

**PROCEEDINGS OF LGMD DAYS MEETING:
Prognosis and Treatment in LGMD**

October 15-17, 2014

**IRCCS “S. Camillo”,
Lido di Venezia (VE), Italy**



**GRUPPO FAMILIARI
BETA-SARCOGLICANOPATIE
ONLUS**



LGMD DAYS

PROGNOSIS AND TREATMENT IN LGMD

15-17 OTTOBRE 2014

IRCCS «S. Camillo»
Via Alberoni 70, Lido di Venezia VE



www.beta-sarcoglicanopatie.it

WEDNESDAY OCTOBER 15th

9.15 Registration of participants

10.00 Annalena Venneri

Welcome greetings

Chairman: Francesco Piccione (Venice)

SESSION I - Introduction

10.15 Luisa Politano (Naples)

Genetics and cardiology of LGMD

10.40 Jordi Diaz Manera (Barcelona)

LGMD subtypes: MRI imaging

11.05 Corrado Angelini (Venice), Elisabetta Tasca (Venice), Marina Fanin (Padua)

Fatigue, gender and NOS in dominant and sporadic LGMD

11.30 Pascal Laforet (Paris)

Frequency and natural History of Sarcoglycanopathies: the experience of Paris-Est Neuromuscular Center

11.55 Discussion

12.20 Lunch

SESSION II - From diagnosis to patient care

14.00 M Savarese

Diagnosis by muscle chip

14.25 Francesca Bevilacqua (Venice), Rita Lorio (Venice)

Neuropsychological tests and QoL

14.50 Andrea Vianello (Padua)

Ventilation: home and hospital care

15.15 Paola Cudia (Venice), Antonio Merico (Venice)

Neurorehabilitation in LGMD

15.40 Tiziana Toso (Padua)

Nutrition guidelines

16.05 Beatrice Vola (Sondrio)

The contribution of association

16.30 Discussion

THURSDAY OCTOBER 16th

9.15 Registration of participants

10.00 Paola Facchin (Padua)

10.10 Massimo Mirandola (Padua)

10.20 Luigi Querini (Padua)

Welcome greetings

Chairmen: Carlo Pietro Trevisan (Padova),
Maurizio Moggio (Milano), Gabriele Siciliano (Pisa)

SESSION I - Therapy and natural History outcome in LGMD

10.30 Doriana Sandonà (Padua)

Small molecule-based therapy for sarcoglycanopathy, a novel perspective.

10.55 Lucia Morandi (Milano)

Clinical features in LGMD

11.20 Elisabetta Gazzo (Genova), Claudio Bruno (Genova)

Improvement of molecular dystrophic process in alpha-sarcoglycan KO mice by blockade of extracellular ATP/P2P axis

11.45 Marija Meznaric (Ljubljana Slovenia)

Histopathology of cardiomyopathy in a patient with α -sarcoglycanopathy

12.10 Claudio Semplicini (Padua)

Follow-up and clinical study in Beta-sarcoglycans

12.35 Gabriele Siciliano (Pisa)

Muscle exercise evaluation in LGMD

13.00 Discussion

13.25 Lunch

15.00 Giacomo Comi (Milan)

Registry of LGMD

15.25 Paola Melacini (Padua)

Heart treatment in LGMD

15.40 C Borsato

LGMD: functional/electrophysiological outcomes

16.05 Corrado Angelini (Venice)

Discussion and closing remarks

16.30 Meeting GFB ONLUS and patient families

FRIDAY OCTOBER 17th

9.30 Vincenzo Nigro (Naples)

NGS and LGMD

10.00 Laura Drigo (Padua)

Discussion by groups of Horizon 2020 Projects

Thursday, 15 October **1st Session h. 10.15-11.55**

Chairman: F. Piccione (Venice)

Genetics Aspects and Heart Involvement in LGMDs

L. Politano

Department of Experimental Medicine, Cardiology and Medical Genetics. Second Napoli University, Italy

Limb-girdle muscular dystrophies (LGMDs) represent a heterogeneous group of genetic rare disorders characterized by progressive deterioration and weakness of proximal limb muscles. A high variability in clinical course and phenotype has been observed, ranging from severe forms with rapid onset and progression, to very mild forms allowing affected people to have fairly normal life expectancy and daily activities.

