Fondazione IRCCS Istituto Neurologico "C. Besta", Milano, Italy;* ² *Mitochondrial Biology Unit, Medical Research Council, Hills Road, Cambridge, CB2 0XY, UK;* ³ *UOD Malattie Muscolari e Rare, Fondazione "Ca' Granda" IRCCS Policlinico Milano, Italy;* ⁴ *UO Neurologia IV, Fondazione IRCCS Istituto Neurologico "C. Besta", Milano, Italy;* ⁵ *UO Neurologia, Fondazione "Ca' Granda" IRCCS Policlinico, Milano, Italy*

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

A. Martinuzzi, M. Vavla, C. de Conti, E. Russo, S. Modolo, E. Trevisi
IRCCS "E. Medea", Conegliano Research Centre, Italy

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

S. Messina^{1,2}, M. Russo², C. Profazio², S. La Foresta², S. Mondello¹, G.L. Vita², M. Sframeli², M.G. Di Stefano¹, M. La Rosa¹, C. Barcellona¹, C. Faraone², C. Rodolico¹, A. Toscano¹, P. Ruggeri³, G. Vita^{1,2}

¹ *Department of Neurosciences,* ² *Nemo Sud Clinical Center,*

³ *Department of Clinical and Experimental Medicine, University of Messina, Italy*

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.

Neuropsychological 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)

O. Musumeci, M. Marino, F. Montagnese, D. Parisi, C. Rodolico, M. Cucinotta, A. Ciranni, A. Toscano
Department of Neuroscience, University of Messina, Italy

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 myopathic 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

P. Negrini¹, M. Cavalleri¹, M. Romei¹, E. Brighina², S. Gandossini², F. Magri³, A.C. Turconi², G. Reni¹, N. Bresolin^{2,3}, M.G. D'Angelo²

¹ IT Engineering Unit, IRCCS E. Medea- Bosisio Parini, Lecco, Italy; ² NeuroMuscular Unit, IRCCS E. Medea-Bosisio Parini, Lecco, Italy; ³ Fondazione Policlinico, Università di Milano, Italy

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

D. Orsucci, M. Mancuso, C. Angelini, E. Bertini, V. Carelli, G.P. Comi, M.A. Donati, A. Federico, C. Minetti, M. Moggio, T. Mongini, F.M. Santorelli, S. Servidei, P. Tonin, A. Toscano, C. Bruno, L. Bello, E. Caldarazzo Ienco, M. Catteruccia, M. Filosto, C. Lamperti, I. Moroni, O. Musumeci, E. Pegoraro, D. Ronchi, D. Sauchelli, M. Scarpelli, M. Sciacco, M.L. Valentino, L. Vercelli, M. Zeviani, G. Siciliano
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

E.M. Pennisi¹, M. Arca², E. Bertini³, C. Bruno⁴, D. Cassandrini⁵, A. D'Amico³, M. Garibaldi², F. Gragnani¹, R. Massa⁶, S. Missaglia⁷, L. Morandi⁸, O. Musumeci⁹, E. Pegoraro¹⁰, F.M. Santorelli⁵, D. Tavian⁷, A. Toscano⁹, C. Angelini¹⁰

¹ "San Filippo Neri" Hospital, Roma, Italy; ² Sapienza Università di Roma, Italy; ³ "Bambin Gesù" Hospital IRCCS, Roma, Italy; ⁴ "Gaslini" Hospital IRCCS, Genova, Italy; ⁵ "Stella Maris" IRCCS, Pisa, Italy; ⁶ Tor Vergata University, Roma, Italy; ⁷ CRIBENS, Università Cattolica del "Sacro Cuore", Milano, Italy; ⁸ "C. Besta" Institute, Milano, Italy; ⁹ Università di Messina, Italy; ¹⁰ Università di Padova, Italy

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 hepatic 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 compromise. Critical aspects for disability and life expectancy in Italian patients are arrhythmic cardiopathy and skeletal 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

I. Pezzini¹, M. Savarese², A. D'Amico³, E. Mercuri⁴, G. Astrea¹, J. Baldacci¹, C. Fiorillo¹, F. Moro¹, A. Rubegni¹, R. Battini¹, S. Messina⁵, C. Bruno⁶, P. Striano⁷, K. Gorni⁸, V. Sansone⁸, E. Pegoraro⁹, A. Berardinelli¹⁰, G. Comi¹¹, M. Moggio¹², M. Mora¹³, L. Morandi¹³, I. Moroni¹³, A. Pini¹⁴, E. Picillo¹⁵, L. Politano¹⁵, F. Ricci¹⁶, T. Mongini¹⁶, A. Malandrini¹⁷, A. Romano¹⁸, R. Tupler¹⁹, F. Muntoni²⁰, V. Nigro²¹, F.M. Santorelli¹

¹ IRCCS Stella Maris, Pisa, Italy; ² Medical Genetics Laboratory, Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Italy; ³ Unit of Neuromuscular and Neurodegenerative Disorders, "Bambino Gesù" Hospital, Rome, Italy; ⁴ Department of Pediatric Neurology, Catholic University, Rome, Italy; ⁵ Dep. of Neuroscience, Psychiatry and Anaesthesiology, University of Messina, Italy; ⁶ Center of Myology and Neurodegenerative Disease-IRCCS Gaslini, Genoa, Italy; ⁷ Pediatric Neurology, IRCCS "Gaslini", Genoa, Italy; ⁸ Centro Clinico NEMO, Milan, Italy; ⁹ Department of Neurosciences,

University of Padua, Italy; ¹⁰ Unit of Child Neurology and Psychiatry, IRCCS "C. Mondino", Pavia, Italy; ¹¹ Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Neurology Unit, IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, Milan, Italy; ¹² Neuromuscular Unit, Department of Neuroscience, IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, University of Milan, Italy; ¹³ IRCCS Neurological Institute Carlo Besta, Milan, Italy; ¹⁴ Child Neurology Unit, IRCCS Institute of Neurological Sciences, Bologna, Italy; ¹⁵ Department of Experimental Medicine, Cardiology and Medical Genetics, Second University of Naples, Italy; ¹⁶ Department of Neuroscience Rita Levi Montalcini, University of Torino, Italy; ¹⁷ Unit of Neurology and Neurometabolic Disorders, Department of Medicine, Surgery and Neurosciences, University of Siena, Italy; ¹⁸ Neuropathology Unit, Institute of Experimental Neurology and Division of Neuroscience, IRCCS "San Raffaele" Scientific Institute, Milan, Italy; ¹⁹ Miogen Lab, Department of Life Sciences, University of Modena and Reggio Emilia, Italy; ²⁰ Dubowitz Neuromuscular Centre, UCL Institute of Child Health & Great Ormond Street Hospital for Children, London, UK; ²¹ Telethon Institute of Genetics and Medicine, Naples, Italy

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 identified predictably 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

R. Piras¹, E. Malfatti^{2,3}, O. Akman⁴, M.A. Maioli¹, A. Mateddu¹, S. Di Mauro⁴, G. Marrosu¹

¹ Centro Sclerosi Multipla, University of Cagliari, ASL8, Cagliari; ² Department of Pathology, Institute of biomedicine, University of Gothenburg, Sweden; ³ Myology Institute, Neuromuscular Morphology Unit, Pierre and Marie Curie University, Pitié-Salpêtrière University Hospital Group, Sorbonne Universities, Paris, France; ⁴ Department of Neurology, Columbia University Medical Center, New York, NY

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

D. Velardo¹, T. Domi¹, E. Porrello¹, A. Capotondo², A. Biffi², R. Tonlorenzi¹, S. Amadio¹, S. Takeda³, M.A. Ruegg⁴, S.C. Previtali¹

¹ Institute of Experimental Neurology (INSPE) and Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milano, Italy; ² Tiget and Division of Regenerative Medicine, IRCCS San Raffaele Scientific Institute, Milano, Italy; ³ Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Ogawa-higashi, Kodaira, Tokyo, Japan; ⁴ Biozentrum, University of Basel, Switzerland

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, joint 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 Schwann 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, synthesized 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

G. Primiano¹, L. Manni², C. Cuccagna¹, D. Bernardo¹, C. Sancricca¹, S. Servidei¹

¹ Department of Geriatrics, Neuroscience and Orthopedics, Institute of Neurology, Università Cattolica del "Sacro Cuore", Roma, Italy;

² Institute of Translational Pharmacology, CNR, Roma, Italy

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

A. Ruggieri^{1,2}, S. Zanotti¹, R. Massa³, C. Terracciano³, L. Maggi¹, B. Pisanis¹, F. Brancati⁴, S. Saredi¹, L. Morandi¹, M. Mora¹, B. Minassian²

¹ Neuromuscular Disease and Immunology Unit, Fondazione IRCCS Istituto Neurologico "C. Besta", Milan, Italy; ² Program in Genetics and Genome Biology, The Hospital for Sick Children and University of Toronto, Canada; ³ Department of Systems Medicine (Neurology), Tor Vergata University, Rome, Italy; ⁴ Medical Genetics, Tor Vergata University, Rome, Italy

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

D. Sauchelli, M. Luigetti, G. Primiano, C. Cuccagna, D. Bernardo, C. Sancricca, S. Servidei
Department of Neurology, Catholic University, Rome, Italy

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

M. Savarese^{1,2}, G. Di Fruscio^{1,2}, O. Farina³, M. Fanin⁴, E. Picillo⁴, L. Passamano⁵, D. Ronchi⁶, C. Fiorillo⁷, G. Astrea⁸, S. Sampaolo³, G.P. Comi⁶, C. Angelini⁴, C. Bruno⁷, FM. Santorelli⁸, G. Di Iorio³, L. Politano⁵, A. Toscano⁹, V. Nigro^{1,2}

¹ Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Naples, Italy; ² Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Italy; ³ Neurology Clinic II, Department of Medical Sciences, Surgery, Neurology, Metabolic Diseases and Geriatrics, Second University of Naples, Italy; ⁴ Department of Neurosciences, University of Padova, Italy; ⁵ Cardiomyology and Medical Genetics, Department of Experimental Medicine, Second Naples University, Naples, Italy; ⁶ "Dino Ferrari" Center, IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, Neuroscience Section, Dipartimento di Fisiopatologia medico-chirurgica e dei trapianti, Università di Milano, Italy; ⁷ Center of Myology and Neurodegenerative Disorders, Istituto Giannina Gaslini, Genova, Italy; ⁸ Neuromuscular and Molecular Medicine Unit, IRCCS "Stella Maris" Foundation, Pisa, Italy; ⁹ Department of Neurosciences, University of Messina, Italy

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

C. Semplicini¹, C. Bertolin¹, S. Vianello¹, L. Bello¹, I. Colombo², G. Sorarù¹, M. Moggi², E. Pegoraro¹

¹ Department of Neurosciences, University of Padova, Italy;

² Department of Neurosciences, University of Milano, Italy

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 disequilibrium.

