

INTRODUCTION TO THE 6th AIM CONGRESS

Since the first national symposium in Myology in Camogli in 2001, we have witnessed considerable advances in our understanding of muscle diseases. This sixth meeting organised in Rome by the Italian Association of Myology (AIM) in collaboration with the Association of Neuropathology, has become the premier forum for the presentation of new research in muscle disorders, presenting advances in the disease processes, that will undoubtedly lead to significant treatment advances in the nearby future.

The goal of the program Committee has been to attract plenary speakers of the highest calibre, who will give presentations on a range of topical themes. There is also a common teaching course that will undoubtedly lead to productive sharing experiences. Various research groups are contributing with over sixty oral communications muscle club cases and posters. Their abstracts are highlighting new insights into diagnostic procedures and new approaches for improved treatments and management strategy.

This 6th AIM meeting is showing the growth of the national society and of the collaboration networks in this research area.

Corrado Angelini, MD

President Italian Association of MyologyProgram

Joint Meetings

Centro Congressi Holiday Inn Rome West – Via Aurelia Km 8.4 (Via Bogliasco, s.n.c.)
Roma 24 – 27 Maggio 2006

42° Congresso

Associazione Italiana di Neuropatologia (AINP)

32° Congresso

Associazione Italiana Ricerca Invecchiamento Cerebrale (AIRIC)

6° Congresso

Associazione Italiana di Miologia (AIM)

Programma

Mercoledì 24 MAGGIO

Aula Olimpia 1

12,30 Registrazione dei partecipanti

MIOLOGIA

14,00 COMUNICAZIONI LIBERE

VASCULAR ENDOTHELIAL GROWTH FACTOR GENE TRANSFER USING ADENO-ASSOCIATED VIRAL VECTORS STIMULATES SKELETAL MUSCLE REGENERATION AND ENHANCES MUSCLE FUNCTION IN *MDX* MICE

S. Messina, M. Aguenouz, A. Bitto, A. Migliorato, M. Giacca, F. Squadrito, G. Vita

FUNCTIONAL AND STRUCTURAL MODIFICATIONS OF DYSTROPHIC MUSCLES AFTER AUTOLOGOUS TRANSPLANTATION OF MUSCLE-DERIVED AC133+ STEM CELLS

Priori, MG. D'Angelo, R. Bottinelli, G. Cossu, N. Bresolin, L. Garcia, Y. Torrente

VARIABLE ALTERATION OF ECM COMPONENTS IN DIFFERENT MUSCULAR DYSTROPHIES

S. Zanotti, C. Cappelletti, P. Bernasconi, C. Angelini, A. Prella, L. Moranti, M. Mora

REFINING GENE EXPRESSION DEREGLATIONS IN DMD MUSCLE

M. Pescatori, E. Bertini, C. Minetti, C. Bruno, G. Tasca, M. Mirabella, E. Mercuri, P. Tonali, E. Ricci

UNUSUAL DYSTROPHIN DELETIONS ASSOCIATED WITH BECKER MUSCULAR DYSTROPHY (BMD)

G. Galluzzi, J. Mela, L. Colantoni, R. Verardo, S. Servidei

BONE METABOLISM ALTERATIONS IN CHILDREN WITH DUCHENNE MUSCULAR DYSTROPHY: RESPONSE TO A FIRST-LINE TREATMENT

S. Saraifoger, A. Dubini, P. Dassi, L. Morandi, M.L. Bianchi

MUSCULAR DYSTROPHIES DUE TO GLYCOSYLATION DEFECTS IN A GROUP OF ITALIAN PATIENTS

A. Ruggieri, I. Moroni, S. Saredi, C. Di Blasi, L. Farina, C. Pantaleoni, G. Uziel, M. Mora, L. Morandi

EXPANDING THE CLINICAL SPECTRUM OF *POMT1* PHENOTYPE

A. D'Amico, A. Tessa, C. Bruno, S. Petrini, R. Biancheri, M. Pane, M. Pedemonte, E. Ricci, A. Falace, A. Rossi, E. Mercuri, F.M. Santorelli, E. Bertini

EFFECT ON COLLAGEN VI EXTRA-CELLULAR ASSEMBLY OF COL6A1 AND COL6A2 C-TERMINAL MUTATIONS IN ULLRICH CONGENITAL MUSCULAR DYSTROPHY

P. Sabatelli, S. Ravaioli, G. Pepe, B. Giusti, L. Lucarini, L. Merlini, E. Bertini, S. Petrini, E. Mattioli, C. Bonneman, S. Squarizoni, and N.M.Maraldi

REDUCED NITRIC OXIDE PRODUCTION IN CRITICAL ILLNESS MYOPATHY

M. Capasso, A. Pandolfi, M. Pace, P. Di Tomo, M. Ragno A. Uncini, A. Di Muzio

16,15 *Coffee break*

16,30 Lettura magistrale: RUOLO DELLE CELLULE STAMINALI NELLA TERAPIA DELLE DISTROFIE MUSCOLARI (G. Cossu, Roma)

17,30 MUSCLE CLUB

19,00 INAUGURAZIONE DELLA RIUNIONE CONGIUNTA

19,15 Lettura Magistrale: LA NEUROLOGIA ITALIANA TRA PASSATO E FUTURO (D. Schiffer, Torino)

20,00 *Cocktail di benvenuto*

Giovedì 25 MAGGIO

Aula Olimpia 1-2

CORSO DI AGGIORNAMENTO

Infiammazione ed Autoimmunità nelle Malattie Neurologiche

8,30 IMMUNITÀ UMORALE E CELLULARE NELLE MALATTIE DEMIELINIZZANTI DEL SISTEMA NERVOSO CENTRALE E PERIFERICO (L. Battistini, Roma)

9,15 MULTIPLE SCLEROSIS PATHOLOGY: EVOLUTION OF PATHOGENIC CONCEPT (H. Lassmann, Vienna)

10,00 INFIAMMAZIONE NELLE MALATTIE DA PRIONI (F. Tagliavini, Milano)

10,45 *Coffee break*

11,00 MECHANISMS OF CELL DEATH IN NEURODEGENERATIVE DISEASES AND THE ROLE OF MICROGLIAL INFLAMMATION (M. Graeber, Londra)

11,45 NUOVE ACQUISIZIONI SULLA PATOGENESI DELLE NEUROPATIE AUTOIMMUNI (N. Rizzuto, Verona)

12,30 IMMUNITÀ UMORALE E CELLULARE NELLE POLIMIOSITI E DERMATOMIOSITI (R. Mantegazza, Milano)

13,15 *Pausa Pranzo*

Aula Olimpia 1-2

CORSO TEORICO-PRATICO

Tissue and Brain Banking

14,30	BIO-BANCHE: ASPETTI REGOLATORI ED ESPERIENZE ITALIANE ED EUROPEE (L.G. Spagnoli, Roma)
15,00	CONSENSO INFORMATO ALLA DONAZIONE DI ORGANI E TESSUTI A FINI SCIENTIFICI E RUOLO DELLE ASSOCIAZIONI NELLA PROMOZIONE DELLA CULTURA DELLA DONAZIONE (GL. Forloni)
15,15	BRAIN BANKING AND BRAIN NETWORKS FOR DEVELOPMENT OF NEUROSCIENCE RESEARCH (H. Kretschmar, Monaco di Baviera)
16,00	<i>Coffee break</i>
16,15	TECNICHE DI PRELIEVO E CONSERVAZIONE DEL TESSUTO NERVOSO (G. Giaccone, Milano)
16,45	BIO-BANCHE DI TESSUTO MUSCOLARE: UN'ESPERIENZA ITALIANA (C. Angelini, Padova)
17,00	CONTROLLI DI QUALITÀ DELLE BIO-BANCHE
17,10	PROTEZIONE DEI DATI BIOLOGICI PERSONALI (C. Venturini, Roma)
17,20	FATTIBILITÀ DI STRUTTURE DI BRAIN-BANKING IN ITALIA (O. Bugiani, Milano)
17,30	"CONCLUDING REMARKS" (L. Santi, Genova)
18,00	ASSEMBLEA SOCI AIM
21,00	<i>Cena sociale</i>

Venerdì 26 MAGGIO

Aula Olimpia 1

MIOLOGIA

8,30	COMUNICAZIONI LIBERE
	EPIDEMIOLOGY OF FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY: ORIGINAL DATA AND REVIEW OF THE LITERATURE E. Pastorello, M.L. Mostacciuolo, G. Vazza, M. Armani, C. Angelini, G. Tomelleri, P. Tonin, G. Galluzzi, R.G. Tupler, C.P. Trevisan
	RAGE-NF-KB PATHWAY ACTIVATION IN RESPONSE TO OXIDATIVE STRESS IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY C. Rodolico, V. Macaione, M. Aguenouz, A. Mazzeo, A. Patti, E. Cannistraci, R. M. Di Giorgio, G. De Luca, G. Vita
	A NOVEL GNE MUTATION CAUSES FAMILIAL RECESSIVE MYOPATHY WITHOUT INCLUSION BODIES R. Del Bo, M. Guglieri, M.G. D'Angelo, S. Ghezzi, F. Magri, L. Napoli, A. Prella, M. Moggio, M. Mora, N. Bresolin, G.P. Comi
	MUTATIONAL ANALYSIS OF DYSFERLIN GENE IN ITALIAN PATIENTS WITH MIYOSHI MYOPATHY (MM) J. Mela, G. Galluzzi, R. DiGiacopo, M. Catteruccia, R. Verardo, S. Servidei
	NOVEL MUTATIONS IN LAMIN A/C GENE V.M. Ventriglia, Giulio Piluso, Francesca D'Amico, Luisa Politano, Vincenzo Nigro
	LIMB-GIRDLE MUSCULAR DYSTROPHIES: CLINICAL FEATURES AND GENETIC FREQUENCY IN A LARGE ITALIAN POPULATION M. Guglieri, F. Magri, R. Cagliani, M.G. D'Angelo, A. Prella, F. Fortunato, S. Lucchiari, S. Salani, R. Del Bo, S. Ghezzi, C. Zecca, C. Lamperti, L. Morandi, M. Mora, M. Moggio, N. Bresolin, G.P. Comi
	DOMINANT AND RECESSIVE INHERITANCE IN CAV3 DEFICIENCY M. Traverso, S. Stringara, S. Assereto, M. Pedemonte, R. Biancheri, S. Scapolan, E. Tonoli, F. Sotgia, M.P. Lisanti, E. Gazzero, R. Frulio, M.A. Donati, F. Zara, C. Minetti
	MRI STUDY OF LIMB GIRDLE MUSCULAR DYSTROPHIES AND INFLAMMATORY MYOPATHIES C. Borsato, R. Dal Borgo, R. Stramare, M. Fanin, E. Pegoraro, C. Angelini