The possibility to benefit of techniques of new generation, has dramatically improved the power of diagnosing these diseases, so that the number of disorders now included in the group of LGMD is almost doubled compared to just 10 years ago. So far, 31 LGMD loci have been identified, 8 autosomal dominant and 23 autosomal recessive. The dominant forms (LGMD 1A-1H) are generally milder and relatively rare, accounting for less than 10% of all Limb Girdle Muscular Dystrophies. The recessive forms (LGMD2A-2W) are more frequent, having a cumulative prevalence of 1:15.000, save some geographical differences. Among them, LGMD2A (calpainopathy) is the most frequent form observed in the Italian population, followed by LGMD2B (dysferlinopathy) and LGMD2C-2F (sarcoglycanopathies).

Differently from other forms of muscular dystrophy (dystrophinopathies, myotonic dystrophies, nuclear envelop cardiomyopathies etc.), in which cardiac involvement is a peculiar feature of the disease, myocardium is spared in the majority of LGMDs with the exception of particular subtypes. In these forms, cardiac abnormalities can range from conduction tissue defects (e.g., atrial fibrillations, flutters, atrio-ventricular blocks, supra-ventricular or ventricular ectopic beats, ventricular tachycardia) as is more frequently observed in LGMD1B, LGMD2M and LGMD2R to dilated cardiomyopathy characteristic of LGMD2C, LGMD2F and LGMD2I. Cardiac problems may precede, overlap with or follow skeletal muscle weakness. Therefore it is important to include cardiological evaluation in the regular follow-up of these patients, to adopt appropriate treatment when necessary.

Muscle MRI studies in patients with sarcoglycanopathies

J. Díaz-Manera^{1 2}

¹ Neuromuscular disorders Unit. Neurology department, Hospital de la Santa Creu I Sant Pau, Barcelona; ² Centro de Investigación en Red en Enfermedades Raras (CIBERER), Valencia, Spain

Muscle MRI is becoming an important tool in the diagnosis and follow-up of patients with muscle dystrophies. Very good quality images from whole body can be obtained using 1.5 Teslas MRI devices that are available in many centers. There are at present several softwares that allow us to study different characteristics of the muscles such as fatty infiltration or edema. Muscle MRI can be used in daily clinics to select damages areas of the muscle increasing the performance of the muscle biopsy to obtaining a diagnosis. In the last 20 years many studies have been published analyzing the different patterns of muscle atrophy that are characteristic of every muscle disease. Although there are not pathognomonic patterns, muscle MRI is in general useful to suggest a diagnosis. Muscle MRI is useful also to follow-up patients over time because it does not irradiate patients and can be repeated safely every year. For this reason, MRI is becoming an interesting tool in natural history studies or in clinical trials.

In the case of sarcoglycanopathies there is not a clear pattern published yet but in my personal experience the patients have fatty infiltration involving deltoids, biceps and periscapular muscles in the upper limbs. Paraspinal and abdominal muscles are severely involved as well as pelvic muscles psoas and the glutei muscles. In the lower limbs there is a severe involvement of the quadriceps, semimebranosus, semitendinosus and biceps. In general, the muscles of the legs are not involved. This pattern is very characteristic and is not commonly found in other muscle dystrophies allowing to an easy detection when is found in patients with an undiagnosed muscle dystrophy. However a similar pattern can be found in patients with an adult onset Pompe disease, that have however a different clinical history and muscle biopsy. In conclusion, muscle MRI is a interesting tool that can be useful for the diagnosis and follow-up of patients with a muscle dystrophy produced by mutation in the sarcoglycan genes, but further studies are needed to know which is the pattern of muscle involvement of the patients.