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

M. Sframeli^{1,2}, A. Sarkozy¹, G. Astrea³, M. Scotto¹, L. Feng¹, R. Mein⁴, M. Yau⁴, R. Phadke¹, C. Sewry¹, S. Robb¹, G. Vita⁵, S. Messina⁵, F. Muntoni¹

¹ Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London, UK; ² Department of Neurosciences and NEMO SUD Clinical Centre, Messina, Italy; ³ Neuromuscular and Molecular Medicine Unit, Department of Developmental Neuroscience, IRCCS "Stella Maris", Pisa, Italy; ⁴ DNA laboratory, GSTS Pathology, Genetics Centre, Guy's Hospital, London, U; ⁵ Department of Neurosciences, University of Messina, Italy

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- $\alpha 2$ related dystrophy (MDC1A) accounting for 40% of cases, followed by dystroglycanopathies (25%), Ullrich-CMD (22%) and CMD related to mutations in SEPNI 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

C. Terracciano^{1,2}, M. Nuccetelli¹, M. Gibellini^{1,2}, E. Rastelli², A. Petrucci³, S. Bernardini¹, R. Massa²

¹ Department of Experimental Medicine and Surgery, Division of Clinical Biochemistry, Tor Vergata University, Rome, Italy;

² Department of Systems Medicine, Division of Neurology, Tor Vergata University, Rome, Italy; ³ Center of Neuromuscular and Neurological Rare Diseases "S. Camillo-Forlanini" Hospital, Rome, Italy

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

E. Viggiano¹, E. Picillo¹, M. Ergoli¹, S. Del Gaudio²,
A. Cirillo³, L. Politano¹

¹ *Cardiomyology and Medical Genetics, Department of Experimental Medicine, Second University of Naples, Italy;*

² *Section of Biotechnology and Molecular Biology "A. Cascino", Department of Experimental Medicine, Second University of Naples, Italy;* ³ *Institute of Bioscience and Bioresources, National Research Council of Italy, Naples, Italy*

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

C. Dato¹, G. Capaldo¹, L. Lombardi¹, O. Farina¹, F. Cipullo¹, G. Costa², S. Mannone², G. Colella³, M.A.B. Melone¹, S. Sampaolo¹, G. Di Iorio¹

¹ Second Division of Neurology, ² Department of Audiology of Phoniatry, ³ Department of Medical, Surgical and Dental Specialities, Second University of Naples, Italy

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

D. Diodato¹, G. Tasca¹, D. Verrigni¹, A. D'Amico¹, T. Rizza¹, G. Tozzi¹, D. Martinelli², M. Verardo¹, F. Invernizzi³, A. Nasca³, E. Bellacchio⁴, D. Ghezzi³, F. Piemonte¹, C. Dionisi-Vici², R. Carrozzo¹, E. Bertini¹

¹ Neuromuscular and Neurodegenerative Diseases Unit, Children Research Hospital "Bambino Gesù", Rome, Italy; ² Division of Metabolism, Children Research Hospital "Bambino Gesù", Rome, Italy; ³ Molecular Neurogenetics Unit, Foundation IRCCS Neurological Institute "C. Besta", Milan, Italy.

AIFM1 is a gene located on the X chromosome, coding for AIF (Apoptosis Inducing Factor), a mitochondrial flavo-protein involved in caspase-independent cell death. AIFM1 mutations have been associated with different clinical phenotypes: a severe infantile encephalopathy with combined oxidative phosphorylation deficiency (COXPD6) with or without ventriculomegaly, and the Cowchock syndrome, an X-linked Charcot-Mary-Tooth disease (CMTX4) with axonal sensori-

motor 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

F. Lupidi, C. Gelardi, P. Cardinaletti, M.G. Danieli, P. Di Bella, L. Provinciali, F. Logullo

Neurological Clinic, Department of Experimental and Clinical Medicine, Marche Polytechnic University, Ancona, Italy

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 myopathic 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 considered 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

C. Panicucci¹, C. Fiorillo¹, F. Moro², F. Trucco¹,
M. Pedemonte¹, G. Brisca³, F.M. Santorelli², C. Minetti¹,
C. Bruno³

¹ UOC Neurologia Pediatrica e Malattie Muscolari, IRCCS Istituto "G. Gaslini", Genova, Italy; ² UOC Medicina Molecolare per Malattie Neurodegenerative, IRCCS Fondazione "Stella Maris", Pisa; ³ UOSD Centro di Miologia e Patologie Neurodegenerative, IRCCS Istituto "G. Gaslini", Genova, Italy

Mutations in guanosine diphosphate mannose (GDP-mannose) pyrophosphorylase B (GMPPB), a key enzyme of the glycosylation 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 glycosylated alpha dystroglycan was present. Extensive genetic analysis 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 glycosylation of alphadystroglycan at muscle biopsy.

MC5. Unusual pR471H Laminopathy

J.L. Rinnenthal¹, H.H. Goebel¹, A. Hucko², M. Schuelke²,
A. Schaenzer³, W. Stenzel¹

¹ Department of Neuropathology, Charite, Berlin, Germany;

² Department of Neuropediatrics, Charite, Berlin, Germany;

³ Department of Neuropathology, Justus Liebig University, Giessen, Germany

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

M.L. Valentino¹, M. Columbaro², P. Avoni¹, L. Pironi³,
R. Liguori¹

¹ UOC Clinica Neurologica, IRCCS Istituto delle Scienze Neurologiche di Bologna, Italy; ² Istituto Ortopedico Rizzoli, Bologna, Italy; ³ Dipartimento Malattie Apparato Digerente e Medicina Interna, Policlinico "S. Orsola-Malpighi", Bologna, Italy

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. Abetalipoproteinaemia, 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 assessmentG. Baranello¹, M.T. Arnoldi¹, C. Bussolino¹, C. Mastella², A. Aliverti³, A. Lo Mauro³¹ "Carlo Besta" Neurological Institute Foundation Milan, Italy;² Sapre Ospedale Policlinico Maggiore "Mangiagalli e Regina Elena" Foundation Milan, Italy; ³ Dipartimento di Elettronica, Informazione e Bioingegneria Politecnico di Milano Milan, Italy

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 ACO2E. Barca^{1,2}, H. Neil³, A. Naini², D. De Vivo⁴, S. Di Mauro²¹ Department of Neuroscience, University of Messina, Italy;² Houston Merritt Center, Columbia University Medical Center, New York, USA; ³ Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; ⁴ Pediatric Neurology, Columbia University Medical Center, New York, USA

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-resolution respirometry 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 mutationsC. Barcellona¹, M. Sframeli², G.L. Vita², M.G. Di Stefano¹,M. La Rosa¹, S. La Foresta², C. Faraone², M. Russo²,C. Rodolico¹, S. Messina^{1,2}, G. Vita^{1,2}¹ Department of Neurosciences, University of Messina, Italy; ² Nemo Sud Clinical Center, Messina, Italy

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

L. Bello^{1,2}, A. Kesari¹, H. Gordish-Dressman¹, A. Cnaan^{1,3}, L.P. Morgenroth¹, J. Punetha^{1,3}, T. Duong¹, E.K. Henricson⁴, E. Pegoraro², C.M. McDonald⁴, E.P. Hoffman^{1,3};
on behalf of the CINRG Investigators

¹ Children's National Medical Center, Washington, DC, USA;

² Dipartimento di Neuroscienze, Università di Padova, Italy; ³ The George Washington University, Washington, DC, USA; ⁴ University of California, Davis, CA, USA

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

D. Bernardo, S. Servidei

Department of Neurology, Catholic University, Rome, Italy

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

F. Biasini¹, S. Portaro¹, A. Mazzeo¹, A. Toscano¹, G.M. Fabrizi², F. Taioli², G. Vita¹, C. Rodolico¹

¹ Department of Neurosciences, University of Messina, Italy;

² Section of Neuropathology, Department of Neurological and Movement Sciences, University of Verona, Italy

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

S. Bortolani, L. Vercelli, V. Ponzalino, S. Boschi, E. Vittonatto, L. Chiadò-Piat, T. Mongini

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

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 myopathic 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

C. Bragato¹, G. Gaudenzi², F. Blasevich¹, G. Pavesi²,

L. Maggi¹, M. Giunta¹, F. Cotelli², M. Mora¹

¹ Neuromuscular Diseases and Neuroimmunology Unit, IRCCS Neurological Institute "C. Besta", Milano, Italy; ² Department of Biosciences, University of Milan, Italy

Mutations in the dynamin-2 gene (*DNM2*) cause autosomal dominant centronuclear myopathy (CNM) and dominant intermediate Charcot-Marie-Tooth neuropathy type B (CMT1B), 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 CMT-mutated.

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* knock-down is a valuable model for dynaminopathies. 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

E. Bugiardini^{1,2}, M.G. Thor¹, R. Cardani², G. Meola², M.G. Hanna¹, R. Mannikko¹

¹ MRC Centre for Neuromuscular Diseases, Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK; ² Department of Neurology, IRCCS Policlinico "San Donato", University of Milan, Italy

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 DII-S4S5 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 inactivation (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

E. Caldarazzo Ienco, D. Orsucci, C. Simoncini, V. Montano, A. Lo Gerfo, L. Chico, L. Petrozzi, M. Brondi, G. Siciliano, M. Mancuso

Department of Clinical and Experimental Medicine, Neurological Clinic, University of Pisa, Italy

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

G. Capaldo, C. Dato, L. Lombardi, F. Tuccillo, S. Sampaolo, G. Di Iorio, M. Melone

Department of Neurological Sciences, Second University of Naples, Italy

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 mitochondrial dysfunction is a common pathogenetic pathway.

Topic: myology, mitochondria.