10,30	<i>Coffee Break</i>
10,45	COMUNICAZIONI LIBERE
	RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF PHENYLBUTYRATE IN SPINAL MUSCULAR ATROPHY E. Mercuri, E. Bertini, S. Messina, A. D'Amico, A. Solari, C. Angelozzi, R. Battini, A. Berardinelli, P. Boffi, C. Bruno, C. Cini, F. Colitto, C. Minetti, T. Mongini, L. Morandi, G. Neri, S. Orcesi, M. Pane, M. Pelliccioni, A. Pini, F. D. Tiziano, M. Villanova, G. Vita, C. Brahe
	CORRELATION BETWEEN THE HAMMERSMITH FUNCTIONAL MOTOR SCALE AND SMN 2 COPY NUMBER IN A MULTICENTRIC STUDY E. Bertini, C. Angelozzi, S. Messina, A. D'Amico, R. Battini, A. Berardinelli, P. Boffi, C. Bruno, C. Cini, F. Colitto, M. Minetti, T. Mongini, L. Morandi, G. Neri, S. Orcesi, M. Pane, M. Pelliccioni, A. Pini, F. D. Tiziano, M. Villanova, G. Vita, E. Mercuri, C. Brahe
	MYOTONIC DYSTROPHY TYPE 1 RE-TESTED AFTER 10 YEARS: DOES SOMATIC INSTABILITY AMPLIFY CTG EXPANSION? V. Romeo, E. Bonifazi, G. Novelli, E. Pegoraro, C. Angelini
	MUSCLEBLIND-LIKE PROTEIN 1 (MBNL1): A NEW HISTOPATHOLOGICAL MARKER OF MYOTONIC DYSTROPHIES G. Meola, R. Cardani, G. Rotondo, V. Sansone, E. Mancinelli
	PROGRESSIVE EXTERNAL OPHTHALMOPLÉGIA (PEO) REWORKED M. Catteruccia, R. DiGiacopo, S. Chiatamone, G. DellaMarca, S. Servidei
	ASSOCIATION OF MYASTHENIA GRAVIS AND MITOCHONDRIAL MYOPATHY: DOUBLE TROUBLE OR PATHOGENICALLY CORRELATED DISEASES? A. Evoli, G. Minicuci, M. Catteruccia, R. Di Giacopo, S. Servidei
	CLINICAL AND GENETIC CHARACTERIZATION IN TWO FAMILIES WITH MUSCLE PHOSPHOFRUCTOKINASE DEFICIENCY O. Musumeci, C. Bruno, C. Rodolico, D. Cassandrini, M. Aguenouz, L. Serlenga, S. Zoccolella, A. Ciranni, A. Amati, G. Vita, A. Toscano.
	SERCA1 AND CALSEQUESTRIN STORAGE MYOPATHY: A NEW SURPLUS PROTEIN MYOPATHY G. Vattemi, L. Palmucci, P. Tonin, T. Mongini, M. Marini, L. Grigoli, R. L'Erario, N. Rizzato, G. Tomelleri
	THE ITALIAN CONTRIBUTION TO THE EUROBioBANK NETWORK: A SUCCESSFUL AND OPERATIONAL MODEL FOR SUPPORTING SCIENTIFIC EXCHANGE AND COOPERATION L. Politano, C. Angelini, M. Moggio, M. Mora

13,00 *Pausa pranzo*

14,00 PRESENTAZIONE POSTER

Aula Olimpia 1

16,00 NETWORK AIM

17,30 Chiusura Congresso AIM

Aula Olimpia 2

8,00 Discussione casi clinici

INVECCHIAMENTO E MALATTIE DEGENERATIVE ED EREDITARIE

8,30 COMUNICAZIONI LIBERE

10,30 *Coffee Break*

10,45 COMUNICAZIONI LIBERE

12,15 Lettura magistrale: CARATTERISTICHE NEUROPATOLOGICHE DELLE TSE ANIMALI (M. Caramelli, Torino)

13,00 *Pausa pranzo*

14,00 PRESENTAZIONE POSTER

Aula Olimpia 2

NEUROPATOLOGIA SPERIMENTALE

16,00 COMUNICAZIONI LIBERE

16,45 Lettura magistrale: LE HUNTINGTINE: UN APPROCCIO INTEGRATO ALLA PATOGENESI DELLA MALATTIA DI HUNTINGTON (E. Cattaneo, Milano)

17,30 *Coffee Break*

17,45 COMUNICAZIONI LIBERE

19,00 Assemblea Soci AINP; Riunione Direttivo AIRIC

Sabato 27 MAGGIO

Aula Olimpia 1

8,00 DISCUSSIONE CASI CLINICI

NEUROPATIE PERIFERICHE E MALATTIE INFIAMMATORIE

8,30-10,45 COMUNICAZIONI LIBERE

10,45 *Coffee break*

CORSO DI AGGIORNAMENTO
NEUROPATOLOGIA PEDIATRICA

11,00 Lettura Magistrale: "NEW CLASSIFICATION OF MALFORMATIONS OF CNS" (H. Sarnat, Calgary-Alberta, Canada)

11,45 HEMIMEGALENCEPHALY (L. Flores Sarnat, Calgary-Alberta, Canada)

12,10 SCLEROSI TUBEROSA (P. Curatolo, Roma)

12,35 SVILUPPO NORMALE E PATOLOGICO DEL CERVELLETTO (A. Simonati, Verona)

13,00 DETERMINANTI GENETICHE NELLE MALFORMAZIONI DELLA FOSSA CRANICA POSTERIORE (E. M. Valente, Roma)

ABSTRACTS

FUNCTIONAL AND STRUCTURAL MODIFICATIONS OF DYSTROPHIC MUSCLES AFTER AUTOLOGOUS TRANSPLANTATION OF MUSCLE-DERIVED AC133+ STEM CELLS

M. Belicchi (1), C. Marchesi (1), R. Benchaoui (1,2), G. D'Antona (3), G. Fagioli (1), C. Lamperti (1), A. Prella (1), M. Guglieri (1), M. Moggi, A. Priori (1), MG. D'Angelo (1), R. Bottinelli (3), G. Cossu (4), N. Bresolin (1), L. Garcia (2), Y. Torrente (1)

(1) *Fondazione IRCCS Ospedale Maggiore Policlinico of Milan, Department of Neurological Sciences, Dino Ferrari Center, University of Milan, Italy;* (2) *G         & CNRS UMR 8115, Evry, France;* (3) *Department of Experimental Medicine, University of Pavia, Human Physiology unit, Pavia, Italy;* (4) *Stem Cell Research Institute, San Raffaele Hospital, Milan, Italy*

We recently reported that human circulating AC133+ progenitor cells can be induced to differentiate into skeletal muscles of scid-mdx dystrophic mice. However, in blood specimens of dystrophic patients there are low numbers of identifiable AC133+ stem cells that are difficult to be expanded in vitro. Here, we show that autologous transplantation of muscle-derived AC133+ cells in Duchenne muscles is safety and feasible. No local or systemic side effects were observed in treated DMD patients included in a randomised double blinded phase I study. Surprisingly, two treated patients had an increased ratio of capillary per muscle fibers with a switch from slow to fast myosin+ myofibers and partial recovery of some contractility force as showed by functional studies. We thus extended this clinical observations combining gene and stem cell treatment by the genetic engineering of AC133+ stem cells using exon-skipping approach which rescue the dystrophin expression. In these experiments the intramuscular and intra-arterial transplantation of engineered DMD stem cells caused a significant amelioration of skeletal muscle structure and function when delivered to scid-mdx dystrophic mice. We speculate that transplantation of DMD engineered AC133 positive stem cells could represent a future treatment for Duchenne muscular dystrophy.

IDIOPATHIC INFLAMMATORY MYOPATHIES: TLR4 IS DIFFERENTIALLY EXPRESSED ON MUSCLE TISSUE

P. Bernasconi, C. Cappelletti, F. Baggi, P. Confalonieri, E. Mariani, S. Saredi, M. Mora, L. Morandi, M. Mirabella (2), Giacomo P. Comi (3), Renato Mantegazza (1)

(1) *U.O. Neurologia IV, Istituto Nazionale Neurologico Carlo Besta, Milan;* (2) *Dipartimento di Neuroscienze, Universit   Cattolica Rome;* (3) *Dipartimento di Scienze Neurologiche, Universit   degli Studi di Milano, Milan*
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Purpose of the study was to investigate the role of innate immunity in the pathogenesis of the major forms of idiopathic inflammatory myopathies (IIM): dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM), an heterogeneous group of inflammatory myopathies characterized by muscle cell infiltrations and specific alterations of muscle fibers. By real-time PCR, immunohistochemistry and immunoblotting we studied the expression of TLRs 1-10 in 24 IM muscle biopsies (8 DM, 9 PM and 7 IBM) and in 9 control muscles. We found a higher expression level of TLR4 in IM muscles than in controls (DM 2.769 $\pm$ 1.283-fold; PM 2.826 $\pm$ 1.927-fold; IBM 5.943 $\pm$ 3.557-fold). Using an antibody which detects TLR4 intracellularly, TLR4 was detected in infiltrating cells surrounding single muscle fibers and in some muscle fibers, close or not to the infiltrate. No TLR4+ signal was detected in controls. Using an antibody recognizing TLR4 on cell surface, in PM/IBM the invading cells were TLR4+, while in DM the positive signal was detected only on few infiltrating cells; in all IM muscle fibers the signal was undetectable. These observations suggest a possible involvement of innate immunity in the inflammatory processes observed in IM muscles, in particular in PM and IBM. This work was granted by Italian Ministry of Health (RF132 to P.B.).

CORRELATION BETWEEN THE HAMMERSMITH FUNCTIONAL MOTOR SCALE AND SMN 2 COPY NUMBER IN A MULTICENTRIC STUDY

E. Bertini (1), C. Angelozzi (2), S. Messina (9), A. D'Amico (1), R. Battini (3), A. Berardinelli (4), P. Boffi (5), C. Bruno (6), C. Cini (3), F. Colitto (2), M. Minetti (6), T. Mongini (5), L. Morandi (7), G. Neri (2), S. Orcesi (4), M. Pane (2), M. Pelliccioni (2), A. Pini (10), F. D. Tiziano (2), M. Villanova (8), G. Vita (9), E. Mercuri (2), C. Brahe (2)

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(7) Division of Neuromuscular Diseases, National Neurological Institute C. Besta, Milano, Italy;

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(9) Neurological Institute, University of Messina, Italy;

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Spinal muscular atrophy (SMA) is a common autosomal recessive disorder caused by depletion of SMN (survival motor neuron) protein due to homozygous absence of the SMN 1 gene. SMN 2, a nearly identical copy gene of SMN 1, produces approximately 10% full-length SMN protein. It has been reported that an overall correlation exists between the forms of SMA and the number of SMN2 copies which progressively reduce in SMA type 3, 2 and 1. It has also been reported however that the number of SMN 2 copies within each form is variable and that it cannot always predict the severity of the phenotype in individual cases. The aim of this study has been to evaluate the number of SMN 2 copies in a large cohort of Italian non ambulant children with SMA 2 in an attempt to establish a possible correlation with the level of functional abilities assessed by the Hammersmith functional motor scale.