Muscle fatigue, nNOS and muscle fiber atrophy in LIMB girdle muscular dystrophy

C. Angelini^{1,2}, E. Tasca², A.C. Nascimbeni¹, M. Fanin¹

¹ Department of Neurosciences, University of Padova; ² IRCCS Fondazione "San Camillo" Hospital, Lido di Venezia, Italy

Muscle fatigability and atrophy are frequent clinical signs in limb girdle muscular dystrophy (LGMD), but their pathogenetic mechanisms are still poorly understood.

We review a series of different factors that may be connected in causing fatigue and atrophy, particularly considering the role of neuronal nitric oxide synthase (nNOS) and additional factors such as gender in different forms of LGMD (both recessive and dominant) underlying different pathogenetic mechanisms.

In sarcoglycanopathies, the sarcolemmal nNOS reactivity varied from absent to reduced, depending on the residual level of sarcoglycan complex: in cases with complete sarcoglycan complex deficiency (mostly in beta-sarcoglycanopathy), the sarcolemmal nNOS reaction was absent and it was always associated with early severe clinical phenotype and cardiomyopathy.

Calpainopathy, dysferlinopathy, and caveolinopathy present gradual onset of fatigability and had normal sarcolemmal nNOS reactivity. Notably, as compared with caveolinopathy and sarcoglycanopathies, calpainopathy and dysferlinopathy showed a higher degree of muscle fiber atrophy.

Males with calpainopathy and dysferlinopathy showed significantly higher fiber atrophy than control males, whereas female patients have similar values than female controls, suggesting a gender difference in muscle fiber atrophy with a relative protection in females. In female patients, the smaller initial muscle fiber size associated to endocrine factors and less physical effort might attenuate gender-specific muscle loss and atrophy.

Frequency and natural history of sarcoglycanopathies: the experience of Paris-Est neuromuscular center

R. Guimaraes-Costa, A. Behin, T. Stojkovic,

R. Ben Yaou, B. Eymard, F. Leturcq¹, P. Laforêt

Centre de Référence de Pathologie Neuromusculaire Paris-Est, Institut de Myologie, GH Pitié-Salpêtrière, APHP, Paris;

¹ Laboratoire de Biochimie et Génétique Moléculaire, Groupe Hospitalier Cochin, APHP, Paris, France

Limb girdle muscular dystrophies (LGMDs) are a heterogeneous group of inherited progressive muscle disorders affecting predominantly the pelvic and scapular girdle muscles. Sarcoglycanopathies are a group of recessive LGMDs caused by mutations in the genes that encode for the components of the sarcoglycan (SG) complex.

Based on molecular and genetic criteria, sarcoglycanopathies are classified as LGMD2D (α -SG), LGMD2E (β -SG), LGMD2C (γ -SG), LGMD2F (δ -SG). Sarcoglycans are tightly bound to each other so that mutation in one normally results in partial or total deficiency of all of them. Alpha and gamma SGs are specific to skeletal muscle and heart, while β -SG is expressed in multiple tissues, although expression is prominent in skeletal and cardiac muscle. Few epidemiological data is available concerning sarcoglycanopathies, prevalence based in biopsy and genetic analyses was estimated to be 5.6×10^{-6} inhabitants.

We performed a retrospective study of all patients with sarcoglycanopathies followed in our center, in a cohort of 63 patients; 39 women, 24 men (32 α -SG; 4 β -SG; 27 γ -SG; none δ -SG). Mean age of disease onset was 6.8 years old (6.9 y. for α -SG; 7.2 y. for β -SG; and 6.6 Y. for γ -SG). Among all patients, 69.8% were wheelchair bounded at last evaluation. Considering severity of each form, 50% of β -SG patients were wheelchairbound, while 68.75% of α -SG and 74.07% of γ -SG were in the same condition. Moreover, ambulation at 18 years old was possible in 100% of β -SG patients, but only 40.6% of α -SG and 48.14% of γ -SG were still ambulatory at same age.