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

R. Cardani¹, A. Botta², R. Valaperta^{3,4}, B. Fossati⁵, G. Rossi², L. Fontana², C. Trevisan⁵, G. Cuomo¹, E. Costa^{3,4}, G. Meola^{1,6}

¹ *Laboratory of Muscle Histopathology and Molecular Biology, IRCCS-Policlinico "San Donato", Milan, Italy;* ² *Department of Biomedicine and Prevention, Tor Vergata University of Rome, Italy;* ³ *Research Laboratories, IRCCS Policlinico "San Donato", Milan, Italy;* ⁴ *Service of Laboratory Medicine 1, Clinical Pathology, IRCCS Policlinico "San Donato", Milan, Italy;* ⁵ *Department of Neurosciences, University of Padua, Italy;* ⁶ *Department of Biomedical Science for Health, IRCCS Policlinico "San Donato", University of Milan, Italy*

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 hyperCKemia, 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 myopathic 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

R. Cardani¹, R. Valaperta^{2,3}, F. Lombardi^{2,3}, B. Rampoldi³, R. Panella³, B. Fossati⁴, E. Brigonzi⁴, S. Cassarino Gallo⁴, M. Piccoli⁵, G. Cuomo¹, R. Rigolini³, P. Gaia³, E. Pusineri⁶, E. Costa^{2,3}, G. Meola^{1,4}

¹ *Laboratory of Muscle Histopathology and Molecular Biology, IRCCS Policlinico "San Donato", Milan, Italy;* ² *Research Laboratories,* ³ *Service of Laboratory Medicine 1, Clinical Pathology,* ⁴ *Department of Biomedical Sciences for Health, University of Milan,* ⁵ *Laboratory of Stem Cells for Tissue Engineering,* ⁶ *Clinical Cardiology Unit and CCU, IRCCS Policlinico "San Donato", Milan, Italy*

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

I. Colombo¹, S. Testolin¹, M. Ripolone¹, R. Violano¹, R. Xhani¹, V. Lucchini¹, N. Grimoldi², F. Tiberio², M. Sciacco¹, G.P. Comi³, M. Moggio¹

¹ Neuromuscular and Rare Disease Unit, Department of Neuroscience, ² Neurosurgery Unit, Department of Neuroscience, ³ "Dino Ferrari" Centre, Department of Pathophysiology and Transplantation Neuroscience Section (DEPT), Neurology Unit, Foundation IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, University of Milan, Italy

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 (\pm 87.3) to 459 m (\pm 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% (\pm 19.2) to 74.5% (\pm 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

C. Cuccagna, F. Fattori, M. Luigetti, G. Primiano, D. Bernardo, D. Sauchelli, M. Catteruccia, E. Bertini, S. Servidei
Department of Neurology, Catholic University, Rome, Italy

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 (DNM2) 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

P. Cudia^{1,2}, P. Kiper², A. Baba², A. Marcante^{1,2}, C. Enrichi², S. Lampa², S. Rossi², L. Ghezzi³, A. Tumbarello³, F. Piccione^{1,2}, C. Angelini^{1,2}.

¹ Neuromuscular Division, ² Neurorehabilitation Division, ³ Neuroradiology Department, IRCCS San Camillo Hospital Foundation, Venice, Italy

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 been 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?

P. D'Ambrosio¹, E. Picillo¹, M. Ergoli¹, A. Torella², L. Passamano¹, V. Nigro^{2,3}, L. Politano¹

¹ *Cardiomyology and Medical Genetics, Department of Experimental Medicine, Second University of Naples, Italy;* ² *Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Italy;* ³ *Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy*

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 hyperCKemia. 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

V. Emmanuele¹, M. Wasserstein¹, J. Pang¹, S. Krishna¹, K. Tanji², P. Nagy², M. Hirano¹

¹ *Columbia University Medical Center, Neurology, New York, USA;* ² *"Mount Sinai" School of Medicine, Genetics and Genomic Science and Pediatrics, New York, USA;* ³ *Columbia University Medical Center, Pathology and Cell Biology, New York, USA*

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 Proven 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

C. Fiorillo¹, F. Fattori², G. Astrea³, A. Rubegni³, J. Baldacci³, A. Tessa³, M. Savarese⁴, E. Bertini², C. Minetti¹, A. D'Amico², C. Bruno¹, F.M. Santorelli³

¹ *U.O.C. Neurologia Pediatrica e Malattie Muscolari, IRCCS Istituto "G. Gaslini", Genova, Italy;* ² *UOC Malattie Neuromuscolari e Neurodegenerative, Ospedale Pediatrico "Bambino Gesù", Roma, Italy;* ³ *UOC Medicina molecolare e Malattie Neuromuscolari, IRCCS "Stella Maris", Pisa, Italy;* ⁴ *Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy*

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 ophthalmoparesis 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

C. Fiorillo, A. Robbiano, F. Madia, L. Pennese, F. Trucco, C. Panicucci, M. Pedemonte, C. Bruno, C. Minetti, F. Zara
UOC Neurologia Pediatrica e Malattie Muscolari, UOSD Centro di Miologia e Patologie Neurodegenerative, IRCCS Istituto "G. Gaslini", Genova

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

B. Fossati¹, J. Lanzone¹, I. Merli¹, E. Cè², S. Rampichini², F. Esposito², G. Meola¹

¹Neurology, IRCCS Policlinico "San Donato", University of Milan, Italy; ²Department of Biomedical Sciences for Health, University of Milan, Italy

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 \pm 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)

D. Ginocchio^{1,2}, F. Bianchi³, E. Alfonsi⁴, E. Alvisi⁴, F. Rao¹, M. Iatomasi¹, A. Lizio¹, A. Schindler², V. Sansone¹

¹NEMO (NEuroMuscular Omnicentre) Clinical Center, Neurorehabilitation Unit, University of Milan, Italy; ²Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, Italy; ³IRCCS Maugeri Foundation, Milan, Italy; ⁴Department of Neurophysiopathology "C. Mondino" National Institute of Neurology, IRCCS, Pavia, Italy

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

K. Gorni¹, G. D'Angelo², A. Berardinelli³, A. D'Amico⁴, F. Racca⁵, C. Mastella⁶, I. Moroni⁷, E. Falcier¹, F. Rao¹, V. Sansone¹

¹ NEMO (NEuroMuscular Omnicomprehensive) Clinical Center, Fondazione Serena ONLUS, Neurorehabilitation Unit, University of Milan, Italy; ² Neuromuscular Unit- IRCCS "E. Medea" Bosisio Parini, Lecco, Italy; ³ Dipartimento di Clinica Neurologica e Psichiatria dell'età evolutiva, Fondazione "C. Mondino", Pavia, Italy; ⁴ Unit of Neuromuscular and Neurodegenerative disorders, "Bambino Gesù" Children's Hospital, Roma; ⁵ SC Anestesia e Rianimazione Pediatrica Azienda Ospedaliera Alessandria, Italy; ⁶ SAPRE (Servizio di Abilitazione Precoce dei Genitori), Fondazione IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, Milano; ⁷ O Neuropsichiatria Infantile Fondazione IRCCS Istituto Neurologico "C. Besta"

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

E. Harris¹, J. Hudson², M. Neri³, A. Ferlini³, K. Bushby¹, H. Lochmüller¹, V. Straub¹, R. Barresi^{1,4}

¹ John Walton Muscular Dystrophy Research Centre, Newcastle University, Newcastle upon Tyne, UK; ² Northern Genetics Service, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK; ³ Section of Medical Genetics and Microbiology, Department of Medical Sciences, University of Ferrara, Italy; ⁴ Rare Diseases Advisory Group Service for Neuromuscular Diseases, Muscle Immunoanalysis Unit, Dental Hospital, Richardson Road, Newcastle upon Tyne, UK

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 (thrombocytopenia, 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

S. Iachettini¹, R. Cardani¹, R. Valaperta^{2,3}, A. Marchesi⁴, A. Perfetti⁵, V. Ricigliano⁶, G. Cuomo¹, L. Vaienti⁴, E. Costa^{2,3}, G. Meola^{1,6}

¹ Laboratory of Muscle Histopathology and Molecular Biology, ² Research Laboratories, ³ Service of Laboratory Medicine 1, Clinical Pathology, ⁴ Department of Plastic and Reconstructive Surgery, ⁵ Laboratory of Molecular Cardiology, ⁶ Department of Biomedical Science for Health, IRCCS Policlinico "San Donato", University of Milan, Italy

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 CIC-1 mutations causing myotonia congenita reduce sensitivity to 9-AC

P. Imbrici¹, C. Altamura¹, A. Toscano², R. Mantegazza³, J.F. Desaphy¹, D. Conte Camerino¹

¹ Department of Pharmacy, Drug Sciences, University "Aldo Moro", Bari, Italy; ² Department of Neurosciences, University of Messina, Italy; ³ Neurology IV, Neuromuscular Diseases and Neuroimmunology Unit, Foundation IRCCS Neurological Institute "Carlo Besta", Milan, Italy

Loss-of-function mutations of CIC-1 are responsible for dominant and recessive myotonia congenita (MC). We recently identified two novel CIC-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 CIC-1 channels. Interestingly, none of the other CIC channels tested so far are as sensitive to 9AC as is CIC-1. It is also known to reduce the macroscopic resting Cl conductance of skeletal muscle (gCl) that is mostly carried by CIC-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.

Supported by Health Ministry (grant GR-2009-1580433).