Seventy children with type 2 SMA were included in the study. Twenty-five patients had 2 copies of SMN2 gene, 42 had 3 and 3 patients had 4 copies. The functional scores ranged between 0 and 34 (mean $\pm$ SD; 7.48 ± 7.54) in the patients with 2 copies, between 1 and 34 (14.14 ± 9.98) in the patients with 3 copies and between 10 and 31 (21.30 ± 10.60). Although patients with higher number of SMN2 copies had overall better functional abilities, it was not always possible to predict the clinical phenotype and the prognosis on the basis of the number of copies in individual cases. Further studies are needed to better elucidate the role of other factors such as modifying genes and external factors in determining clinical severity.

THE 9-BP DELETION IN REGION V OF MTDNA: A NEW POSSIBLE CAUSE OF SENSORINEURAL HEARING LOSS?

E. Borgione, S.A. Musumeci, G. Rizzo, F. Castello, M. Elia, G. Calabrese, C. Scuderi

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A deletion of one of the two copies of the intergenic 9-bp repeat sequence (CCCCCTCTA), located between the cytochrome oxidase II and lysine tRNA genes of mitochondrial DNA, has previously been used as a polymorphic anthropological marker (MIC9D) for people of east Asian origin, and - to a lesser extent - in Oceanian and African populations. Although this polymorphism is common in Africa and Asia, it has not been reported in Northern Europe. We report the presence of the 9-bp deletion, identified by RFLP and confirmed by sequence analysis, in homoplasmies in lymphocytes of four patients (1 proband and his mother, two other unrelated females) with sensorineural hearing loss and variable neurological signs such as mental retardation, epilepsy, retinitis pigmentosa and myopathy. This deletion was absent in 100 controls.

Although additional evidence is needed, our results suggest that the MIC9D polymorphism may play a pathogenetic role in some ethnicities.

MRI STUDY OF LIMB GIRDLE MUSCULAR DYSTROPHIES AND INFLAMMATORY MYOPATHIES

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(2) Department of Radiology, University of Padua, Italy

Objective: To determine whether MR muscle imaging might reveal early and specific patterns in limb-girdle dystrophies (LGMDs) and inflammatory myopathies (IMs).

Methods: Patients with a defined molecular diagnosis of LGMD2A and 2B and patients with histopathological diagnosis of polymyositis were included. Muscle involvement was studied by MRI: fibro-fatty replacement was scored on T1 sequences by the Mercuri score, muscle oedema was evaluated on STIR sequences by a semiquantitative score. Both scores were compared with functional GSGC score and the muscle strength MRC score.

Results: There was a linear correlation between muscle strength and functional performance. The fibro-adipose substitution was in linear correlation with functional performance and muscle strength. In LGMDs patients, T1 sequences revealed that fibro-fatty replacement involved particularly the posterior compartment of limbs, while STIR sequences revealed muscle oedema in the anterior compartments. Patients affected by polymyositis presented altered signal in T1 and STIR sequences in anterior and posterior compartments of limbs.

Conclusions: MR muscle imaging (T1 and STIR sequences) provides a non invasive diagnostic tool in LGMDs and IMs and may be useful in serial follow up evaluation in myopathic patients. Muscle oedema on STIR sequences seems to be more closely correlated with early pathogenetic

events both in LGMDs and IMs, but anterior compartment is more selectively involved in LGMDs.

NEPRILYSIN PARTICIPATES IN SKELETAL MUSCLE REGENERATION AND IS ACCUMULATED IN ABNORMAL MUSCLE FIBERS OF INCLUSION BODY MYOSITIS

A. Broccolini, T. Gidaro, R. Morosetti, C. Gliubizzi, T. Servirei, M. Pescatori, P. A. Tonali, E. Ricci, M. Mirabella

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Neprilysin (NEP, EP24.11), a metallopeptidase originally shown to modulate signaling events by degrading small regulatory peptides, is also an amyloid- β (Ab) degrading enzyme. We investigated a possible role of NEP in inclusion body myositis (IBM) and other acquired and hereditary muscle disorders, and found that in all myopathies NEP expression was directly associated with the degree of muscle fiber regeneration. In IBM muscle, NEP protein was also strongly accumulated in Ab-bearing abnormal fibers. In vitro, during the experimental differentiation of myoblasts, NEP protein expression was regulated at the post-transcriptional level with a rapid increase in the early stage of myoblasts differentiation followed by a gradual reduction thereafter, in coincidence with the progression of the myogenic program. Treatment of differentiating muscle cells with the NEP inhibitor DL-thiorphan resulted in impaired differentiation that was associated with abnormal regulation of Akt and ERK1/2 activation. Therefore, NEP may play an important role during muscle cells differentiation, possibly through the regulation, either directly or indirectly, of the IGF-I-driven myogenic program. In IBM muscle increased NEP may be instrumental in a) reducing the Ab accumulation in vulnerable fibers, and b) promoting a repair/regenerative attempt of muscle fibers possibly through the modulation of IGF-I-dependent pathways.

REDUCED NITRIC OXIDE PRODUCTION IN CRITICAL ILLNESS MYOPATHY

M. Capasso, A. Pandolfi, M. Pace, P. Di Tomo, M. Ragno, A. Uncini, A. Di Muzio

Chieti, Ascoli Piceno

Critical illness myopathy (CIM) is associated with sepsis, multiorgan failure, steroids and neuroblocking agents. Increased inducible NO synthase (NOS2) expression and peroxynitrite generation from NO and superoxide have been reported to possibly induce muscle dysfunction in septic patients. On the other hand, sepsis reduces constitutive nitric oxide synthase (NOS1) muscular expression in an animal model.

We investigated NOSs expression and peroxynitrite production in patients with sepsis and CIM.

Protein nitrotyrosination, a marker of peroxynitrite, was detected through immunohistochemistry and quantified through ELISA. Immunohistochemistry and western blot were employed to localize and quantify NOS1, NOS2 and NOS3.

We studied three patients and seven normal controls. Two patients with inclusion body myositis and muscle sections treated with a solution inducing nitrotyrosine were studied as positive controls. Specificity of nitrotyrosine staining was verified by pre-treating sections with a solution reducing nitrotyrosine to aminotyrosine.

In all patients we found no staining for nitrotyrosine, markedly reduced or no staining for NOS1 and normal expression of NOS2 and NOS3. Statistically significant reduction of nitrotyrosine and NOS1 was found in patients compared to controls.

NO regulates many muscle functions including resting potential and excitation-contraction coupling. Our findings suggest that reduced NO production and not peroxynitrite generation may occur in muscles of patients with sepsis and CIM. If NO deficiency also has a pathogenic role in the myopathy remains to be determined.

STRATEGIES OF OXIDATIVE STRESS REDUCTION IN MITOCHONDRIAL MYOPATHIES

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In mitochondrial myopathies (MM) OXPHOS genome mutations generate impairment of respiratory chain, a key event to imbalance intracellular ATP production and trigger apoptotic pathway. In this context, metabolically deranged mitochondria are a potent source of reactive oxygen species (ROS), a process targetable by putative effective therapies. Aim of the study has been to analyze the exercise-related circulating levels of oxidative stress markers of 10 MM patients in relation to a 10 week aerobic training program and to 30 day administration of a cysteine donor stimulator of endogenous glutathione synthesis. Exercise was performed by a step by step cicloergometer intermittent incremental powerload protocol till 70% of maximal contraction power output.

Compared to 7 matched controls, MM patients showed significantly ($p < 0.05$) increased levels of oxidative stress products, such as lipoperoxides, advanced oxidation proteins products, and reduced levels of ferric reducing ability. Aerobic training was able to significantly reduce ($p < 0.05$) exercise related ROS production, this being modulated by the antioxidant dietary regime utilized. Our results confirm, in vivo, the pathogenic role of oxidative stress in mitochondrial diseases and indicate possible strategies to revert the deleterious effects of respiratory chain deficiency in these diseases.

PROGRESSIVE EXTERNAL OPHTHALMOPLÉGIA (PEO) REWORKED

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PEO phenotype is the best example of the genetic heterogeneity of mitochondrial disorders. We review 90 cases in which PEO was the clinical relevant aspect, excluding complex mitochondrial encephalomyopathies. All patients had un-

equivocal mitochondrial abnormalities on muscle biopsies. Mitochondrial DNA analysis demonstrated 54% single deletions, 18% multiple deletion, 7% A3243G-MELAS and 3% A8344G-MERRF mutations all with positive family history only for PEO, 4% rare mutations. In 14% of patients, sporadic cases mostly with mild clinical manifestations, no mtDNA changes were identified. Age of onset was between 5 and 70 years. The most commonly associated symptom was neurosensory hearing loss (83%). Neuropathy was present in 10% of the cases. Cardiac abnormalities were mainly manifested with minor clinical signs, except in one patient that needed PM implantation, and one patient with severe hypertrophic cardiomyopathy. Even though in absence of clinical signs of CNS involvement, cerebral/ cerebellar atrophy was present in 23% of the cases and white matter abnormalities in 20%. EEG was abnormal in 19% and did not correlated with MRI findings. 50% of the patients presented slight, subclinical sleep-related breathing abnormalities, mostly of obstructive type and six patients had severe sleep apnoeas that required CPAP therapy. Three patients died for acute respiratory failure. PEO is not always a benign disease, and the most life threatening aspect is the presence of respiratory involvement and sleep breathing disorders that requires careful monitoring.

ULTRASTRUCTURAL APPROACH TO MOLECULARLY DEFINED FKRP-RELATED MUSCULAR DYSTROPHY

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MDC1C AND LGMD2I are two allelic conditions due to mutations in Fukutin-related protein gene, FKRP, encoding for a putative glycosyltransferase involved in α -dystroglycan processing. Defects in the dystroglycan-dystrophin complex play a crucial role in both basement membrane organization and link with intracellular actin. FKRP locates possibly in a sub-compartment of the ER closely associated to the nuclear envelope in normal and regenerating muscle. The retention of mutant FKRP in the ER along with a reduced enzymatic function of the protein have recently been suggested as a possible mechanism of disease in MDC1C and LGMD2I. We examined at ultrastructural level four muscle biopsies from patients in whom a LGMD2I was previously molecularly diagnosed (by immunohistochemistry, Western blot and DNA molecular assay) to define the relationship between molecular pathogenetic mechanisms and submicroscopical alterations. In all FKRP-mutated patients muscle fibers showed: focally thinning of basal lamina, swollen ER cisternae with membrane re-arrangement and numerous both intermyofibrillary and subsarcolemmal lipid vacuoles sometimes associated with mitochondrial aggregates. Our data suggest that the observed ultrastructural alterations may be related to a malfunctioning FKRP. The presence of lipid vacuoles may be interpreted as a secondary pathological marker related to glycoprotein dysfunction.