Concerning systemic involvement, cardiomyopathy was observed in 24.07% of all patients (in 50% of β -SG cases, 39.13% of γ -SG cases, and in only 7.69% of α -SG cases). On the other hand, respiratory involvement was present in 37.7% of patients and a different distribution was observed among each subtype. None of β -SG patients was on non-invasive ventilation, but 26.08% of γ -SG and 52% of α -SG patients needed non-invasive ventilation (1 additional γ -SG had tracheotomy). Four patients with γ -SG died (2 men; 2 women, mean age 30 years old).

Genetic study showed that in α -SG, heterozygous R77C mutation was the most frequent mutation (9/32 cases). Regarding γ -SG we found the following mutations: homozygous del521T (13 patients), homozygous del525T (9 patients), heterozygous del521T (1 patient) and heterozygous del525T (2 patients). Each $\alpha\beta$ -SG patient presented a distinct mutations being impossible to establish a more frequent mutation.

Our results point to a higher frequency of γ -SG, followed by α -SG and lastly by β -SG. Sarcoglycanopathy seems to be a severe form of muscular dystrophy with almost 70% of patients being wheelchair-bounded at long-term evaluation. Among these non ambulatory patients, 75% have lost ambulation before 18 years old and could be considered a "Duchenne-like" form. The remaining 25% of patients could then be considered a "Becker-like" form. In this large series of patients with sarcoglycanopathies, clinical and genetic data collected may enable search for correlations between mutations type, protein deficiency and clinical severity.

2nd Session h. 14.00-16.30**Large screening of a NMD cohort of patients by MotorPlex, an innovative strategy of targeted resequencing**

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A. Garofalo^{1,2}, T. Giugliano^{1,2}, C. Fiorillo, G. Tasca⁴,
C. Pisano², F. Del Vecchio Blanco², G. Piluso²,
O. Musumeci⁵, M. Mora⁶, L. Morandi⁶, E. Ricci⁴,
T. Mongini⁷, L. Santoro⁸, L. Politano^{9,13}, C. Angelini¹⁰,
G.P. Comi^{11,12}, C. Bruno^{3,13}, V. Nigro^{1,2}

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The identification of causative mutations in neuromuscular disorders is a crucial issue for the future possibility of a differentiated treatment on a genetic basis. However, the large genetic heterogeneity of neuromuscular disorders and the phenotypic overlap between the different forms hamper a rapid and cheap diagnosis, based on a gene-by-gene approach. In addition, interfamilial and intrafamilial phenotypic variability of patients sharing the same pathogenic variants suggests that additional modifier genes may be involved.

In the last few years, the Next-generation sequencing (NGS) approaches have received unlimited consideration as universal test for almost all Mendelian conditions. In particular, NGS has demonstrated to be an excellent and cost-effective solution in highly heterogeneous diseases.

We have developed a NGS-based platform, named MotorPlex, to test 93 disease genes causing a muscular phenotype.

We have analyzed 391 patients with a clinical diagnosis of neuromuscular disorders. The patients were classified, according to their clinical phenotype, as being affected by Limb girdle muscular dystrophies (48.1%), by congenital myopathies (36.1%) or by other clinical conditions (15.8%), comprising, among others, distal myopathy (17 cases), isolated hyperCKemia (16 cases) and metabolic myopathy (7 cases). The molecular

diagnosis has been found in 158 cases (40.4%), while 131 cases were judged incomplete, because these samples showed a single heterozygous variant in recessive genes fitting with the phenotype or with variations of likely pathogenicity in genes unrelated to the observed clinical condition.

In conclusion, MotorPlex is an ideal first tier test: it is cost-effective and thus applicable to a large number of patients and/or unaffected individuals; it is easily reproducible; it has high values of specificity and sensitivity; it includes all relevant NMD genes; and it is easily upgradable with novel discovered genes.