Severe rhabdomyolysis in a patient with "Heat Stroke"

M. La Rosa¹, M. Sframeli², G.L. Vita², M.G. Distefano¹, C. Barcellona¹, F. Granata³, M. Aguenouz¹, C. Rodolico¹, A. Toscano¹, S. Messina^{1,2}, G. Vita^{1,2}

¹ Department of Neurosciences, University of Messina, Italy; ² Nemo Sud Clinical Centre, Messina, Italy; ³ Department of Clinical and Experimental Medicine, University of Messina, Italy

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)

A. LoMauro¹, M. Romei², S. Gandossini², E. Marchi², A.C. Turconi², N. Bresolin^{2,3}, A. Aliverti¹, M.G. D'Angelo²

¹ Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Italy; ² IRCCS E Medea, Neuromuscular Unit, "Bosisio Parini", Lecco, Italy; ³ Fondazione Policlinico, Università di Milano

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

L. Lombardi, S. Sampaolo, G. Capaldo, C. Dato, G. Di Iorio
Department of Medical, Surgical, Neurological, Aging and Metabolic Sciences, Second University of Naples, Naples, Italy

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 $\mu\text{mol/h/L}$ 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 phosphatase-positive 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

L. Losito¹, L. Gennaro¹, M. De Rinaldis¹, D. De Matteis², A. Trabacca¹

¹ Scientific Institute IRCCS “Eugenio Medea”, “La Nostra Famiglia”, Unit for severe disabilities of developmental age and young adults (Developmental Neurology and Neurorehabilitation), Brindisi Research Centre, Brindisi, Italy; ² Rehabilitation Center “La Nostra Famiglia” Association, Lecce, Italy

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

F. Magri¹, M. Savarese^{2,3}, G. Di Fruscio^{2,3}, A. Govoni¹, M. Moggio¹, R. Brusa¹, M.G. D'Angelo⁴, M.D. Piga¹, D. Ronchi¹, R. Del Bo¹, F. Fortunato¹, G.P. Comi¹, V. Nigro^{2,3}

¹ Dino Ferrari Center, IRCCS “Ca' Granda” Ospedale Maggiore Policlinico, Neuroscience Section, Dipartimento di Fisiopatologia medico-chirurgica e dei trapianti, Università di Milano, Italy and Neuromuscular Unit, “Dino Ferrari” Centre IRCCS “Ca' Granda” Ospedale Maggiore Policlinico, Neuroscience Section, Milan, Italy;

² Laboratorio di Genetica Medica, Dipartimento di Biochimica, Biofisica e Patologia generale, Seconda Università di Napoli, Italy; ³ Telethon Institute of Genetics and Medicine, Napoli, Italy;

⁴ “E. Medea” Scientific Institute, Bosisio Parini, Lecco, Italy

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 biophysical 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

M. Marino,¹ O. Musumeci¹, G. Paleologo², S. Romeo¹, A. Migliorato¹, C. Rodolico¹, A. Toscano¹

¹ Department of Neuroscience, University of Messina, Italy;

² Department of Cardiovascular Diseases, Papardo, Piemonte Hospital, Messina, Italy

Danon disease is an X-linked multisystem disorder, due to the primary deficiency of Lysosome-Associated Membrane Protein 2 (LAMP2). The classic clinical presentation is 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, whereas echocardiography 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 a molecular 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)

F. Montagnese¹, F. Granata², O. Musumeci¹, C. Rodolico¹, S. Mondello¹, M. Cucinotta¹, A. Ciranni¹, M. Longo², A. Toscano¹

¹ Department of Neurosciences, ² Department of Radiological Sciences, University of Messina, Italy

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

O. Musumeci¹, M. Gaeta², S. Mondello¹, P. Ruggeri³, F. Montagnese¹, M. Cucinotta¹, S. Vinci², D. Milardi², A. Toscano¹

¹ Department of Neurosciences, ² Department of Biomedical Sciences and of Morphologic and Functional Images, ³ Department of Specialist Medical-Surgical Experimental Sciences and Odontostomatology, University of Messina, Italy

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

A. Nikolic¹, G. Ricci^{1,2}, J. Daolio¹, F. Mele¹, M. Govi¹, L. Ruggiero³, L. Vercelli⁴, E. Bucci⁵, C. Fiorillo⁶, L. Villa⁷, M. Cao⁸, L. Maggi⁹, L. Santoro³, T. Mongini⁴, G. Antonini⁵, C. Bruno⁶, M. Moggio⁷, C. Angelini⁸, L. Morandi⁹, V. Sansone¹⁰, M.A. Maioli¹¹, G. D'Angelo¹², A. Di Muzio¹³, C. Rodolico¹⁴, G. Siciliano², G. Tomelleri¹⁵, A. Berardinelli¹⁶, R. Tupler¹

¹ University of Modena and Reggio Emilia, Italy; ² University of Pisa, Italy; ³ University "Federico II" of Naples, Italy; ⁴ University of Turin, Italy; ⁵ Sapienza Università di Roma, Italy; ⁶ "G. Gaslini" Institute of Genoa, Italy; ⁷ University of Milan, Italy; ⁸ University of Padua, Italy; ⁹ IRCCS Foundation "C. Besta" Institute Milan, Italy; ¹⁰ University of Milan, Clinical center "Nemo", Italy; ¹¹ University of Cagliari, Italy; ¹² IRCCS Institute "E. Medea" Bosisio Parini, Italy; ¹³ University of Chieti, Italy; ¹⁴ University of Messina, Italy; ¹⁵ University of Verona, Italy; ¹⁶ IRCCS "C. Mondino" Foundation Pavia, Italy

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 ($\leq 25\%$) and normal level of methylation ($\geq 35\%$), 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

M. Pane^a, E. Mazzone^a, L. Fanelli^a, R. De Sanctis^a, C. Palermo^a, S. Sivo^a, A. D'Amico^b, S. Messina^c, L. Politano^d, R. Battini^e, S. Frosini^e, M. Pedemonte^f, E. Pegoraro^h, A.L. Berardinelliⁱ, G. D'Angelo^j, A. Pini^k, G. Baranello^l, E. Mercuri^a
^a Child Neurology and Psychiatry Unit, Rome; ^b Department of Laboratory Medicine, Unit of Molecular Medicine, Bambino Gesù Hospital, Rome; ^c Department of Neurosciences, Psychiatry and Anaesthesiology, University of Messina; ^d Department of experimental Medicine, University of Naples; ^e Department of Developmental Neuroscience, Stella Maris Institute, University of Pisa; ^f Neuromuscular Disease Unit, G. Gaslini Institute, Genoa; ^g Neuromuscular Center, SG. Battista Hospital, University of Turin; ^h Department of Neurosciences, University of Padua; ⁱ IRCCS "C. Mondino" Foundation, University of Pavia; ^j IRCCS Eugenio Medea, Bosisio Parini; ^k Child Neurology and Psychiatry Unit, Maggiore Hospital, Bologna; ^l Pediatric Neurology and Neuroradiology Units, Neurological Institute C. Besta, Milan, Italy

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

R. Papa¹, F. Madia¹, D. Bartolomeo¹, F. Trucco¹,
M. Pedemonte¹, M. Traverso¹, P. Broda¹, C. Bruno², F. Zara¹,
C. Fiorillo¹, C. Minetti¹

¹ UOC Neurologia Pediatrica e Malattie Muscolari, ² UOSD Centro di Miologia e Patologie Neurodegenerative, IRCCS Istituto "G. Gaslini", Genova.

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.

Fourteen 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

V. Papa¹, D. Cordelli², C. Graziano³, R. Rinaldi⁴,
L. Badiali De Giorgi⁵, R. Costa¹, G. Cenacchi¹

¹ Department of Neuromotor and Biomedical Sciences, Alma Mater University, Bologna, Italy; ² UO Infantile Neuropsichiatria, ³ UO Medical Genetic, ⁴ UO Neurology, ⁵ UO Pathology, S. Orsola-Malpighi Hospital, Bologna, Italy

Infantile neuroaxonal dystrophy (INAD) is an autosomal recessive degenerative disease with onset in the first or second year of life. Mutation in the *PLA2G6* 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 expres-

sive 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 electromyography. 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. Preliminary 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).

D. Parisi, O. Musumeci, F. Montagnese, M. Marino,
A. Migliorato, A. Ciranni, C. Rodolico, A. Toscano
Department of Neurosciences, University of Messina, Italy

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 pre-symptomatic 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 stained by May-Grünwald/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 euophysiological and swallowing protocol

M.B. Pasanisi¹, E. Raimondi², M. Zardoni², F. Gianelli²,
M. Colombo², C. Nascimbene², L. Maggi¹, A. Schindler³,
M. Osio², L. Morandi¹

¹ Department of Neuromuscular disorders and Neuroimmunology, Neurological Institute IRCCS "Carlo Besta", Milan, Italy;

² Department of Clinical Neurophysiology, University Hospital "Luigi Sacco", Milan, Italy; ³ Department of Otolaryngology, University Hospital "Luigi Sacco", Milan, Italy

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 morbidity 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 cohort of Italian patients.

Italian Registry of NLSDs. Clinical and genetical characterization

E.M. Pennisi¹, M. Arca², E. Bertini³, C. Bruno⁴, D. Cassandrini⁵, A. D'Amico³, M. Garibaldi², F. Gragnani¹, R. Massa⁶, S. Missaglia⁷, L. Morandi⁸, O. Musumeci⁹, E. Pegoraro¹⁰, F.M. Santorelli⁵, D. Tavian⁷, A. Toscano⁹, C. Angelini¹⁰

¹ "San Filippo Neri" Hospital, Rome, Italy; ² Sapienza Università di Roma, Italy; ³ "Bambino Gesù" Hospital IRCCS, Rome, Italy; ⁴ "G. Gaslini" Hospital IRCCS, Genova, Italy; ⁵ "Stella Maris" IRCCS, Pisa, Italy; ⁶ Tor Vergata University, Rome, Italy; ⁷ CRIBENS, Catholic University of the Sacred Heart, Milan; ⁸ "C. Besta" Institute-Milan, Italy; ⁹ University of Messina, Italy; ¹⁰ University of Padua, Italy

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 hepatic 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 compromise. Critical aspects for disability and life expectancy in Italian patients are arrhythmic cardiopathy and skeletal 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.

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

R. Petillo¹, M. Scutifero¹, M. Lanza², M. De Bernardo³, A. Taglia¹, N. Rosa³, L. Politano¹

¹ Cardiology and Medical Genetics, Dept. of Experimental Medicine, Second University of Naples, Italy; ² Eye Department, Second University of Naples, Naples, Italy; ³ Department of Medicine and Surgery, University of Salerno, Salerno, Italy

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 $44,7 \pm 11,2$ years. Forty were males (31,2%, mean age $42,8 \pm 9,8$) and 29 were female (25,2%, mean age $47,3 \pm 12,6$ 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

R. Piras¹, M. Iascone², M.A. Maioli¹, A. Mateddu¹, L. Pezzoli², M.E. Sana², G. Marrosu¹

¹ Centro Sclerosi Multipla, University of Cagliari, ASL8, Cagliari, Italy; ² Lab Genetica Molecolare, USSD LGM, AO "Papa Giovanni XXIII", Bergamo, Italy

Alpha dystroglycanopathy due to POMT2 (Protein O-mannosyltransferase 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 with 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 several years showed normal immunostaining for Desmin and α -DG (anti- α -DG I1H6C4 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 IHC, 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 biophysical criteria and the possible misdiagnosis based on newer methods.