GENETIC HETEROGENEITY OF ITALIAN MEB

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The congenital muscular dystrophies (CMD) with glycosylation defects are inherited disorders linked to six proven or putative glycosyltransferase genes, including POMT1, POMT2, POMGnT1, fukutin, fukutin-related protein and LARGE. CMD can be associated with different phenotypes ranging from Walker-Warburg syndrome (WWS) to limb girdle muscle dystrophies. Muscle-eye-brain phenotype (MEB) is characterised by structural brain abnormalities, eye involvement and mental retardation and is caused by mutations in POMGnT1. Villanova et al. reported a peculiar milder phenotype variant overlapping with MEB in a series of Italian patients. This phenotype –subsequently termed 'Italian-MEB'–, is characterised by hypertrophy of the legs, macroglossia, microcephaly, mental retardation and cerebellar hypoplasia. Two of these reported patients presented severe psychomotor retardation, floppiness and difficulties in swallowing from birth and brain MRI showed mega cisterna magna with vermis hypoplasia. Muscle biopsies demonstrated dystrophic features with reduced expression of merosin and α -dystroglycan. In both POMGnT1 mutations were excluded. In one case we identified mutations in POMT2. The girl, at the age of 11 years, is unable to sit and had tracheotomy and gastrostomy. POMT1 mutations were found in a second patient, who died at the age of 3 years for acute chest infection. Both these genes have been associated with WWS, and POMT1 mutations have also been identified in patients with LGMD and mental retardation. We have considered these genes as plausible candidates for other phenotypes related to α -dystroglycanopathies. Our results further confirm the genetic heterogeneity of α -dystroglycanopathies demonstrating that a specific phenotype can be associated with mutations in different genes.

EXPANDING THE CLINICAL SPECTRUM OF POMT1 PHENOTYPE

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The congenital muscular dystrophies (CMD) with glycosylation defects are a heterogeneous group of inherited disorders often involving brain, eyes and muscle, characterized by an abnormally glycosylated α -dystroglycan (α -DG) and mutations in proven or putative glycosyltransferases. The complex heterogeneity of CMD with reduced α -DG is both genetic and

clinical. Mutations in the same glycosyltransferase gene can be associated with different clinical phenotypes and patients with the same clinical diagnosis may harbor mutations in different glycosyltransferases. Overlap between established nosographic entities is also possible. Mutations in POMT1 have originally been identified in patients with Walker-Walburg syndrome (WWS), $\frac{3}{4}$ the most severe form of the genetically-known CMD $\frac{3}{4}$, characterized by cobblestone lissencephaly, neuronal migration defects and other structural brain changes, eye abnormalities, and death in early infancy. More recently a subgroup of Turkish patients with limb girdle muscle dystrophy, mental retardation, microcephaly and normal MRI have been shown to carry a new POMT1 mutation (p.Ala200Pro), proposing further clinical heterogeneity. We report POMT1 mutations in three patients with a distinct CMD phenotype characterized by early onset, severe motor disability, microcephaly, and mental retardation not associated with structural brain changes on neuroimaging. Our findings expand the spectrum of POMT1-associated phenotypes.

CASE REPORT: SEPTIN GENE MUTATION IN A SPORADIC CASE OF HEREDITARY NEURALGIC AMYOTROPHY (HNA)

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Hereditary neuralgic amyotrophy (HNA) is an autosomal dominant recurrent neuropathy affecting the brachial plexus with muscle weakness and atrophy preceded by severe pain in the affected arm.

We report the case of a 44-year-old man admitted to our Division in December 2005, who complained of several recurrent episodes of sore throat followed by hoarse voice, pain, sensory disturbances and arms weakness. This episodes were mostly triggered by mild infection. The first episode, at the age of 19, was characterized by paresthesia, severe pain, followed by paresis, proximal muscular atrophy with winged scapula which involved his right shoulder. This resolved over the following 4 months. Similar recurrent episodes affected both arms. These attacks resulted in permanent sensory deficit, weakness, muscular atrophy with left winged scapula. Recurrent laryngeal nerve was involved during two attacks with complete recovery. Neurological examination revealed left proximal weakness, left periscapular hypotrophy and hypoesthesia, left medial forearm hypoesthesia, dysmorphic features such as hypotelorism and right palpebral ptosis. CSF analysis was normal. Intercritical EMG examination showed chronic neurogenic changes in trapezius and infraspinatus muscles of the left shoulder. Diagnosis of HNA was definitely established through DNA analysis: a new missense mutation in septin gene (SEPT9) on chromosome 17q25 was found. Conclusions: even sporadic cases matching HNA diagnostic criteria can be confirmed by molecular diagnosis.

A NOVEL GNE MUTATION CAUSES FAMILIAL RECESSIVE MYOPATHY WITHOUT INCLUSION BODIES

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Five brothers belonging to an Italian family were affected with a long lasting history of progressive distal and proximal muscle weakness.

Four males and one female born from non-consanguineous parents presented with distal lower limb weakness, followed by slowly progressive proximal upper and lower limb involvement, beginning from the ages of 25 to 40 years. CPK levels were moderately increased. EMG demonstrated a chronic neurogenic pattern in two patients, and a mixed myogenic/neurogenic pattern in one. Muscle biopsy showed unspecific myopathic findings in two brothers, with normal expression of the dystrophin-glycoprotein complex, dysferlin and calpain-3. Molecular analysis for SMN gene deletion, Calpain3 and FKRP gene mutation was negative.

Sequence analysis of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine-kinase (GNE) gene showed in all affected members a state of compound heterozygosity for two new GNE mutations located within the epimerase domain of the protein: a c.5A>G resulting in a Glu2Gly amino acid change, and a c.1105G>A resulting in a Gly351Ser change. based on the results of GNE gene analysis.

This autosomal recessive myopathy is due to mutations of GNE gene. The finding of an AR-hereditary Inclusion Body Myopathy without rimmed vacuoles and quadriceps sparing, suggests that the clinical pictures associated with GNE gene may be more variable than previously observed.

HYPOKALEMIC MYOPATHY SECONDARY TO ALDOSTERONOMA

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Primary hyperaldosteronism due to aldosteronoma is a rare cause of persistent hypokalemia but it has not been associated to hypokalemic myopathy. Muscle damage in hypokalemic myopathy is characterized by a vacuolar myopathy with necrotic fibres and in some cases tubular aggregates. We report a 40-year old woman who, since she was 30, suffered for hypertension. During anti-hypertensive treatment, she experienced numerous episodes characterized by myalgias, cramps, muscle weakness at four limbs and persistent severe exercise intolerance. Neurological examination showed proximal weakness, brisk tendon reflexes and fatigue to repetitive exercise. Laboratory investigations revealed a slightly elevated CK levels (253 U/L; n.v. < 200), low potassium levels (2.0 mmol/L; n.v. 3.5-5.2), high aldosterone levels with normal renin suggesting a primary hyperaldosteronism. EMG study showed a myopathic pattern. Muscle MRI demonstrated an

adipose substitution of proximal muscles at lower limbs. Sural glands MRI demonstrated a neoplasm of left sural gland. A vacuolar myopathy was present in the muscle biopsy with lipid storage; carnitine was normal in plasma, muscle and urines as well CPT II. After potassium supplementation she evidenced a clinical marked improvement. EMG and serum CPK levels normalized. A second muscle biopsy showed diffuse muscle regeneration and a reduction of lipid storage. Unilateral adrenalectomy revealed a right adrenocortical adenoma. After surgery, potassium, aldosterone levels normalized in few weeks. Our data support the secondary nature of a hypokalemic myopathy due to a rare evidence of a hyperaldosteronism secondary to a sural neoplasm. An early diagnosis of a sural disorder might prevent a severe myopathic involvement.

FAMILIAR PARTIAL LIPODYSTROPHY OF THE DUNNINGAN TYPE (FPLD) AND MYOPATHY DUE TO LAMIN A/C GENE (LMNA) G465D MUTATION

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Mutations in LMNA cause a variety of apparently unrelated disorders called laminopathies involving muscle (EDMD 2-3, LGMD1B), nerve (CMT2B1), heart (CMD1A), adipose tissue (FLPD) or determining progeria. The lipodystrophic and myopathic phenotypes are thought to be mutually exclusive due to different sites involved by the mutations; however, a few cases of association have been reported, mainly with the R482W FLPD common mutation.

We describe a 35-year-old woman affected by FPLD, mild myopathy and hypertensive cardiopathy. She had lipoatrophy of limbs and trunk, fat accumulation in the cervico-facial area, broad shoulders, short legs, thick hands with spindle-shaped fingers, android appearance, hirsutism, diffuse muscle hypertrophy and mild proximal weakness with easy fatigability. She suffered from hypertension, impaired glucose tolerance with insulin resistance and hypertriglyceridemia. The father and one sister were also affected.

CK were mildly elevated; muscle biopsy demonstrated aspecific myopathic changes, and reduction of calpain on WB. Genetic analysis revealed a missense mutation, G465D, in exon 8 that occurs in the globular C-terminal portion of the lamin A/C protein, reported only once in a typical FLPD. Lamins are nuclear envelope proteins that may have a role in the regulation of gene expression; alterations in the nuclear envelope may disrupt proteins interactions or modify the expression of genes involved both in striated muscle (i.e. calpain) and adipocyte function.

ASSOCIATION OF MYASTHENIA GRAVIS AND MITOCHONDRIAL MYOPATHY: DOUBLE TROUBLE OR PATHOGENICALLY CORRELATED DISEASES?

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The association of myasthenia gravis (MG) and mitochondrial myopathies (MM) has only occasionally been reported.

We describe five patients with MM and MG: three were antiMusk-positive (1, 2, 3), one anti-AchR-positive (4) and one seronegative (5). All had chronic PEO, dysphonia, dysphagia, proximal muscle weakness, and a various degree of respiratory dysfunction. Symptoms showed fluctuations, and partially responded to anticholinesterases, steroids and plasmapheresis or e.v. immunoglobulin. Muscle fatigability far exceeded muscle weakness. In all patients aerobic exercise test demonstrated precocious and abnormal increase of lactic acid. Exercise test was normal in 20 other MG patients, independently of the severity of the disease. EMG showed myopathic changes. Repetitive stimulation was positive in pt 2, while Single Fiber EMG was positive in all five patients. Muscle biopsies demonstrated marked mitochondrial abnormalities; in pt 4 and 5 biochemistry revealed dysfunction of respiratory chain enzymes and mitochondrial DNA analysis demonstrated the presence of the G8344A-MERRF mutation. Family history was positive in pt 2 and 5.

Although a casual association of the two diseases may not be excluded, this seems unlikely. Rather,

a) due to reduced energy supply, abnormal mitochondria may impair neuromuscular transmission directly or affecting presynaptic or postsynaptic organization of the neuromuscular junction and/or

b) abnormal mitochondria may induce the formation of antibodies that cross-react with AChR or Musk epitopes.