Respiratory assessment in children and adults with neuromuscular disorders (NMD)

F. Rao

Centro Clinico NeMO, Fondazione Serena onlus, Ospedale Niguarda Ca' Granda, Milano

Neuromuscular diseases (NMD) in childhood are the most frequent causes of chronic respiratory failure (CRF), which requires the use of home mechanical ventilation (HMV). The respiratory care of the child and adults with NMD represents a major challenge for professionals involved and for the family. The key points in the treatment of the patient with NMD are the introduction and the monitoring of non invasive ventilation (NIV) and the management of airway secretions. A good care of these aspects allows a better control of the disease and the prevention of respiratory exacerbations which, if not recognized and promptly treated, weigh heavily on the clinical course of these illnesses. In this paper we examine every single aspect of the diagnostic respiratory functional assessment and its programs of periodic monitoring, the techniques of airway clearance required and their management, aiming to an integrated program involving all the professionals that work with the patient, starting from the physical therapist, in collaboration with the patient and family. First we considered the indications of NIV, its modalities and some criticisms such as the choice of masks, the management of leaks and the prevention of skin lesions associated to the use of interfaces. Another important feature of neuromuscular patients is represented by airway obstruction by secretions: we examined the standard of evaluation of ineffective cough and the ways of its improvement. Lastly, we stressed the importance of multidisciplinary approach to the neuromuscular patient during the evolution of the disease.

Nutrition Guidelines

T. Toso

Ambulatorio Nutrizionale, Padova

The clinical heterogeneity of the various forms of LGMDs identified until today makes the outline of precise nutritional guidelines highly complex. This is especially true when the guidelines have to be associated with the variability of the clinical course, especially with regards to the level of physical activity and the presence/absence of respiratory and cardiac complications. Moreover, the different proteins involved in the various patterns of LGMDs are responsible for different pathogenetic mechanisms, to which can correspond specific deficiency and/or needs of micro and macronutrients.

It would be desirable, since the diagnosis, a constant monitoring of the weight, with the aim of avoiding overweight and obesity, which could negatively influence the motor autonomy of the subjects affected by the limb-girdle muscular dystrophy.

The acquisition of the sole body weight, which is a composited data, is not sufficient in order to identify the variances in the body composition, whose evaluation should be granted by impedenziometry and anthropometry techniques, with particular attention to the lean body mass and its preservation.

In the absence of specific guidelines for the LGMDs, general guidelines must be adopted. More specifically, the recommendations which increasingly come from the scientific research cannot be ignored, especially those which suggest to base the feeding mainly on food coming from vegetable sources (Healthy Eating Plate – Harvard Medical School 2011; World Cancer Research Found & American Institut for Cancer Research 2007).

In this context the abundance of antioxidants, which is a typical feature of the vegetable food, could be useful not only in the reduction of the oxidative stress, but also by playing a preventive role with respect to the progression of the disease.

Recent in vitro studies and of muscular biopsies, in fact, have showed an increase of the oxidative stress in the deficit of calpain 3 and dysferlinopathies, which can consequently be considered an important factor in the evolution of the muscular damage.

Beta-sarcoglycanopathy: any longer an “orphan” disease?

B. Vola, P. Bonetti¹

Family Group of Betasarcoglycanopathy Onlus (Gfb Onlus) and Italian Union Against Muscular Dystrophy (UILDM), Talamona, Italy; ¹ Fondazione Istituto Italiano di Tecnologia IIT, Center for Genomic Science, Milan, Italy

Limb girdle muscular dystrophy (LGMD) is a group of rare genetic diseases, including 31 different forms, inherited both in an autosomal dominant and recessive manner, and clinically characterized by a progressive involvement of limb musculature, proximal more than distal.

LGMD2E, also known as beta-sarcoglycanopathy, is an autosomal recessive dystrophy caused by mutations in the gene encoding beta-sarcoglycan, a cell plasma membrane that forms a tetrameric complex with other three types of sarcoglycans (alfa, gamma, delta).