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

V. Ponzalino, L. Vercelli, S. Bortolani, T. Mongini
Center for Neuromuscular Diseases, Department of Neurosciences 'Rita Levi Montalcini', University of Torino, Italy

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 scleroderma-like 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/m² weekly for four weeks, then 375 mg/m² one and two months after the last administration), followed by a "maintenance phase" (Rituximab 375 mg/m² 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

S. Portaro, D. Parisi, S. Sinicropi, F. Biasini, A. Ciranni, P. Girlanda, A. Toscano, G. Vita, C. Rodolico
Department of Neurosciences, University of Messina, Italy

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 immunosup-

pression; 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

S. Ravaglia, L. Maggi, M. Sapuppo, L. Morandi, P. Bernasconi, M. Filosto
Pavia, Milano, Brescia

Non-dystrophic myotonias include Myotonia Congenita (MC), either dominant or recessive, caused by skeletal muscle chloride channel-1 gene (CLCN1) mutations, as well as Paramyotonia 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 indicates 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-onset type II glycogenosys (GSDII)

S. Ravaglia, G. Piaggi, V. Rossi, A. Malovini, P. De Filippi, A. Pichiecchio, C. Danesino, A. Carlucci
Pavia

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) $\geq 30\%$ 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

G. Ricci¹, A. Logerfo¹, A. Servadio², G. Dell'Osso³, A. Rocchi¹, C. Lamperti⁴, M.A. Donati⁵, M. Mancuso¹, G. Siciliano¹

¹ Department of Clinical and Experimental Medicine, Neurological Unit, University of Pisa, Italy; ² Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Italy; ³ Orthopedic Clinic, University of Pisa, Italy; ⁴ Unit of Molecular Neurogenetics, The Foundation "Carlo Besta" Institute of Neurology IRCCS, Milan, Italy; ⁵ Metabolic and Neuromuscular Unit, Meyer Hospital, Florence, Italy

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, the age, 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 a 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

F. Rinaldi, A. Todeschini, S. Rota, E. Pari, I. Volonghi, A. Padovani, M. Filosto

Clinical Neurology, Section for Neuromuscular Diseases and Neuropathies, University Hospital "Spedali Civili", Brescia, Italy

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

J. Roussel¹, P. Imbrici¹, A. Farinato¹, L. Maggi², P. Bernasconi², R. Mantegazza², D. Conte Camerino¹, J.-F. Desaphy¹

¹ Department of Pharmacy, Drug Sciences, University "Aldo Moro", Bari, Italy; ² Neurology IV, Neuromuscular Diseases and Neuroimmunology Unit, Foundation IRCCS Neurological Institute "Carlo Besta", Milan, Italy

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 μ M intracellular Ca^{2+} or of the co-expressed β 1 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?

A. Rubegni¹, G. Astrea¹, I. Pezzini¹, F. Moro¹, D. Cassandrini¹, E. Picillo², M. Evoli², P. D'Ambrosio², A. D'Amico³, F.M. Santorelli¹, L. Politano²

¹ IRCCS "Stella Maris", Pisa, Italy; ² Cardiomiologia e Genetica Medica, Dipartimento di Medicina Sperimentale, Seconda Università di Napoli, Italy; ³ IRCCS "Bambino Gesù", Roma, Italy

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 polymyogryia-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 Dystrophy (BMD): a retrospective study

C. Sancricca, M.E. Lombardo, G. Vasco, D. Sauchelli, G. Primiano, M. Pelliccioni, S. Servidei

UILDM, Unione Italiana Lotta Distrofia Muscolare ONLUS, Sezione Laziale, Istituto di Neurologia, Università Cattolica, Roma, Italy

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 orthosis were also collected.

The aim of our study was to establish clinical characteristics, natural history and genotype-phenotype correlation in a large cohort of patients with BMD. 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 BMD 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

V.A. Sansone¹, C. Gagnon², M.T. Rogers³, B. Gallais^{2,4}, B. Schoser⁵, P. Wijkstra⁶, D. Duboc⁷, E. Falcier¹, F. Rao¹, D. Orlikowski⁸, M. Phillips⁹, A. Atalaia¹⁰, M. Boentert¹¹, C. Heatwole¹², M. Iatomasi¹; ENMC

¹ Centro Clinico NEMO, University of Milan, Italy; ² Université de Sherbrooke, Quebec, Canada; ³ Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK; ⁴ Institut de Myologie, Paris, France; ⁵ Friedrich-Baur-Institut Neurologische Klinik, IDZ Neuromuskuläre Erkrankungen, Ludwig Maximilians Universität München, Germany; ⁶ University Medical Centre Groningen, The Netherlands; ⁷ Cardiology, Hop Cochin and Myology Institute, Paris France; ⁸ Intensive Care and Home Ventilation Unit, reference centre for neuromuscular disease Hôpital Raymond Poincaré, Garches, France; ⁹ Rehabilitation Medicine, University of Nottingham, UK; ¹⁰ MRC Neuromuscular Centre, Newcastle, UK; ¹¹ Department of Sleep Medicine and Neuromuscular Disorders, Münster University Hospital, Münster, Germany; ¹² University of Rochester, NY, USA

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

V.A. Sansone, F. Racca, G. Ottonello, A. Vianello, A. Berardinelli, G. Crescimanno, C. Agosto, P. Banfi, G. Baranello, E. Bignamini, M.B. Chiarini, R. Cutrera, A. D'amico, M. Dusio, F. Fanfulla, G. Garuti, R. Giretti, K. Gorni, C. Gregoret, L. Luccoli, S. Messina, M. Pane, M. Pavone, M. Pedemonte, M.C. Pera, M. Piastra, C. Profazio, F. Rao, I. Salvo, R. Testa, M. Villanova, J.L. Casiraghi; on behalf of SMA Family Association
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 agree-

ment 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

M. Scarlato¹, F. Viganò², P. Carrera³, S.C. Previtali¹, A. Bolino²

¹ Department of Neurology, INSPE and Division of Neuroscience, ² Human Inherited Neuropathies Unit, INSPE and Division of Neuroscience, ³ Unit of Genomics for Diagnosis of Human Pathologies, Division of Genetics and Cell Biology, and Laboratory of Clinical Molecular Biology, IRCCS "San Raffaele" Scientific Institute, Milan, Italy

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

M. Scarpelli¹, L. Carreno², T. Pinos², A. Russigna¹, A. Ariatti⁴, L. Verriello⁴, E. Garcia-Arumi², J. Marti², P. Tonin¹

¹ Section of Neurology, Department of Neurological and Movement Sciences, University of Verona, Italy, ² Mitochondrial Disorders Unit, Vall d'Hebron Institut de Recerca, Barcelona, Spain; ³ Department of Neurosciences and Department of Onco-Haematology, University Hospitals of Modena & Reggio Emilia, Italy; ⁴ Department of Neurology, University of Udine, Italy

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 *tRNAlys* 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

C. Scuderi, E. Borgione, M. Lo Giudice, F. Castello, S. Santa Paola, F. Di Blasi, S.A. Musumeci
Oasi Institute for Research on Mental Retardation and Brain Aging, Unit of Neuromuscular Diseases, Troina, Italy

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?

C. Simoncini¹, G. Ricci¹, A. Servadio², L. Chico¹, V. Zampa³, M. Giorgetti⁴, G. Comi⁵, G. Siciliano¹

¹ Department of Clinical and Experimental Medicine, Neurological Unit, University of Pisa, Italy; ² Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Italy; ³ Department of Diagnostic and Interventional Radiology, University of Pisa, Italy; ⁴ Department of Musculoskeletal and Skin Diseases, and Hand Surgery, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy; ⁵ Neuromuscular Unit, Fondazione IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, "Dino Ferrari" Centre, University of Milan, Milan, Italy

In our Center for Neuromuscular Diseases, we routinely use forearm ischemic exercise testing for blood lactate and am-

monium 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 ammonium 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

E. Tasca, V. Pegoraro, A. Merico, C. Angelini
Fondazione Ospedale "San Camillo" IRCCS, Lido Venice, Italy

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

V. Tegazzin¹, C. Tripepi¹, E. Pastorello², C. Angelini², C.P. Trevisan²

¹CMF Unità Chirurgica, Padova, Italy; ²Università di Padova, Italy

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

R. Trovato¹, A. Rubegni¹, A. Petrucci², M. Savarese³, G. Astrea¹, D. Cassandrini¹, L. Lispi², R. Massa⁴, V. Nigro^{3,5}, F.M. Santorelli¹

¹IRCCS Stella Maris, Pisa, Italy; ²Center for Neuromuscular and Neurological Rare Diseases "S. Camillo-Forlanini" Hospital, Roma, Italy; ³Seconda Università di Napoli, Italy; ⁴Tor Vergata University, Roma, Italy; ⁵Telethon Institute of Genetics and Medicine, Napoli, Italy

Autosomal dominant (AD) eccentric core disease (ECD) is a congenital myopathy characterized by eccentric core disease (ECD) is 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 of eccentric 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 of eccentric 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

F. Trucco¹, C. Panicuicci¹, C. Fiorillo¹, A. Andaloro², F. Becchetti², C. Bruno¹, M. Pedemonte¹, C. Minetti¹

¹UOC Neurologia Pediatrica e Malattie Muscolari, ²Centro Scoliosi, UOC Ortopedia, IRCCS Istituto "G. Gaslini", Genova, Italy

Scoliosis surgery in neuromuscular (NM) patients may be associated with a high morbidity and even mortality. We report on our center's 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

F. Trucco¹, R. Papa¹, C. Fiorillo¹, C. Panicucci¹, M. Derchi², F. Cairello², C. Bruno¹, M. Pedemonte¹, C. Minetti¹

¹ UOC Neurologia Pediatrica e Malattie Muscolari, ² UOC Cardiologia, IRCCS Istituto "G. Gaslini", Genova, Italy

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 to deletion 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

L. Vercelli, V. Ponzalino, S. Bortolani, T. Mongini
Center for Neuromuscular Diseases, Department of Neuroscience "Rita Levi Montalcini", University of Torino, Italy

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 autopsic 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 (ICI-Q 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