XP21 CONTIGUOUS GENE SYNDROME: A CASE REPORT

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Duchenne Muscular Dystrophy (DMD), glycerol kinase (GK) and congenital adrenal hypoplasia (AHC) are distinct loci separated by a few megabase on the short arm of chromosome X.

In particularly Xp21 contiguous gene syndrome is contemporaneously characterized by an association of dystrophinopathy and glycerol kinase complex deficiency. We present a neonatal case of total dystrophin deficiency with a hypogonadotropic hypogonadism.

After the metabolic onset the patient has been treated with cortisol therapy. At 9 months of age for the marked hypotonia and for elevated CK levels (10 times the normal) he performed a muscle biopsy that showed a marked fibers variations with inflammation signs.

The immunohistochemical study showed a total deficiency of all three dystrophin domains while the immunoreactions

for sarcoglycan complex were normal. The genetic study has revealed of a deletions involving RP11-339E16 and RP-593P4. The gene dystrophin gene performed by PCR showed a wild deletion involving several exons.

Mass spectrometry of the urine revealed a large increase in glycerol elimination which was quantified by enzymatic assay. It is interesting to report a neonatal onset of GK complex deficiency associated to Duchenne muscular dystrophy (DMD) for the rarity of condition.

SKIN INVOLVEMENT IN NEUROMUSCULAR DISORDERS: PEDIATRIC REPORTS

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The skin and the muscular skeletal tissues despite have a different embryological development (ectodermic for the first and mesodermic for the second) can represent a clinical expression of neuromuscular disorders (NMDs). In particularly skin alterations can be expressions of primitive or secondary muscular disorders. The purpose of this study is to evaluate retrospectively those patients with NMDs that have had in any phases of the diseases any kind of skin involvement (SI). In a period of time from January 2000 and February 2006 we have selected 14 patients with NMD and SI. The median age was 10.3 and the ratio female/male was 1.8. The NMDs has been diagnosed by electrophysiological study and needle muscle biopsy.

A summary of the results obtained showed the association NMD plus SI in 5 cases with a dermatomyositis (DM), in 3 cases congenital indifference to pain (CIP), in 2 cases with a Sjogren syndrome (SS) and, and in 1 case respectively with muscular polyarteritis nodosa (PN), Bethlem myopathy (BM), reflex sympathetic dystrophy (RSD) and scleroderma (SD).

The atypical clinical association between skin anomalies and muscle disorders include different pathologies and in the pediatric field the most frequent is the dermatomyositis.

Conversely we have not seen other NMDs that have an skin expression such as the laminopathies and epidermolysis bullosa simplex associated with muscular dystrophy.

A NEW MITOCHONDRIAL tRNA^{LEU(CUN)} TRANSITION CAUSES A FACIO-SCAPULO-PERONEAL SYNDROME

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Point mutations in mtDNA-encoded tRNA genes causing deficiencies of mitochondrial respiratory chain enzyme activities are a common cause of mitochondrial multisystem

disorders or isolated myopathies. While gene coding for tRNA^{Leu(UUR)} is a "hot spot" for pathogenic point mutations, tRNA^{Leu(CUN)} gene is less common site of mutational changes. We studied a 50-year-old woman affected with progressive generalized muscle weakness and exercise intolerance from 35 years. Neurological examination showed weakness and muscle atrophy with a facio-scapulo-peroneal distribution. No clinical or instrumental evidence of multisystem involvement was present.

On muscle biopsy numerous ragged red fibers, COX negative fibers and fibers with subsarcolemmal accumulation of mitochondria were found. mtDNA analysis identified a new heteroplasmic mutation in the TYC stem of the tRNA^{Leu(CUN)} gene. It is the first change located in this site associated to a mitochondrial myopathy with no involvement of the external ocular muscle.

DIAGNOSTIC CONTRIBUTION OF MUSCLE MRI IN A LGMD2A CASE WITH MRNA ALTERATION

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Limb girdle muscular dystrophy (LGMD) type 2A (LGMD2A) is due to mutations in the CAPN3 gene on chromosome 15, encoding for calpain-3, a muscle specific protease. Over 200 mutation have been described, among which few have been identified only at mRNA level. Muscle MRI in LGMD2A shows a very selective involvement of lower limbs muscles, therefore resulting useful in diagnosis of difficult cases.

We describe the case of two patients, brother and sister, with a clinical picture of limb girdle muscle dystrophy (LGMD) and a biochemical defect of calpain-3 at Western Blot (WB) in which we could not find any genomic alteration through denaturing high-performance liquid chromatography (DHPLC).

Before performing mutation analysis of genes involved in the secondary form of calpain defect (ie dysferlin or titin), patients underwent muscle MRI of lower limbs.

The pattern of muscle degeneration appeared very similar to a control patient with LGMD2A, with a selective involvement of posterior muscles and relative sparing of the anterior compartment, as already described in literature. Thus, in order to exclude hidden mutation, we carried out muscle mRNA analysis by RT-PCR and we identified a heterozygous point mutation at exon 8 (R355W) and an additional amplified fragment of lower weight, resulting in a large deletion of exon 3 and generation of premature stop codon.

The correct identification of the different forms of LGMDs cases can be challenging and new tools for leading genetic analysis to the most eligible diagnosis are necessary. Muscle MRI can be of some utility in these cases.

UNUSUAL DYSTROPHIN DELETIONS ASSOCIATED WITH BECKER MUSCULAR DYSTROPHY (BMD)

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Deletions in the dystrophin gene are the most common disease causing mutations in Xp21-Linked muscular dystrophies: out of frame deletions cause Duchenne phenotype while deletions maintaining the reading frame, are associated with BMD.

Among dystrophinopathies we found three patients with unusual dystrophin deletions reported only once in literature.

An in frame deletion of the region encompassing exon 35 to 44 was found in two oligosymptomatic brothers with high CK (now 18 and 14 year-old) that both had an isolated episode of myoglobinuria at age 12. Western blot demonstrated a smaller and slightly reduced muscle protein. The second unusual deletion was detected in a severe BMD patient (current age 17) that showed reduction of dystrophin immunostaining in muscle specimen with N-terminus and rod domain antibodies and a total absence with C-terminus, suggesting a deletion in the distal region of the gene. By DNA analysis we found the deletion of the penultimate exon (78): the mutation results in a frame shift which creates a novel open reading frame in exon 79 and substitutes the normal C-terminus 14 amino acids with 32 new ones. This deletion, although out of frame, due to the particular position at the 3' end of the gene allows the production of an abnormal protein still able to assembly on the membrane and interact with the other dystrophin-associated-proteins.

NCAM IS HYPOSIALYLATED IN HEREDITARY INCLUSION BODY MYOPATHY DUE TO GNE MUTATIONS

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Autosomal recessive hereditary inclusion-body myopathy is associated with mutations in the GNE gene that codes for a bifunctional enzyme with a critical role in sialic acid biosynthesis. Sialic acid is involved in many biological functions including stabilization of glycoproteins, cellular adhesion and signal transduction. The Neural Cell Adhesion Molecule (NCAM) is a member of the superfamily of adhesion molecules and binds homopolymers of α 2,8-linked sialic acid residues, thus forming polysialic acid (PSA)-NCAM. We studied 5 patients with HIBM due to GNE mutations, 6 patients with a quadriceps sparing myopathy with a possible autosomal recessive inheritance but lacking mutations in the GNE gene (NG-HIBM). Additional controls were inflammatory myopathies, ALS, DMDs and normal muscles. Increased NCAM immunoreactivity was found in regenerating muscle fibers. In HIBM and NG-HIBM biopsies, NCAM expression

was also increased in abnormal non-regenerating muscle fibers in the form of a diffuse cytoplasmic staining, presence of cytoplasmic granular deposits, or both. By western blot we found that NCAM is hyposialylated in hereditary inclusion body myopathy muscle, as suggested by its decreased molecular weight. This abnormality represented the only pathologic feature differentiating HIBM due to GNE mutations from other myopathies with similar clinical and pathologic characteristics.

LIMB-GIRDLE MUSCULAR DYSTROPHIES: CLINICAL FEATURES AND GENETIC FREQUENCY IN A LARGE ITALIAN POPULATION

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Limb-Girdle muscular dystrophies include six rare autosomal dominant (LGMD1) and eleven autosomal recessive (LGMD2) forms, characterised by a wide clinical variability.

We studied 140 patients (76 males, 64 females) with a clinical phenotype of LGMD, Disease-onset ranged from 2 to 55 years of age.

The following muscle proteins were analysed by immunohistochemistry and Western Blot analysis: all sarcoglycans, Dysferlin, Caveolin3, Calpain3, Telethonin, Myotilin and alpha-Dystroglycan. According to the identified protein defect(s) the corresponding gene was analysed.

All LGMD1 patients presented mutations in the Caveolin3 gene (9/139=6,5%).

Variable calpain 3 deficiency is the commonest observed abnormality (79/139 = 56,8%). About 50% of these patients had CAPN3 mutations.

28 out of 29 dysferlin-deficient patients had DYSF gene mutations (29/139 = 20,1%).

19 patients presented a sarcoglycan deficiency (19/139 = 13,7%). 15 carry mutations in a SG gene. 4 patients presented FKRP gene mutations (4/139 = 2,9%).

Protein defects in dysferlin, SG and caveolin 3 strongly predicts primary dysferlinopathy, sarcoglycanopathies and caveolinopathy. No correlation between the degree of protein deficiency and the presence of mutations has been found in calpainopathy. FKRP patients are less common in Italian LGMD patients.

NEUROPATHOLOGICAL STUDY OF SKELETAL
MUSCLE, HEART, LIVER, AND BRAIN IN A
NEONATAL FORM OF ANDERSEN DISEASE
ASSOCIATED WITH A NEW MUTATION IN GBE GENE

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Glycogen storage disease type IV (GSDIV) is an autosomal recessive disorders due to a deficiency of glycogen brancher enzyme 1 (GBE1), resulting in an accumulation of amylopectin-like polysaccharide in muscle and liver. Typically the presentation is with liver involvement in childhood until liver cirrhosis. The neuromuscular form varies in onset (congenital, perinatal, juvenile and adult) and in severity. The congenital form is rare, and only 6 cases have been genetically determined. This form is characterized by polyhydramnios and neonatal hypotonia; liver involvement is uncommon, and the baby usually die between 4 weeks and 4 months of age.

We report the case of an infant with congenital form of GSDIV who presents severe hypotonia, dilatative cardiomyopathy, hepatopathy and hemorrhagic lesions at the CNS, who died at one month of life because of heart failure. Muscle biopsy, heart and liver autopsy showed many vacuoles filled with PAS positive-diastase resistant materials. A morphological study of the autaptic brain has been also performed. The GBE biochemical activity was absent in muscle, liver and heart. The iodine spectrum showed a typical amylopectin-like absorption. GBE 1 gene sequence analysis revealed a novel homozygous stop codon mutation in exon 4. This homozygous null mutation correlates with the lacking enzyme activity and with the ubiquitous, severe neonatal involvement.