Thanks to their genetic structure, formed by a few exons, sarcoglycanopathies in general may be suitable for the adenovirus-based gene therapy, and in fact a phase II clinical trial gene therapy for alfa -sarcoglycanopathy is on-going in the USA.

In 2013 the volunteer organization *Family Group of Beta-sarcoglycanopathy ONLUS (GFB ONLUS www.lgmd2e.org)* was founded aimed at: a) contacting the highest number of patients affected by LGMD and their families; b) collecting data and informations available on LGMD2E; c) stimulating both basic and clinical research. In the last two years the *GFB ONLUS* has involved 64 patients affected by all types of sarcoglycanopathies: 21 LGMD2E, 30 LGMD2D, 10 LGMD2C and 3 by a not well defined Sarcoglycanopathy.

Family Group of Beta-sarcoglycanopathy ONLUS is not a unique association; other six associations specifically dealing with other types of LGMDs are present: Coalition to care Calpain 3 (LGMD2A in the US), the Jain Foundation (LGMD2B in the US), Kurt + Peter Foundation (LGMD2C in the US), LGMD2D Foundation (in the US), Stichting Spierkracht (in the Netherlands) and LGMD2I Fund (in the US). In February 2014 five of these associations formed the “Consortium of LGMD Family Foundations”.

The *GFB ONLUS* also promotes scientific research on LGMD2E, by creating collaborations with researchers to organize both informative and scientific meetings and, eventually, supporting those who are interested in the study of this disease. In this light, *GFB ONLUS* organised on April 19th 2013 October in Milan the first scientific Meeting and on 15th-16th-17th October 2014 in Venice the second scientific meeting, which will allow a fruitful confrontation between national and international researches.

Friday, 16 October

3rd Session h. 10.30-11.55

Chairmen: C.P. Trevisan (Padova), M. Moggio (Milano),
G. Siciliano (Pisa)

Small molecule-based therapy for sarcoglycanopathy, a novel perspective

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D. Sandonà

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¹ *Department of Comparative Biomedicine and Food Science,*

² *Institute of Neuroscience, Padova, National Research Council of Italy*

Sarcoglycanopathy, the collective name of four types of Limb Girdle Muscular Dystrophy (LGMD 2C-2F), is a rare genetic disorder mainly affecting the proximal musculature. It is well known that defects in any one of the genes coding for α -, β -, δ - or γ -sarcoglycan (SG), the components of a complex essential for the sarcolemma integrity of striated muscles, lead to the severe reduction or even the complete loss of SG-complex. Most of the mutations associated to sarcoglycanopathy are missense mutations. We have proven that the primary event in these cases is the premature degradation of a folding-defective mutant and the secondary loss of the wild-type partners, operated by the Endoplasmic Reticulum-Associated Degradation system. Interestingly, the entire complex can be rescued at the proper cellular site by reducing the degradation rate of the mutated-SG, opening a new perspective for the therapy of this neglected disease.

We have designed two small molecule-based strategies aimed at either “save” mutants from degradation or “assist” mutants in the folding process.

The pharmacological inhibition of the E3 ligase HRD1, key element of the sarcoglycan degradative route, leads to the quantitative and functional rescue of the mutant both in a cell model and in primary myotubes derived from a patient suffering of LGMD2D.

Regarding the “protein assisting” strategy, we are testing several small molecules, known as protein-folding correctors, both in a cell model and in patient-derived primary myotubes. By helping α -SG mutants to reach a native/native-like conformation, these treatments preserve the mutant from degradation allowing the assembly into a functional complex that properly localizes at the plasma membrane.

Altogether our results constitute the proof of concept for the development of novel pharmacological therapies for sarcoglycanopathy.