L. Villa¹, L. Peverelli¹, P. Ciscato¹, A. Woods², M. Moggio¹, M. Sciacco¹

¹ UOD Malattie Neuromuscolari e Rare, Dipartimento di Neuroscienze, Fondazione IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, Università di Milano, Italy; ² Clinical Laboratory Immunology, Oxford University Hospitals NHS Trust, Oxford UK

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 UI/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 UI/l) and mild improvement of muscle strength. Azathioprine was administered 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

G.L. Vita¹, A. Catalano³, N. Morabito³, M. Sframeli¹, M.G. Distefano², C. Barcellona², M. La Rosa², G. Vita^{1,2}, S. Messina^{1,2}

¹ NEMO SUD Clinical Centre, Messina, Italy; ² Department of Neurosciences, University of Messina, Italy; ³ Department of Clinical and Experimental Medicine, University of Messina, Italy

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 maintain 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, especially 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

B. Vola¹, R. Maggi², M. Cerletti³, Y. Torrente⁴

¹ Chairman of Family Group of Beta-sarcoglycanopathy (GFB Onlus); ² Department of Endocrinology, Physiopathology and Applied Biology, University of Milan, Italy; ³ Department of Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, USA; ⁴ Laboratory of Stem Cells of the Department of medical-surgical Pathophysiology and Transplantation, University of Milan, Foundation IRCCS "Ca' Granda" Ospedale Maggiore Policlinico in Milan, Italy

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 sarcoglycan 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

S. Zanotti¹, D. Kapetis², S. Gibertini¹, L. Morandi¹, F. Molteni³, M. Mora¹

¹ Neuromuscular Disease and Immunology Unit, Fondazione IRCCS Istituto Neurologico "C. Besta", Milano, Italy; ² Bioinformatics Unit, Fondazione IRCCS Istituto Neurologico "C. Besta", Milano, Italy; ³ Department of Rehabilitation Medicine, "Villa Beretta" Rehabilitation Center, Ospedale Valduce, Costa Masnaga, Italy

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 fibroblast-enriched 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

M.G. Distefano¹, F. Cavallaro², G.L. Vita², M. Sframeli², C. Barcellona¹, M. La Rosa¹, C. Donato², C. Consulo², V. Di Bella², F. Pavone², S. Messina^{1,2}, G. Vita^{1,2}

¹ Department of Neurosciences, University of Messina, Italy;

² Centro Clinico Nemo Sud, Messina

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

E. Campione¹, M. Gibellini^{2,3}, M.V. Cannizzaro¹, E. Rastelli³, A. Petrucci⁴, A. Botta⁵, R. Zenobi², A. Orlandi⁶, S. Chimenti¹, S. Bernardini², R. Massa³, C. Terracciano^{2,3}

¹Department of Systems Medicine, Division of Dermatology, Tor Vergata University, Rome, Italy; ²Department of Experimental Medicine and Surgery, Division of Clinical Biochemistry, Tor Vergata University, Rome, Italy; ³Department of Systems Medicine, Division of Neurology, Tor Vergata University, Rome, Italy; ⁴Center of Neuromuscular and Neurological Rare Diseases S.Camillo-

Forlanini Hospital, Rome, Italy; ⁵Department of Biomedicine and Prevention, Division of Medical Genetic, Tor Vergata University, Rome, Italy; ⁶Department of Biomedicine and Prevention, Division of Anatomic Pathology, Tor Vergata University, Rome, Italy

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 basaloma 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.

AUTHOR INDEX

- | | | | |
|------------------------------|------------------------------|-------------------------------|--------------------------|
| Agosto C., 81 | Astrea G., 40, 54, 57, 59, | Baresi S., 51 | Biasini F., 65, 78 |
| Aguennouz M., 50, 72 | 60, 69, 80, 83 | Barp A., 51 | Biffi A., 58 |
| Akman O., 57 | Atalaia A., 81 | Barresi R., 71 | Bignamini E., 81 |
| Alboini P.E., 49 | Aureli M., 53 | Bartolomeo D., 76 | Blaservitch F., 54, 66 |
| Alfonsi E., 70 | Avoni P., 63 | Battini R., 40, 57, 75 | Boentert M., 81 |
| Aliverti A., 64, 72 | Baba A., 68 | Beccaria M., 50 | Bolino A., 81 |
| Altamura C., 50, 72 | Badiali De Giorgi L., 76 | Becchetti F., 83 | Bonato S., 55 |
| Alvisi E., 70 | Baggi F., 49 | Bellacchio E., 62 | Bordoni A., 52 |
| Amadio S., 58 | Baldacci J., 57, 69 | Bello L., 51, 59, 65 | Borgione E., 82 |
| Andaloro A., 83 | Baldanzi S., 50 | Berardinelli A., 53, 57, 71, | Boriani G., 44 |
| Angelini C., 40, 45, 50, 51, | Ballabio A., 53 | 75, 81 | Bortolani S., 66, 78, 84 |
| 54, 56, 57, 59, 68, 75, 77, | Balottin U., 40 | Berardinelli A.L., 40, 41, 75 | Boschi S., 66 |
| 82, 83 | Banfi P., 81 | Bernardini S., 60, 86 | Botta A., 67, 86 |
| Annunziata A., 43 | Banfi S., 53 | Bernardo D., 59, 65, 68 | Bozzoni I., 53 |
| Antonini G., 75 | Baranello G., 53, 64, 75, 81 | Bernasconi P., 44, 78, 80 | Bragato C., 66 |
| Arca E.M., 57 | Baratto S., 50 | Bertini E., 47, 51, 53, 56, | Brancati F., 58 |
| Arca M., 77 | Barbaro S., 52 | 57, 62, 68, 69, 77 | Bresolin N., 55, 56, 72 |
| Ariatti A., 81 | Barca E., 64 | Bertolin C., 59 | Brighina E., 40, 56 |
| Arnoldi M.T., 64 | Barcellona C., 55, 64, 72, | Bevilacqua F., 50 | Brigonzzi E., 67 |
| Assereto S., 50 | 84, 85 | Bianchi F., 70 | Brisca G., 54, 63 |

- Broda P., 76
 Brondi M., 66
 Bruno C., 50, 56, 57, 59, 63, 69, 70, 75, 76, 77, 83, 84
 Brusa R., 73
 Bucci E., 75
 Bugiardini E., 66
 Bushby K., 71
 Bussolino C., 64
 Cairello F., 84
 Caldarazzo E., 56, 66
 Camerino G.M., 50
 Camia M., 40
 Campadello P., 51
 Campione E., 86
 Cannizzaro M.V., 86
 Cao M., 75
 Capaldo G., 62, 67, 73
 Capanni C., 52
 Capotondo A., 58
 Cappelletti I C., 44
 Carboni N., 44
 Cardani R., 66, 67, 71
 Cardinaletti P., 62
 Carlucci A., 79
 Carrara F., 55
 Carreno L., 81
 Carrera P., 81
 Carrozzo R., 62
 Casiraghi J.L., 81
 Cassandrini D., 54, 57, 77, 80, 83
 Cassarino Gallo S., 67
 Castello F., 82
 Castellotti B., 53
 Catalano A., 84
 Catteruccia M., 40, 53, 56, 68
 Cavalcante P., 44
 Cavallaro F., 85
 Cavalleri M., 56
 Cecchi P., 50
 Cè E., 70
 Cenacchi G., 45, 76
 Cenni V., 52
 Cerletti M., 85
 Cerutti R., 52
 Chiadò-Piat L., 66
 Chiarini M.B., 81
 Chico L., 66, 82
 Chimenti S., 86
 Cinnante C.M., 52
 Cipullo F., 62
 Ciranni A., 56, 74, 76, 78
 Ciscato P., 52, 84
 Civati F., 40
 Civiletto G., 52
 Cnaan A., 65
 Colella G., 62
 Colia G., 40
 Colombo I., 52, 59, 68
 Colombo M., 76
 Columbaro M., 63
 Comi G., 55, 57, 82
 Comi G.P., 48, 52, 59, 68, 73
 Compagnucci C., 51
 Consulo C., 85
 Conte Camerino D., 72, 80
 Cordelli D., 76
 Cornelio F., 54
 Corti S., 41
 Cosottini M., 50
 Costa E., 67, 71
 Costa G., 62
 Costa R., 76
 Cotelli F., 66
 Crescimanno G., 81
 Cuccagna C., 59, 68
 Cucinotta M., 56, 74
 Cudia P., 68
 Cuomo G., 67, 71
 Curcio M., 54
 Cutrera R., 81
 Dallapiccola B., 51
 Damato V., 49
 D'Ambrosio P., 46, 69, 80
 D'Amico A., 40, 43, 44, 51, 53, 54, 57, 62, 69, 71, 75, 77, 80, 81
 Danesino C., 79
 D'Angelo G., 53, 71, 75
 D'Angelo M.G., 40, 56, 72, 73
 Danieli M.G., 62
 Daolio J., 75
 Dato C., 62, 67, 73
 De Bernardo M., 77
 De Cegli R., 53
 de Conti C., 55
 De Filippi P., 79
 Del Bo R., 73
 Dell'Osso G., 79
 De Matteis D., 73
 Derchi M., 84
 De Rinaldis M., 73
 De Sanctis R., 75
 Desaphy J.-F., 50, 72, 80
 De Vivo D., 64
 Di Bella P., 62
 Di Bella V., 85
 Di Bernardo D., 53
 Di Blasi F., 82
 Di Fruscio G., 53, 59, 73
 Di Iorio G., 62, 67, 73
 Di Mauro S., 57, 64
 Di Muzio A., 75
 Diodato D., 51, 62
 Dionisi-Vici C., 51, 62
 Di Siorio G., 59
 Distefano M.G., 72, 84, 85
 Di Stefano M.G., 55, 64
 Domi T., 58
 Donati M.A., 79
 Donato C., 85
 Duboc D., 81
 Duchi S., 52
 Duong T., 65
 Dusio M., 81
 Emmanuele V., 69
 Enrichi C., 68
 Ergoli M., 69
 Ergoli M., 46
 Esposito F., 70
 Evangelisti C., 44
 Evoli A., 49
 Evoli M., 80
 Fabbri S., 50
 Fabrizi G.M., 65
 Fagiolarì G., 52
 Fagiolarì G., 55
 Falcier E., 71, 81
 Fanelli L., 75
 Fanfulla F., 81
 Fanin M., 45, 54, 59
 Faraone C., 55, 64
 Farina O., 59, 62
 Farinato A., 80
 Fattori F., 68, 69
 Feng L., 60
 Ferlini A., 71
 Filocamo M., 53
 Filosto M., 53, 56, 78, 79
 Fiorentino G., 43
 Fiorillo C., 54, 57, 59, 69, 70, 75, 76, 83, 84
 Fiorillo C., 63
 Foisner R., 52
 Fontana L., 67
 Fortunato F., 52, 73
 Fossati B., 67, 70
 Frosini S 75
 Gaeta M., 74
 Gagnon C., 81
 Gaiani A., 40
 Gaia P., 67
 Galletta L., 50
 Gallais B., 81
 Gambineri A., 44
 Gandossini S., 56, 72
 Garcia-Arumi E., 81
 Garibaldi M., 57, 77
 Garuti G., 81
 Gaudenzi G., 66
 Gazzerò E., 50
 Gelardi C., 62
 Gellera C., 53
 Gennaro L., 73
 Ghezzi D., 55, 62
 Ghezzi L., 68
 Giacobini C., 40
 Gianelli F., 76
 Gibellini M., 60, 86
 Gibertini S., 54, 85
 Gimigliano F., 43
 Gimigliano R., 43
 Ginocchio D., 70
 Giorgetti M., 82
 Giretti R., 81
 Girlanda P., 78
 Giunta M., 66
 Goebel H.H., 63
 Gordish-Dressman H., 65
 Gorletta T., 52
 Gorni K., 57, 71, 81
 Govi M., 75
 Govoni A., 73
 Gragnani F., 57, 77
 Granata F., 72, 74
 Graziano C., 76
 Gregoretto C., 81
 Griffo G., 40
 Grimoldi N., 68
 GSDII Italian group of 47
 Hanna M.G., 66
 Harris E., 71
 Heatwole C., 81
 Henricson E.K., 65
 Hirano M., 69
 Hoffman E.P., 65
 Hucko A., 63
 Hudson J., 71