MODIFICATIONS OF BRAIN TISSUE VOLUMES IN
FACIOSCAPULOHUMERAL DYSTROPHY

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Facioscapulohumeral muscular dystrophy (FSHD) can be associated to several CNS disorders, including sensorineural hearing loss, epilepsy and mental retardation.

Aim of our study was to verify if brain tissue volumes measured by segmentation of MRI studies, are altered in FSHD.

Volumes of gray matter (GM), white matter (WM), and cerebro-spinal fluid (CSF) were compared, both globally (by multiple regression analysis) and regionally (by voxel-based morphometry - VBM) in thirty patients with FSHD and 39 normal subjects.

FSHD patients had significantly lower GM volumes and significantly higher CSF volumes ($P < 10^{-4}$). At VBM three clusters of GM loss ($P < 0.05$ corrected for multiple comparisons at cluster level) were detected, in the left precentral cortex (Brodmann areas 6, 2 and 44), in the anterior cingulate

(Brodmann areas 33, 24 and 11) and in the right fronto-polar region (Brodmann area 10).

To the best of our knowledge, this is the first study to demonstrate a reduction in GM volume in the brain of patients with FSHD.

GM loss showed a borderline correlation with disease severity as assessed by Score R ($P < 0.05$).

Brain tissue volumes did not correlate with disease duration, size of the genetic deletion, age at onset and the presence at MRI of WM hyperintensities (detected in 4/22 patients).

We hypothesize that localized GM loss in FSHD is the consequence of a selective involvement of specific CNS structures.

COENZYME Q10 DEFICIENCY AND ISOLATED
MYOPATHY. REPORT OF AN ITALIAN CASE

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Primary coenzyme Q10 (CoQ(10)) deficiency is rare. The pure myopathic form, without central nervous system involvement, has been described in only four cases. The objective of the present work is to report an Italian patient, a 26-yr old man who came to our attention for muscle fatigue, exercise intolerance and occasional episodes of myoglobinuria, with CoQ10 deficiency and isolated myopathy. Blood CK levels were mildly increased (from 300 to 500 U/L), whereas lactate was normal. Skeletal muscle histochemical evaluation and respiratory chain enzyme analyses were normal. The CoQ10 concentration in skeletal muscle was 51% of the normal reference mean. The patient began taking 600 mg/d of a CoQ10 supplement. Follow-up of the patient in 3 months demonstrated significant clinical improvement, no more episodes of myoglobinuria and normalization of creatine kinase levels. Our case confirms the existence of a pure myopathic form of CoQ10 deficiency, expanding the clinical phenotype of this treatable mitochondrial disorder. The dramatic response to the exogenous CoQ10 supplementation highlights the importance of early identification and treatment of this disorder.

MUTATIONAL ANALYSIS OF DYSFERLIN GENE IN
ITALIAN PATIENTS WITH MIYOSHI MYOPATHY
(MM)

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We analyzed 18 MM Italian patients from 13 families. We documented various mutations in 33/36 2p13 chromosomes. A common change, the 4872_4876delinsCCCC identified in the original Libyan Jewish cluster with LGM2B/MM (Argov et al, 2000), was found in 5 unrelated individuals from non consanguineous families, all coming from a small area of central Italy around Rome. Four patients were homozygous

and one patient a compound heterozygote carrying in the second allele a new missense mutation in exon 51. 4872_4876 delinsCCCC causes a premature stop codon and, accordingly, dysferlin protein was absent on Western blot in all homozygous patients. Onset affecting calf muscles varied from 14 to 20 years and progression was slow. Interestingly, three patients had at the beginning episodes of painful enlarged calves after efforts that resolved with steroids. At this stage muscle MRI demonstrated inflammatory abnormalities only on distal leg muscles and normalized after therapy.

The presence of the 4872_4876delinsCCCC in two geographical clusters of populations strongly suggests the existence of a founder effect for this mutation and the importance of identifying common mutations to avoid time consuming genetic analyses in complex genes such dysferlin. The transient inflammatory calves enlargement after effort suggests an abnormal response to mechanical stress of dysferlin-deficient muscles supporting the hypothesis of a central role of dysferlin on repair process of muscle fibers.

MUSCLEBLIND-LIKE PROTEIN 1 (MBNL1): A NEW HISTOPATHOLOGICAL MARKER OF MYOTONIC DYSTROPHIES

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Myotonic dystrophies (DM) are autosomal dominant multi-systemic disorders caused by two noncoding repeats expansion mutations. Myotonic dystrophy type 1 (DM1) is caused by a CTG expansion in the DMPK gene on chromosome 19, whereas myotonic dystrophy type 2 (DM2) is caused by a CCTG expansion in ZFN9 gene on chromosome 3. Both mutations produce pathogenic RNA molecules that accumulate in nuclear foci called ribonuclear inclusions. It has been suggested that CUG/CCUG containing transcripts interact with RNA-binding proteins normally involved in the regulation of alternative splicing, leading to an abnormal splicing of a number of genes that are related to DM pathophysiology. Among these proteins, MBNLs colocalize with ribonuclear inclusions and appear to be involved in DM molecular pathology. In this immunofluorescence study we have examined the in vivo distribution of MBNL1 in biceps brachii muscle biopsies from genetically confirmed DM1 (n=7) and DM2 (n=8) patients, with non-dystrophic myotonic disorders (5 chloride channelopathies, 2 sodium channelopathies), non-DM1/DM2 patients (n=3) and healthy subjects (n=5) used as control. The immunofluorescence study reveal that nuclear accumulations of MBNL1 as protein foci are present only in DM1 and DM2 muscle. This might indicate that MBNL1 is an histopathological marker for the DM pathology. Moreover, as alternative splicing factors, MBNLs might be important targets for therapeutic interventions to correct some of the specific features of DM disease.

RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF PHENYLBUTYRATE IN SPINAL MUSCULAR ATROPHY

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Several studies in the last years have suggested a possible therapeutical role of histone hyperacetylating agents, such as phenylbutyrate (PB), in spinal muscular atrophy (SMA). The aim of the study was to perform a randomized, double-blind, placebo-controlled trial of PB in non ambulant patients with SMA 2. One hundred and seven patients were randomized to two groups: placebo or PB 500 mg/kg/day divided in five doses with an intermittent schedule (7 days on/7 days off). Patients were treated for 3 months with assessments at baseline and weeks 5 and 12. The primary outcome was the 12-week change in Hammersmith functional motor scale. We also measured changes at 12 weeks in strength by myometry and in forced vital capacity (FVC). PB was well tolerated; adverse effects (n=2) included rash and drowsiness with hallucinations. Ninety patients completed the study. The mean changes in Hammersmith functional motor scale score were not significantly different between groups (mean±SD: PB 0.7±1.7; placebo 0.6±2.0). Similarly, there were no differences in mean FVC (PB 0.03±0.2; placebo 0.04±0.2) and myometry change (arm-megascor: PB 2.2±7.8; placebo -1.2±9.9; leg-megascor: PB 3.8±8.3; placebo 2.6±10.6). Our results suggest that, at the regime, schedule and duration of the study, PB did not significantly improve function, strength and respiratory function in patients with SMA.

THE POMPE REGISTRY: CENTRALIZED DATA
COLLECTION TO OUTLINE THE NATURAL COURSE
OF POMPE DISEASE

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Background: Pompe disease is a rare, progressive, and often fatal muscular disease. The underlying pathology is a deficiency of acid alpha-glucosidase (GAA) that hydrolyzes lysosomal glycogen. Pompe disease is a single disease which manifests as a clinical spectrum that varies with respect to age at onset, rate of disease progression, and extent of organ involvement.

Methods: to gain a better understanding of the natural course of Pompe disease, a global, observational Registry was developed to collect anonymous, longitudinal data on Pompe patients.

Preliminary Data Overview: as of January 11, 2006, 150 patients have been enrolled of which the majority (54.0%) is of Caucasian ethnicity. 18.0% of the reported patients have infantile Pompe disease (typically with cardiomyopathy, profound skeletal and respiratory muscle weakness, and death within first year of life). The median age of the infantile diagnosis is 6.3 months. 54.0% of the reported patients have late-onset Pompe disease (typically without cardiac involvement but with progressive skeletal and respiratory muscle weakness and longer survival). The median age of the late-onset diagnosis is 31.4 years. Age of onset was unknown in 28.0% patients. The (median) range of time from symptom onset to diagnosis is 6.7 months for infantile patients and 7.5 years for late-onset patients. Out of 45 late-onset Pompe patients investigated for genotype, in 32 (71.1%) the IVS1-13T>G mutation was found.

Summary: the Pompe Registry attempts to increase the understanding of this rare disease and to potentially improve patient management. Preliminary data show that the (median) range of time from symptom onset to diagnosis is similar to published literature, suggesting the need for greater disease awareness.

VASCULAR ENDOTHELIAL GROWTH FACTOR GENE
TRANSFER USING ADENO-ASSOCIATED VIRAL
VECTORS STIMULATES SKELETAL MUSCLE
REGENERATION AND ENHANCES MUSCLE
FUNCTION IN MDX MICE

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Vascular endothelial growth factor (VEGF) is a major regulator of blood vessel formation during development and in the adult organism. Several evidences support its role in myogenesis and in myoblast migration and survival. Recently it has been reported that the delivery of VEGF using adeno-associated-virus (AAV) vectors reduces muscle damage and

promotes muscle regeneration in experimental models of muscle necrosis in mouse.

We tested whether this effect was reproducible in mdx mice evaluating and we also measured the effect on muscle function by using a grip meter, CK level and morphological and biochemical parameters.

Ten 4-week old mdx and ten wild type mice were treated with intramuscular administration of AAV-VEGF (3 x 10⁸ viral particles) or AAV-LacZ (3 x 10¹¹ viral particles) into the biceps and tibialis anterior (TA) muscles. Evaluations were performed one month after injection.

VEGF-AAV treatment increased the expression of VEGF.

Mdx mice treated with AAV-VEGF showed a significantly higher forelimb strength than AAV-LacZ controls (+19.5%, $p < 0.05$) as well as higher strength normalised to weight (+14.9%, $p < 0.05$). There were no differences in CK levels between VEGF- and LacZ-treated mdx mice. Quantitative morphological evaluation of VEGF-treated biceps and TA muscles showed a reduction of necrotic area ($p < 0.05$) and an increase of regenerating area ($p < 0.05$). We report herein the novel observation of a pro-regenerative role of VEGF in mdx mice and of its beneficial effects on functional parameters. Further studies are needed to better clarify the mechanisms underlying the VEGF-induced benefit in mdx mice and to investigate possible therapeutic implications in Duchenne muscular dystrophy.