DNAJB6 myopathy

M.B. Pasanisi, L. Maggi, S. Zanotti, S. Saredi,
F. Salerno, L. Morandi, M. Mora

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DNAJB6 gene mutations cause an autosomal dominant limb-girdle muscular dystrophy (LGMD1E). The disease usually manifests in adulthood and may be associated with distal muscle weakness. Muscle pathology is characterized by rimmed vacuoles and myofibrillar abnormalities. *DNAJB6* belongs to the J-proteins (Hsp40) family, a class of co-chaperones characterized by a J-domain, and participates in autophagic and proteasomal turnover of proteins. We present clinical, pathological and molecular findings in four unrelated patients. Two females manifested early onset (mean 8.5 years) with distal lower limb muscles weakness, followed several years later by proximal upper and lower limb involvement. Both patients became wheelchair bound (mean age 38.5 years). One of them developed respiratory failure and dysphagia after 50 years of age, now (at 55 years) she is on non-invasive ventilation and has a percutaneous endoscopic gastrostomy for nutrition. The other patient manifested a moderate respiratory involvement at the age of 40. A third female patient presented difficulty climbing stairs at 16 years of age, then she developed slowly progressive proximal weakness, with loss of ambulation after about 20 years from onset. Now, at 40 years, she refers occasional dysphagia, and her neurological evaluation shows both proximal and distal limb weakness. The fourth patient is a 56-year old male who began to complain of mild difficulty climbing stairs at the age of 36. At present he refers difficulty getting up from a low chair and climbing stairs, he has predominantly proximal lower limbs weakness. In all patients morphological evaluation showed some rimmed vacuoles with acid phosphatase positivity around them. Electromyographic study demonstrated a myopathic pattern along with spontaneous activity (fibrillation and positive sharp waves); CK values were normal or only slightly increased. In all patients muscle imaging, by CT or MRI, showed fatty infiltration of biceps femoris and adductor magnus with a relative preservation of gracilis in thighs, and involvement of medial gastrocnemius with preservation of soleus in legs, in agreement with findings reported by Sandell et al. Molecular analysis detected two novel *DNAJB6* mutations, and two known mutations, one of them recently reported by Jonson et al.

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

E. Gazzo, S. Baldassari, S. Assereto, C. Panicucci, C. Fiorillo, C. Minetti, E. Traggiai¹, F. Grassi², C. Bruno
Department of Neuroscience, Istituto "G. Gaslini", Genova, Italy; ¹ Novartis IRB, Basel, CH; ² IRB, Bellinzona, CH

Limb-girdle muscular dystrophy 2D (LGMD2D), caused by mutations in the gene encoding alpha-sarcoglycan (a-SG), is a rare disorder characterized by progressive weakness and degeneration of skeletal muscle. Pathological features of muscle biopsies from these patients show myofiber degeneration and necrosis, endomysial fibrosis, and reactive inflammatory response. In this scenario, extracellular ATP (eATP), a molecule released from the cytosol of dying cells, contributes to the initial phase of the immune response and later to the amplification of the inflammasome reaction. Excessive eATP causes protracted P2X receptors activation with alteration in muscle intracellular calcium homeostasis as well as recruitment of inflammatory cells.

Intriguingly, a-SG binds eATP and displays an ecto-ATPase activity, thus controlling eATP concentration at the surface of cells expressing P2X receptors and attenuating the magnitude and/or the duration of eATP-induced signals.

In order to evaluate the role of eATP in the *in vivo* inflammatory response and progression of the degenerative process associated to a-SG deficiency, we analyzed the consequences of P2X7 pharmacological inhibition in a-sarcoglycan (Sgca-null mice) muscle function and morphology and on molecular markers of innate and adaptive immune response.

For this purpose we treated a-SK null mice with periodate-oxidized ATP (oATP), a compound that irreversibly antagonizes P2X receptors and which has been shown to ameliorate the phenotype of animal models with different inflammatory diseases. We determined that pharmacological inhibition of P2X purinergic receptors improved muscular function and morphology in a-SG knock-out mice. The beneficial effect exerted by purinergic blockade was associated with a reduction of the number and area of the inflammatory infiltrates and to a decrease of muscle transcript levels of Il1 and Il6.