- Iachettini S., 71
 Iascone M., 77
 Iatomasi M., 70, 81
 Ienco 56, 66
 Imbrici P., 50, 72
 Invernizzi F., 54, 62
 Iolascon G., 43
 Iorio R., 49
 Kapetis D., 85
 Kesari A., 65
 Kiper P., 68
 Krishna S., 69
 La Foresta S., 55, 64
 Lampa S., 68
 Lamperti C., 52, 54, 55, 56, 79
 Lanza M., 77
 Lanzone J., 70
 La Rosa M., 55, 64, 72, 84, 85
 Lattanzi G., 44, 52
 Liguori R., 63
 Lispi L., 83
 Lizio A., 70
 Lochmüller H., 71
 Logerfo A., 79
 Lo Gerfo A., 66
 Lo Giudice. M., 82
 Logullo F., 62
 Loi1 M., 52
 Lo Mauro A., 64, 72
 Lombardi F., 67
 Lombardi L., 62, 67, 73
 Lombardo M.E., 40, 80
 Longo M., 74
 Lopez-Otin C., 52
 Lorio R., 50
 Losito L., 73
 Lucchini V., 68
 Luccoli L., 81
 Luigetti M., 59, 68
 Lupidi F., 62
 Madia F., 70, 76
 Maggi L., 44., 58, 66, 75, 76, 78, 80
 Maggi R., 85
 Magliano L., 40
 Magri F., 52, 56, 73
 Maioli M.A., 57, 75, 77
 Malandrini A., 57
 Malfatti E., 57
 Malovini A., 79
 Mancuso. M., 56, 66, 79
 Mannikko R., 66
 Manni L., 58
 Mannone S., 62
 Mantegazza R., 44, 49, 54, 72, 80
 Marcante A., 68
 Marchesi A., 71
 Marchet S., 52, 54
 Marchi E., 72
 Marino M., 56, 74, 76
 Marrosu G., 57, 77
 Martì, 81
 Martinelli D., 51, 62
 Martinuzzi A., 55
 Martone J., 53
 Massacesi M., 50
 Massa R., 57, 58, 60, 77, 83, 86
 Mastella C., 64, 71
 Mateddu A., 57, 77
 Mazzeo A., 65
 Mazzone E., 75
 McDonald C.M., 65
 Mein R., 60
 Melchionda L., 55
 Mele F., 75
 Melone M., 67
 Melone M.A.B., 62
 Meola G., 66, 67, 70, 71
 Mercuria E., 75
 Mercuri E., 53, 57
 Merico A., 82
 Merli I., 70
 Messina S., 40, 55, 53., 57, 60, 64, 72, 75, 81, 84, 85
 Migaleddu G., 50
 Migliorato A., 74, 76
 Milardi D., 74
 Minassian B., 58
 Minetti C., 50, 56, 63, 69, 70, 76, 83, 84
 Missaglia S., 57, 77
 Modolo S., 55
 Moggio M., 47, 52, 55, 57, 59, 68, 73, 75, 84
 Molteni F., 85
 Mondello S., 55, 74
 Mongini T., 42, 56, 57, 66, 75, 78, 84
 Montagnese F., 47, 56, 74, 76
 Montano V., 66
 Morabito N., 84
 Mora M., 45, 54, 57, 58, 66, 85
 Morandi L., 44, 53, 54, 55, 57, 58, 75, 76, 77, 78, 85
 Morandi. L., 57
 Morgenroth L.P., 65
 Moro F., 57, 63, 80
 Moroni I., 57, 71
 Motta M.C., 40
 Muntoni F., 57, 60
 Musumeci O., 47, 50, 56, 57, 74, 76, 77
 Musumeci S.A., 82
 Mutarelli M., 53
 Nagy P., 69
 Naini A., 64
 Nasca A., 62
 Nascimbene C., 76
 Negrini P., 56
 Neil H., 64
 Neri M., 71
 Niceta M., 51
 Nigro V., 45, 46, 53, 54, 57, 59, 69, 73, 83
 Nikolic A., 75
 Nuccetelli M., 60
 Orlandi A., 86
 Orlikowski D., 81
 Orsini C., 46
 Osio M., 76
 Ottonello G., 81
 Padovani A., 53, 79
 Pagliarani S., 52
 Paleologo G., 74
 Palermo C., 75
 Panella R., 67
 Pane M., 40, 53, 54, 75, 81
 Pang J., 69
 Panicucci C., 63, 70, 83, 84
 Papa R., 76, 84
 Papa V., 76
 Parenti G., 46
 Pari E., 53, 79
 Parisi D., 56, 76, 78
 Pasanisi B., 58
 Pasanisi M.B., 76
 Passamano L., 59, 69
 Pastorello E., 83
 Patalano M., 40
 Pavesi G., 66
 Pavone F., 85
 Pavone M., 81
 Pedemonte M., 63, 70, 75, 76, 81, 83, 84
 Pegoraro E., 44, 51, 57, 59, 65, 75, 77
 Pegoraro V., 45, 82
 Pelliccioni M., 80
 Pennese L., 70
 Pennisi E.M., 57, 77
 Pera M.C., 81
 Perfetti A., 71
 Petillo R., 46, 77
 Petrozzi L., 66
 Petrucci A., 50, 60, 83, 86
 Peverelli L., 84
 Pezzini I., 57, 80
 Pezzoli L., 77
 Phadke R., 60
 Phillips. M., 81
 Piaggi G., 79
 Piastra M., 81
 Piccione F., 68
 Piccoli M., 67
 Pichieccchio A., 79
 Picillo E., 46, 57, 59, 69, 80
 Piemonte F., 62
 Piermarini E., 51
 Piga M.D., 73
 Pini A., 57, 75
 Pinos T., 81
 Pinzan E., 45
 Piras R., 57, 77
 Pironi L., 63
 Politano L 75
 Politano L., 40, 43, 44, 46, 57, 59, 77, 80
 Ponzalino V., 66, 78, 84
 Porrello E., 58
 Portaro S., 50, 65, 78
 Previtali S., 53
 Previtali S.C., 52, 58
 Prevital S.C., 81
 Primiano G., 59, 68, 80
 Profazio C., 55, 81
 Provinciali L., 62
 Punetha J., 65
 Pusineri E., 67
 Racca F., 71, 81
 Raimondi E., 76
 Rampichini S., 70
 Rampoldi B., 67
 Rao F., 70, 71, 81
 Rastelli. E., 60, 86
 Ravaglia S., 78, 79
 Reni G., 56
 Reyes A., 55