ANALYSIS OF MYOTUBULARIN-RELATED 1
(MTMR1) PRE-MRNA SPLICING IN CONGENITAL AND
ADULT-ONSET DM1 AND IN DM2 MUSCLE BIOPSIES

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The MTMR1 gene belongs to a conserved family of phosphatases, whose founder is the MTM1 gene, mutated in X-linked myotubular myopathy. During skeletal muscle differentiation there is an increased expression of the MTMR1 adult C isoform mRNA with a parallel decrease in fetal A and B isoforms. Aberrant MTMR1 mRNA splicing was recently documented in myotubes of congenital DM1 patients, with increased of the fetal A and B levels compared with the adult C isoform, and it was proposed that such aberrant splicing could play a pathogenic role in the dysmaturative muscle features characteristic of congenital DM1. In order to verify this issue we analyzed by RT-PCR MTMR1 alternative splicing in muscle biopsies from 2 congenital and 26 adult onset DM1 patients, 5 DM2 patients and 16 controls. RT-PCR analysis showed low levels of fetal A and B MTMR1 RNA isoforms in all DM (either congenital DM1, adult-onset DM1 and DM2 patients) not evident in controls. However, no significant differences between patients and controls were detected in the levels of the C isoform, which appeared always predominant. These findings suggest that MTMR1 aberrant splicing is not specific of congenital DM1, but possibly reflects the impairment of correct pre mRNA splicing processes generally occurring in myotonic dystrophies. Muscle cultures studies are needed to definitively assess its pathogenic role on muscle differentiation.

CLINICAL AND GENETIC CHARACTERIZATION IN TWO FAMILIES WITH MUSCLE PHOSPHOFRUCTOKINASE DEFICIENCY

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Muscle phosphofructokinase deficiency (PFK) is a relatively rare metabolic myopathy characterized by early muscle fatigue, proximal weakness, compensated hemolytic anaemia and hyperuricemia.

We report 2 unrelated families with PFK deficiency. Family 1: the proband (pt1), a 45 year-old man who, since he was 25, complained of episodic diffuse myalgias, vomiting and dark urines after mild exercise. He had a mild hypertrophic cardiomyopathy. Neurological examination was unremarkable. Family history was negative but his older 12 year-old son showed elevated CK levels (max 600 IU/l). Laboratory tests revealed mild reticulocytosis (2%; v.n.0-1.5 %), increased serum bilirubin (1.8 mg/dl) and hyperuricemia 8.5 (n.v. 5-7 mg/dl). Ischaemic forearm test showed a normal lactate increase. Family 2: a 16 year-old woman (pt2) since childhood experienced exercise intolerance, myalgias but no pigmenturia. A sister suffered of myalgias. Neurological examination was normal.

Muscle biopsy in pt1 and pt2 was normal. Biochemical studies on muscle homogenate revealed a 4% (pt1) and 1% (pt2) of PFK residual activity. Pt1 was compound heterozygous with a novel point mutation D591A and a previously reported IVS6-2A>C. His two sons and four brothers harboured the heterozygous D591A except his son with hyperCKemia who was heterozygous for IVS6-2A>C. In the pt2 we found a homozygous intronic variant IVS6 -3 A>G which lead to an AG insertion in the cDNA. Both asymptomatic parents were heterozygous for the same mutation. Despite of absence of chronic muscle weakness and muscle glycogen storage in both families biochemical and molecular pattern confirmed the presence of PFK deficiency.

MEROSIN-DEFICIENT CONGENITAL MUSCULAR DYSTROPHY

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Merosin deficient congenital muscular dystrophy (MD-CMD) is an autosomal recessive muscle disorder caused by mutations in the laminin alpha2 chain gene (LAMA2). The distinctive pattern of abnormalities that characterizes MD-CMD includes: neonatal hypotonia, weakness, markedly delayed motor development, normal or subnormal mental development, abnormalities in the cerebral. Recent case studies reported that some patients have several structural abnormalities such as abnormal cerebral cortical gyration, hypoplasia of cerebellum and pons, dilation of ventricles. Diagnosis of

MD-CMD was based on their clinical and dystrophic muscle biopsy findings. We evaluate the different aspects of two patients with MD-CMD: one girl who presented at birth with marked generalized hypotonia and normal mental development; one boy had moderate weakness and mental retardation and epilepsy. The clinical picture, biochemical findings, neurophysiological investigations, biopsy findings and extensive abnormalities of white matter or magnetic resonance imaging (MRI) found in these cases are presented. One case had abnormal white matter in the cerebrum, with sparing of the corpus callosum, internal capsule; one case had dilation of ventricles and neuronal migration anomalies. All patients had diffuse white matter alterations similar to those seen in cases of leukodystrophy, periventricular and subcortical white matter were involved. The cases confirmed the large phenotypic variability in the merosin deficiency congenital muscular dystrophy.

NEUROPSYCHOLOGICAL ASSESSMENT IN CHILDREN WITH DUCHENNE DE BOULOGNE MUSCULAR DYSTROPHY

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Impairment of intelligence in Duchenne muscular dystrophy (DMD) was described by Duchenne de Boulogne himself in 1868. The literature shows that about 30-40% of DMD subjects are mentally retarded. The non-progressive, cognitive deficit mainly affects verbal abilities, therefore memory and language functions are the most severely impaired; the scales of patients with DMD show low scores in verbal and visuo spatial memories, comprehension, arithmetic and vocabulary. Memory deficit mainly involves long-term memory; short-term memory impairment was also present, even if less frequently. Attention deficit was also reported. Learning disabilities in arithmetic, writing and reading were also found; the level of reading disorder has been compared to that of children with developmental dyslexia. The present study investigated the neuropsychological performance in a group of DMD (25 subjects); neuropsychological tests included: WISC-R, Rey's complex figure test; Benton's visual retention form D; Frostig's developmental test of visual perception. The children were selected on the basis of physical examination, muscle biopsy, histochemical analysis, Western blot analysis, genetic testing and on their having regular and normal schooling. Testing for dystrophin in muscles did not show immunoreactivity in 18 cases, very weak immunoreactivity was detected in 7 cases. Molecular genetic testing detected a deletion in the central and proximal high frequency deletion regions in all subjects. These patients did not show any brain anomaly evident on neuroimaging. Only 8 subjects had a total IQ below average. In 10 subjects, a mild and non-significant impairment in the long-term verbal memory was detected as was minor attention deficit; this patient also had slight difficulties in the immediate auditory memory, and in recent memory. No impairment of intellectual function was detected in 7 of the children we examined. The study was therefore aimed to assess, through a battery of multisectorial tests, some neuropsychological functions and the presence of sectorial defects in DMD.

INFANTILE AUTISM AND DUCHENNE DE BOULOGNE MUSCULAR DYSTROPHY

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Autism is a neurodevelopmental disorder of genetic origins, characterized by impairments in three core domains: social interaction, language development and patterns of behavior. It is now clear that autism is not a disease but a syndrome characterized by phenotypic and genetic complexity. Research indicates that autism is largely caused by genetic factors that lead to abnormal brain development. A considerable number of cases of autism are linked to specific syndrome that can be identified according to their clinical characteristics, or by means of some biological marker. Duchenne muscular dystrophy (DMD) is an X-linked condition, it is caused by the absence or disruption of the protein dystrophin, which is found in a variety of tissues, most notably skeletal muscle and neurones in particular regions of the CNS. Experimental evidence suggests that in adult, dystrophin normally modulates synaptic terminal integrity, distinct forms of synaptic plasticity and regional cellular signal integration. The identification of novel dystrophins in the brain has recently implicated its absence or malfunction etiologically in mental retardation (Kumagai et al, 2001). DMD and autism spectrum disorder occur with a greater than random frequency (Wu et al, 2005). In DMD, the individual profiles of cognitive and behavioral disorders appear to depend on complex profiles of transcriptional regulation associated with individual dystrophin mutations that result in the corresponding presence or absence of individual brain dystrophin isoforms that normal exhibit developmental, regional and cell-type specific expression and functional regulation (Mahler, 2003). We report two children with autism and DMD. In this study examining the role of genetic factors in the brain abnormalities and association between these two conditions. Furthermore these cases highlight the importance of a thorough neuromotor examination for all children with autism.

REPRODUCTIVE FITNESS IN STEINERT DISEASE

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease - caused by CTG repeat-expansion in the 3' untranslated region of DMPK - characterized by myotonia, multisystemic lesions and hypogonadism. The most significant abnormality in males is testicular atrophy, with primary tubular degeneration, fibrosis and hyalinization often leading to male infertility. In women, the relationship between MD and infertility remains controversial.

Aim of the study was to investigate and compare the reproductive fitness in DM1 female and male patients. The pedigrees from 30 DM1 families including 334 individuals have been evaluated. Among them 219 were adults in the reproductive age (112 males and 107 females). Offspring from 99 affected individuals, 58 males and 41 females was analysed.

Results. Forty-seven (81%) males and 40 (97.6%) females had sons while 11 (11%) males and 1 (2.4%) female did not have any. The differences are statistically significant (c2 test: $P < 0.005$).

Although there was a higher number ($n=151$) of offspring from males compared with females ($n=107$), the difference was not statistically different.

Discussion. Data here reported while confirming a reduction of fertility in MD males, are against infertility in MD females. A possible explanation of the different reproductive fitness observed in DM1 patients can reside on the different role played by gametes in the fecundation process. In fact spermatozoa carrying an abnormal size of triplets CTG, are unfavourable in their run to the cell eggs.

EPIDEMIOLOGY OF FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY: ORIGINAL DATA AND REVIEW OF THE LITERATURE

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Few epidemiological studies are available on FacioScapuloHumeral Muscular Dystrophy (FSHD), an autosomal dominant disease associated with a deletion on chromosome 4q35. These surveys, mainly clinical-based, indicated widely variable prevalence rates ranging 2.2 to 66.9 cases $\times 10^{-6}$.

A genetic epidemiological investigation on FSHD is in progress for the 4,490,586 inhabitants of Veneto Region. All subjects were evaluated by molecular analysis of EcoRI mutation.

Preliminary study concerning the Padua province (845,603 inhabitants, on January 1st 2003) estimated a prevalence rate of FSHD at 45 cases $\times 10^{-6}$, identifying 38 subjects with a 4q35 fragment ranging 14 to 38 Kb (mean 22). They were 24 males and 14 females with mean age of 49 years (range 9-84). Age of onset and clinical course had not clear-cut correlation with the EcoRI fragment size; however, it was noteworthy that five patients with 4q35 fragment larger than 30 Kb (32,38), showed mild facial-sparing phenotype. In four of them (familial cases) linkage analysis in chromosome 4q35, by microsatellites D4S426, D4S2930, D4S1523, D4S139, corroborated the relationship of mild FSHD with mild EcoRI fragment deletion. On the whole, our data indicate that FSHD is among the most frequent neuromuscular diseases. Furthermore, we have evidence that mild facial-sparing phenotype is correlated with 4q35 fragment larger than 30 Kb.

