PROCEEDINGS of the XII CONGRESS OF MEDITERRANEAN SOCIETY OF MYOLOGY

Naples, Italy

May 18-20, 2015
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. Andrès: 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. 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
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 functionally 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 SMNΔ7 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.

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 characterized 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 deramatomyositis (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 (TNFα, LTβ, BAFF), Interferons (IFNα/β/γ), 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-TNFα treatment in IM are conflicting and new onset DM/PM has been described after administration of anti-TNFα agents to treat other diseases. Treatment with anti-IFNα 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**

**2.0.1 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 intramyofiber vacuoles and multigranulite aggregates, and endomyal fibronuclear-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 bioply-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.

**2.0.2 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 naturally 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.

**2.0.3 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. Iglesias1, C. Jou3, J.-J. Martin3, J. Weiss4, Sandrine Herbelet1, J. De Bleecker 1

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 (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
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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. Savarese1,2, G. Di Fruscio1,2, A. Torella1,2, M. Mutarelli2, C. Bruno3, C. Fiorillo3, M. Fanin4, L. Ruggiero5, A. Garofalo1,2, T. Giugliano1,2, M. Dionisi5, O. De Concilio1,2, F. Del Vecchio Blanco1,2, G. Piluso1,2, O. Musumeci6, G. Siciliano7, M. Mora8, L. Morandi9, 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. Poliziano18, V. Nigro1,2

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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**

3.0.1 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.

3.0.2 Huge variability in a huge gene: TTN

variants identified in a large NGS-resequencing project


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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 dystrophias (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 Myotónica 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 subepicardium 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 postoperative 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 an 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 stepforward for the treatment of these patients with otherwise no therapeutic option.

ORAL COMMUNICATIONS

4.0.1 Severe and early onset cardiomyopathy in females with Danon disease
Departments of Pathologya, Paediatricsc, Molecular and Clinical Medicineb, University of Gothenburg, Gothenburg, Sweden, Regional Molecular Genetics Laboratoryd, 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 myocardiut 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 cardiomyo-
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 autogluco-sylating 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 ubiquinated and labeled with sequestosome/p62 in both conditions. In spite of similar light and electron microscopic 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
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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 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
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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

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, Limbgirdle 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 TGFbeta. 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


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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 recently 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 morosin positive congenital muscular dystrophy, who presented at the age of 9 a first degree A-V block and died from a fatal supraventricular paroxysm 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


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Emery-Dreifuss muscular dystrophy (EDMD), is a skeletal myopathy characterized by progressive muscular weakness, joint contractures, and cardiac disease. LMNA gene, encoding lamin 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 multifaceted approach**

**INVITED LECTURES**

**6.1 Clinical aspects of mucopolysaccharidosis**

G. Andria

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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 successful 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 cranialacunia, 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 (platyspondyilia, 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**

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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
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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
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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

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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 ad 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 pharmaceutical treatment of neuromuscular diseases challenging the current dogma that limits to satellite cells the target of interventions toward stimulating endogenous regeneration of diseases 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

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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 ΔΨm 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
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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
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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 wheelchair 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
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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 BAG3 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
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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...
Fasciitis frequently accompanies myopathy in acute critical illness muscle wasting: evidence from qualitative ultrasound and muscle biopsy analysis


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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 than those 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) 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° ± 4.0 to 5.5° ± 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

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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 anamnisis 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 neurologic 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 cardiological involvement, revealed the presence of a new missense mutation c.290A>C in exon 1 (p.LYS97THR).
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Russian Neuromuscular Children’s Centre experience of Emery-Dreifuss muscular dystrophy

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 cardiological involvement were found.

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

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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.

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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-
opened 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

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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. Paroxysmal 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 paroxismal atrial fibrillation.