- Riccardi G., 43
 Ricci F., 57
 Ricci G., 44, 50, 75, 79, 82
 Riccio I., 43
 Riccio V., 43
 Ricigliano V., 71
 Rigolini R., 67
 Rinaldi F., 53, 79
 Rinaldi R., 76
 Rinnenthal J.L., 63
 Ripolone M., 68
 Rizza T., 51, 62
 Robbiano A., 70
 Robb S., 60
 Rocchi A., 79
 Rodolico C., 44, 48, 55, 56, 64, 65, 72, 74, 75, 76, 78
 Rogers M.T., 81
 Romano A., 57
 Romei M., 56, 72
 Romeo S., 74
 Ronchi D., 59, 73
 Rosa N., 77
 Rossi G., 67
 Rossi S., 68
 Rossi V., 79
 Rota S., 53, 79
 Roussel J., 80
 Rubegni A., 57, 69, 80, 83
 Ruegg M.A., 58
 Ruggeri P., 55, 74
 Ruggieri A., 58
 Ruggiero L., 44, 54, 75
 Russigna A., 81
 Russo E., 55
 Russo M., 55, 64
 Sagliocchi A., 40
 Salerno F., 54
 Salvo I., 81
 Sampaolo S., 59, 62, 67, 73
 Sana M.E., 77
 Sanerica C., 59, 80
 Sansone V., 57, 70, 71, 75
 Sansone V.A., 81
 Santa Paola S., 82
 Santorelli F.M., 57, 59, 63, 69, 77, 80, 83
 Santoro L., 75
 Sapuppo M., 78
 Saredi S., 58
 Sarkozy A., 60
 Sauchelli D., 56, 59, 68, 80
 Savarese M., 46, 53, 54, 57, 59, 69, 73, 83
 Scalise R., 40
 Scarlato M., 81
 Scarpelli M., 81
 Schaenzer A., 63
 Schindler A., 70
 Schiumarini D., 53
 Schoser B., 81
 Schuelke M., 63
 Sciacco M., 52, 68, 84
 Scorrano L., 52
 Scotto M., 60
 Scuderi C., 82
 Scutifero M., 40, 77
 Semplicini C., 40, 51, 59
 Servadio A., 79, 82
 Servidei S., 56, 59, 65, 68, 80
 Sewry C., 60
 Sferra A., 51
 Sframeli M., 40, 55, 60, 64, 72, 84, 85
 Siciliano G., 50, 56, 66, 75, 79, 82
 Simoncini C., 50, 66, 82
 Singhmarwah V., 53
 Sinicropi S., 78
 Sivo S., 75
 Sorar G., 51
 Sorarù G., 59
 Squarzone S 52
 Stenzel W., 63
 Straub V., 71
 Striano P., 57
 Taglia A., 46, 77
 Taioli F., 65
 Takeda S., 58
 Tanji K., 69
 Tartaglia M., 51
 Tasca E., 45, 82
 Tasca G., 51, 54, 62
 Tavian D., 57, 77
 Tegazzin V., 83
 Terracciano C., 58, 60, 86
 Tessa A., 69
 Testa R., 81
 Testolin S., 52, 68
 Thor M.G., 66
 Tiberio F., 68
 Tiziano F.D., 41
 Tizzano E., 42
 Todeschini A., 53, 79
 Tomelleri G., 75
 Tonin P., 81
 Tonlorenzi R., 58
 Torella A., 69
 Torrente Y., 85
 Toscano A., 47, 50, 55, 56, 57, 59, 65, 72, 74, 76, 77, 78
 Tozzi G., 62
 Trabacca A., 73
 Traverso M., 76
 Trevisan C., 67
 Trevisan C.P., 83
 Trevisi E., 55
 Tripepi C., 83
 Trovato R., 54, 83
 Trucco F., 63, 70, 76, 83, 84
 Tuccillo F., 67
 Tumbarello A., 68
 Tupler R., 57, 75
 Turconi A.C., 56, 72
 Vaienti L., 71
 Valaperta R., 67, 71
 Valentino M.L., 63
 Valsecchi M., 53
 Varanita T., 52
 Vasco G., 80
 Vavla M., 55
 Velardo D., 52, 58
 Verardo M., 62
 Vercelli L., 44, 54, 66, 75, 78
 Verriello L., 81
 Verrigni D., 62
 Vianello A., 81
 Vianello S., 59
 Viganò F., 81
 Villa L., 75, 84
 Villanova M., 81
 Vinci S., 74
 Violano R., 68
 Viscomi C., 52
 Vita G., 40, 55, 60, 64, 65, 72, 78, 84, 85
 Vita G.L., 55, 64, 72, 84, 85
 Vittonatto E., 66
 Vola B., 85
 Volonghi I., 79
 Volpi L., 50
 Wasserstein M., 69
 Wijkstra P., 81
 Woods A., 84
 Xhani R., 68
 Yau M., 60
 Zaccaro A., 40
 Zampa V., 82
 Zanni G., 51
 Zanotti S., 54, 58, 85
 Zara F., 50, 70, 76
 Zardoni M., 76
 Zenobi R., 86
 Zeviani M., 52, 56

NEWS FROM AROUND THE WORLD

MSM

The 12th Congress of the Mediterranean Society of Myology will be held in Naples, Italy on May 2015, from 18th to 20th. The congress will be chaired by Prof. Giovanni Nigro, President of the Mediterranean Society of Myology. The Scientific Committee, is chaired by Prof. Giovanni Nigro, cooperated by the Board of the Society: Lefkos Middleton (London); George Serratrice (Marseille); Yeuda Shapira (Jerusalem); Luisa Politano (Naples); Ekram Abdel-Salam (Cairo); Marinos Dalakas (Athens); Fayçal Hentati (Tunis); Giovanni Meola (Milan); Gabriele Siciliano (Pisa); Eduardo Tizzano-Ferrari (Barcelona); Antonio Toscano (Messina); Janez Zidar (Ljubljana) and by Vincenzo Nigro (Naples); Giuseppe Novelli (Rome); Reinhardt Rüdel (Ulm).

The symposium will be in the traditional two-days MSM format with 6 selected topics:

- Spinal Muscular Atrophies
- Nuclear Envelop Diseases
- Heart involvement in NeuroMuscular Disorders
- Inflammatory Myopathies
- Next Generation Sequency and NeuroMuscular Disorders
- New therapeutic approach in NeuroMuscular Disorders

During the General Assembly of the Society, the new Board of the Society will be elected. Further information is available in the website of the Organizing Secretariat www.fclassevents.com.

The proceedings of the congress are reported in the present issue of *Acta Myologica*.

GCA

During the Gala dinner of the 12th Congress of the Mediterranean Society of Myology to be held in Naples, Italy on May 19th, the 2015 Gaetano Conte Prizes will be assigned.

AIM

The 15th Congress of the Italian Association of Myology will be held in Naples, Italy on May 2015, from 20th to 23rd, closely linked to the Congress of the Mediterranean Society of Myology. The Scientific Committee is chaired by Prof. Giovanni Nigro, cooperated by the Board of the Society: Maurizio Moggio (Milan); Antonio Toscano (Messina); Claudio Bruno (Genoa); Paola Tonin (Verona); Angela Berardinelli (Pavia); Massimiliano Filosto (Brescia); Giovanni Marrosu (Cagliari); Lucia Ovidia Morandi (Milano); Elena Pegoraro (Padua); Gabriele

Siciliano (Pisa) and by Luisa Politano and Vincenzo Nigro (Naples).

The local Organizing Committee is chaired by Luisa Politano and Vincenzo Nigro with the cooperation of Liberato Berrino, Gerardo Nigro, Orlando Paciello, Alberto Palladino, Luigia Passamano, Raffaele Russo, Cira Solimene, Paola D'Ambrosio, Manuela Ergoli, Chiara Orsini, Roberta Petillo, Esther Picillo, Marianna Scutifero, Antonella Taglia and Emanuela Viggiano.

The symposium will be in the traditional three-days AIM format with the following selected topics:

- Spinal Muscular Atrophies
- Rehabilitative aspects in MD
- Laminopathies: a clinical-molecular update
- LGMD: Update on the new phenotypes
- Advances in the treatment of Lysosomal Disorders
- Advances in the treatment of Muscular Dystrophies
- Neuromuscular Junction Diseases

A session dedicated to the meeting with Patient's or Scientific Associations is also included.

Further information are available in the website of the Society www.miologia.org and of the Organizing Secretariat www.fclassevents.com.

The proceedings of the congress are reported in the present issue of *Acta Myologica*.

THE ITALIAN NETWORK FOR LAMINOPATHIES

After six years of intense research and dissemination activity, the Italian Network for Laminopathies involves neurologists, pediatricians, geneticists and basic researchers from 14 Italian regions. The goals of the Network, at its establishment in 2009, were to share knowledge about such complex diseases as laminopathies and to face their investigation through a multidisciplinary approach. These goals have been achieved and we have now an established collaboration among Italian centers involved at diverse levels in Emery-Dreifuss muscular dystrophy, familial partial lipodystrophy and progeroid syndrome research. Yet, as we approach laminopathy research from an interdisciplinary point of view, new complex and unexplored issues emerge, such as how the metabolic disorders caused by LMNA mutations impact on muscular dystrophy or vice versa how muscle wasting contributes to adipose tissue abnormalities. To attempt a comprehensive view of these aspects, Network partners are investigating the secretome of laminopathic patients and new unexpected findings are emerging.

LGMD-EuroNet

An informal meeting of the LGMD EuroNET is planned to be in Naples, May 22nd, during the Congress of the Italian Association of Myology. Further information will be available on the website www.lgmd2e.org.

WMS

The 20th International WMS Congress will be held in Brighton, UK from 30th September to 4th October, 2015.

The Congress will be held in the traditional WMS format with three selected topics. One day of the symposium will be dedicated to each of the selected topics addressing emerging discoveries in the field of:

- Muscle metabolism in health and disease
- Immune mediated Peripheral Nerve, Neuromuscular Junction, and Muscle Disorders
- Advances in the treatment of Neuromuscular Disorders

Abstracts will also be welcome on advances across the neuromuscular field. Further information is available in the website of the Society www.wms2015.com.

FORTHCOMING MEETINGS

2015

May 5-9

2015 ISBER Annual Meeting. Phoenix, Arizona.
Information: website: www.isber.org

May 18-20

12th Mediterranean Society of Myology Congress. Naples, Italy. *Information:* giovanni.nigro@unina2.it; luisa.politano@unina2.it; vincenzo.nigro@unina2.it; www.msm-aim.org

May 20-23

15th Congress of the Italian Society of Myology.
Information: giovanni.nigro@unina2.it; luisa.politano@unina2.it; vincenzo.nigro@unina2.it; www.msm-aim.org

June 6-9

The European Human Genetics Conference, Glasgow, United Kingdom. *Information:* website: www.esgh.org

June 8-12

IDMC10 – International Myotonic Dystrophy Consortium Meeting. Paris, France. *Information:* website: www.idmc10.org; email: contact@idmc10.org

June 29 - July 1

Orphan Drugs & Rare Diseases Global Congress 2015 Europe. London, UK. *Information:* website: <http://www.paradigmglobalevents.com/events/orphan-drugs-rare-diseases-global-congress-2015-europe/>

September 29 - October 2

2015 ESBB Annual Conference. London, UK. *Information:* website: <http://www.esbb.org/london/>; Programme: <http://www.esbb.org/london/programme.html>

September 30th - October 4th

20th World Muscle Society Congress. London/Brighton, UK.
Information: website: <http://www.worldmusclesociety.org>

October 6-10

American Society of Human Genetics ASHG Annual Meeting. Baltimore, MD, USA. *Information:* website: www.ashg.org

2016

March 17-20, 2016

The 10th World Congress on CONTROVERSIES IN NEUROLOGY. Lisbon, Portugal. *Information:* website: www.comtecmed.com/cony

April 3-7

The European Human Genetics Conference. Kyoto, Japan. *Information:* website: www.esgh.org

September 4-9

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

October 20-24

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

October (to be announced)

21st World Muscle Society Congress. Granada, Spain.
Information: website: <http://www.worldmusclesociety.org>

2017

October 17-21

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

2018

October 16-20

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

2019

October 22-26

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

2020

October 27-31

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