Histopathology of cardiomyopathy in a patient with α -sarcoglycanopathy

M. Meznaric, E. Kralj¹, C. Angelini², M. Fanin³
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The clinical phenotype of sarcoglycanopathies, caused by mutations in α -, β -, γ - or δ -sarcoglycan genes,

is characterised by the limb-girdle distribution of muscle weakness of variable severity and may include also cardiomyopathy. Frequency of cardiomyopathy varies among individual subtypes and has rarely been reported in α -sarcoglycanopathy.

We report on autopsy heart examination of a 36-year old male patient, homozygous for α -sarcoglycanopathy gene mutation in exon 3 (c. 229C>T, p.Arg77Cys), who died suddenly. He had a Duchenne-like muscular dystrophy.

Characteristic was the outer – subepicardial localisation of focal lesions in the free left ventricular wall, most pronounced in the posterobasal segment of the left ventricle. Histopathological changes consisted of myocardial degeneration without inflammation, increased variability of cardiomyocytes diameters, fibrosis and fatty replacement of the myocardium. Dilated cardiomyopathy was not developed at the time of death which is in agreement with slow progression of cardiomyopathy in α -sarcoglycanopathy.

Follow-up and clinical study in Beta-sarcoglycans

C. Semplicini
University of Padova, Department of Neurosciences, Padova, Italy

Not arrived

Limb girdle muscle dystrophies and exercise

G. Siciliano, C. Simoncini, S. Giannotti¹, G. Ricci
Department of Clinical and Experimental Medicine, Neurological Clinic; ¹ Orthopaedic Clinic, University of Pisa, Italy

In limb-girdle muscular dystrophies (LGMD) different genetic mutations are responsible, by distinct pathogenic mechanisms, of muscle fibers degeneration and strength loss. Depending on that, 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 hamper 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 LG-

MD2I, 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.

4th Session h. 15.00-16.30

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

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Limb girdle muscular dystrophies (LGMD) are highly heterogeneous disorders characterized by predominant limb girdle weakness. Molecular analysis and clinical-genetic correlations are fundamental for genetic counselling, definition of natural history and insight into pathogenesis.

To verify the basic requirements for an national LGMD registry, we collected detailed clinical, biochemical, histological and molecular data of 466 Italian LGMD patients, belonging to 8 neuromuscular Italian centres, listed at the end of this summary.

Among them 309 patients are molecularly defined,

111 (24%) are still un-diagnosed and 46 (10%) carry heterozygous mutations in genes determining autosomal recessive forms. Relative frequency was as follows: 5.5% LGMD1B, 11% LGMD1C, 25.2% LGMD2A, 27% LGMD2B, 9.2% LGMD2I, 9.1% LGMD2D, 6% LGMD2E, 4% LGMD2C, 2.1% LGMD2L, 0.3% LGMD2F, LGMD2R (0,3%) and LGMD2S (0,3%). Onset spans from the first decade to adulthood; LGMD2E being the most precocious (6.2 ± 5.3 years) and LGMD2L the latest (36.6 ± 7.1 years). Creatine-kinase values were generally increased, especially in sarcoglycanopathies, LGMD2B, LGMD1C. Cardiomyopathy was more frequent in LGMD1B (100%), LGMD2E (47%) and LGMD2I (50%) and restrictive pulmonary involvement in LGMD2I (53%) and LGMD2E (47%). 30% of patients was wheelchair-bound.

Overall this study defined the relative frequency of Italian LGMD and improved the knowledge about clinical, morphological and molecular spectrum as far as their natural history. Furthermore the study of undiagnosed patients will potentially lead to identification of new LGMD causative genes.

LGMD: functional/electrophysiological outcomes

C. Borsato
 Not arrived

Saturday, 17 October 4th Session h. 10.00-14.00

Chairman: C. Angelini (Padova)

NGS and LGMD

V. Nigro^{1,2}

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See M. Savarese et al.

Discussion by groups of Horizon 2020 Projects

L. Drigo (Padua)

Discussion and closing remarks

C. Angelini (Padova)