CHANARIN DORFMAN DISEASE (MTSD) AT ADULT ONSET, CLINICAL AND THERAPEUTIC APPROACH- FOUR CASES FROM TWO FAMILIES

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Jordan in 1953 described multisystemic triglyceride storage disorder (MTSD) accumulation of neutral lipid in leukocytes, muscle and epidermis, frequently associated with ichthyosis.

Chanarin and Dorfman (1975) suggested a genetic autosomic recessive transmission in two patients with also cataract hearing loss and nystagmus.

Fatty acid transport into mitochondria was normal and lipase acid activity was normal, hypothesizing a long-chain fatty acid beta oxidation defect (Angelini 1980).

The biochemical and genetic defect is still not completely understood: there are reports of mutations in a recently identified gene, the CGI58 (Lefevre 2001).

The prevalence of this disorder is unknown, in our center we observed four cases in ten years. There is not therapy but some observation reported that a medium chain triglyceride diet and L-carnitine reversed hepatosplenomegaly and ameliorated muscle strength.

We observed two families in which four subjects resulted affected by MTSD with prevalent myopathic involvement. In our families, there were no consanguinity between parents of affected subjects.

In first family we studied three generations: in second generation three subjects were affected, one female, her brother and one female cousin that have lipid storage myopathy; in the previous generation, one maternal uncle had hyperCKemia.

In second family, we studied two generations: in the first parents did not have Jordan anomaly, nor myopathy or other organ involvement, two of three sons presented myopathy, Jordan anomaly and one had cardiomyopathy.

All myopathic subjects presented progressive diffuse weakness and asymmetric muscle atrophy, prevalent in proximal arms and distal legs; the first family patients had clinical myotonia and myotonic discharges at EMG evaluation, the second family patients had myotonic features only at the EMG.

In all affected subjects myopathy was clinically appreciable only in the adult age, in two cases after age of thirty, in the other two after fifty.

Carnitine therapy was ineffective in one patient.

All patients started steroid therapy in association with medium chain fatty acid diet Strength and peripheral blood lipid storage were evaluated after six months. Only one patient shows clinical improvement.

REFINING GENE EXPRESSION DEREGULATIONS IN DMD MUSCLE

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In the last years, Genechip analysis of dystrophic tissues has been used to characterize the alterations that distinguish a malfunctioning DMD muscle from a normal one in patients older than 5 years. By studying the expression profiles (Affymetrix-U133A) of 20 DMD patients age 1.5-24 months we have characterized this initial asymptomatic phase of the disease. Despite the different degree of muscle dysfunction experienced, younger patients showed abnormal expression of most of the genes differentially expressed in symptomatic patients, implying that the deregulation is established long before the onset of a perceptible inability. Most of these alterations can be assigned to one of four key aspects of DMD

pathophysiology: inflammation, muscle regeneration, energy metabolism, and extracellular matrix remodelling. Gene profiling studies succeeded in defining a specific gene expression pattern that consistently characterizes DMD muscle, from early postnatal life on. Nonetheless, they have been so far unable to shed light over the regulatory mechanisms responsible for the activation of pro-fibrotic pathways and the failure of muscle regenerative capacity progressively experienced by DMD muscle. To identify genes modulated along with disease progression we included in the analysis 15 patients age 3-9 years. By running a correlation analysis across patient's age, we provide evidence that a number of genes are progressively induced or repressed in the natural history of the DMD.

CONFOCAL AND IMMUNOELECTRONMICROSCOPY ANALYSES IN AN ULLRICH PATIENT WITH A SECONDARY COLVI DEFECTS

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Ullrich congenital muscular dystrophy (UCMD) is a merosin-positive CMD clinically characterized by severe muscle weakness, proximal contractures and distal hyperlaxity generally associated with COL6 genes mutations. Absence or partial reduction of collagen VI (ColVI) is detectable in UCMD cultured fibroblasts and muscle. Recent reports on UCMD patients without COL6 mutations have suggested genetic heterogeneity. We report detailed comparative morphological findings on 2 patients with a typical UCMD phenotype. One patient had a homozygous missense mutation in COL6A2 while the other showed no mutations in all the COL6 genes with normal ColVI mRNAs levels, and exhibiting a reduced ColVI only behind the basal membrane of muscle. In this latter patient immunohistochemistry of the muscle biopsy with antibodies against proteins that potentially bind to ColVI (NG2, perlecan, alpha-dystroglycan, biglycan, decorin, tenascin) showed a normoexpression, whereas alpha7beta1 integrin complex was altered. Further, immunofluorescence and rotary-shadowing electronmicroscopy (REM) of cultured fibroblasts produced a normal amount and structure of secreted ColVI microfibrils, whereas muscle ultrastructure revealed alterations in 10% of myofibers. Confocal microscopy and REM are useful to identify UCMD patients with a secondary ColVI defect.

PYOMYOSITIS: A FATAL CASE IN A DIABETIC PATIENT

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Background: bacterial pyomyositis is common in tropical climates and rare in temperate countries where it occurs mainly in hosts who are immunocompromised or debilitated

by diseases such as diabetes, malignancy or rheumatologic condition. Purpose: to describe the clinical and histopathological findings of pyomyositis in a diabetic patient. Case report: a 60-year-old man, with a history of diabetes mellitus, was admitted for diffuse and intense myalgia starting about two weeks before. He had no fever and had been assuming oral FANS to treat muscular pain for ten days. His muscles (especially the quadriceps) were very painful with palpation; mild stiffness and proximal muscular weakness were present. Serological studies revealed mild leukocytosis (neutrophils), elevated ESR (44), and CPK five times the upper limit (<195); HBV, HCV and HIV screenings were negative. Electrodiagnostic studies suggested inflammatory myositis. During the same night of admission, septic shock developed with delirium, anuria and marked elevation of serum CPK (60,000). Corticosteroid and antibiotics were given but patient died 2 days later. Staphylococcus aureus was cultured from blood. Autopsy examination detected numerous small abscesses (diameter: around 1 mm) in many striated muscles and heart. The abscesses contained polymorphonuclear leukocytes in all stages. Conclusion: Pyomyositis is often a challenging diagnosis and requires a high degree of suspicion. The condition is usually diagnosed late and for this reason it sometimes has a significant mortality rate.

OXIDATIVE STRESS IN MYOTONIC DYSTROPHY TYPE 1

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Myotonic Dystrophy type 1 (MD), the most frequent muscular dystrophy in adulthood, is an autosomal dominant inherited multisystem disease. Although characterized in its genetics, molecular cell damage mechanisms are still unknown. Recently attention has been paid to mechanisms of messenger RNA interfering by mutated transcripts. In this context increased oxidative stress has been suggested to have a pathogenetic role in MD. Aim of this study has been to analyze the occurrence of oxidative stress in MD and to relate this to clinic aspects of the disease.

Circulating significantly increased levels of advanced oxidation protein products and decreased levels of glutathione were found in 10 MD patients compared to controls. Values were related to extramuscular involvement of the disease, in particular occurrence of cataract and cardiac conduction defect and were partially relieved by dietary induction of endogenous glutathione synthesis. Our results, in indicating oxidative stress as putative pathogenetic factor in MD, suggest that abnormal production of reactive oxygen species can be one of the targeted effects of causative mutation in myotonic dystrophy type 1.

NEUROPSYCHOLOGICAL PROFILE IN CHILDREN WITH DUCHENNE MUSCULAR DYSTROPHY: GENERAL AND SPECIFIC DEFICITS

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Duchenne muscular dystrophy (DMD) has long been recognised as a cause of some degree of mental retardation. However, recent studies show that patients with DMD have specific cognitive deficits.

A neuropsychological assessment has been conducted in a group of 35 boys with DMD, aged 5.6 to 14 years, in order to evaluate the presence and the type of specific cognitive impairments. Diagnosis was based on clinical findings and dystrophin analysis by immunohistochemical and western-blot, and by DNA analysis. All patients received steroids (deflazacort) since 2 to 8 years.

Confirming literature results, our data show that in DMD patients mean Intellectual Quotient (IQ) score is shifted down approximately 1 standard deviation from the normal population. In addition, comprehension, memory and digit span tests have been poorly performed, suggesting a selective impairment of short term memory playing a critical role in the verbal disturbance in these patients. As PET studies showed temporal lobe and cerebellar hypometabolism in DMD patients, neuropsychological profiles found in our patients may be related to a metabolic impairment in these cortical areas. A more complete knowledge of the cognitive deficits in boys with DMD has clear practical implications, both for rehabilitation and for children and families quality of life. Long term steroids therapy seems to have no influence on cognitive development in DMD.

POSSIBLE ROLE OF ENDOTHELIN-1 IN MITOCHONDRIAL MYOPATHIES

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Background. Endothelin-1 (ET-1), interacting with G-protein-coupled receptors, induces multiple effects (vasoconstriction, growth promotion...). Interestingly, it acts as survival factor against apoptosis in cardiomyocytes, smooth muscle cells, fibroblasts and several cancer cells. It is unclear how ET-1 regulates apoptosis: probably inducing overexpression of antiapoptotic molecules as Bcl2. Gene expression of pre-endothelin-1, was described in cardiomyocytes. Mitochondrial perturbation, potentially pro-apoptotic, arises also in skeletal muscle cells (mitochondrial myopathies), but apoptosis is often absent.

Aim. To evaluate ET-1 expression in mitochondrial-affected fibers and its role as anti-apoptotic factor through Bcl-2 overexpression.

Methods. Nine primary and 16 secondary mitochondrial disorders were evaluated. Muscle samples were processed for standard histology. Immunohistochemistry was performed with antibodies versus ET-1 and Bcl-2 (Ventana Medical System). The presence of apoptotic nuclei was determined with FRAGEL apoptosis detection kit (Oncogene), following data sheet instructions. The apoptotic index was obtained comparing the number of apoptotic nuclei with the totality of nuclei in 5HPF.

Results. ET-1 resulted specifically expressed in the affected fibers of 4/9 primary mitochondrial diseases examined. The same cases showed an overexpression of Bcl-2. We observed a more intense reaction in MERFF myopathy compared with CPEO diseases. None of the case with secondary mitochondrial alterations presented Bcl-2 or ET-1 positivity. In these cases the apoptotic index ranged from 22 to 53% (media: 32), whereas the ET-1 and Bcl-2 positive cases showed values less than 20%.

Conclusion. These data suggest an involvement of ET-1 only in primary mitochondrial diseases, with a specific correlation to Bcl-2 expression and its antiapoptotic effect.

THE ITALIAN CONTRIBUTION TO THE EUROBIOBANK NETWORK: A SUCCESSFUL AND OPERATIONAL MODEL FOR SUPPORTING SCIENTIFIC EXCHANGE AND COOPERATION

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EuroBioBank (EBB) is a European network of biological banks established in 2001. The Network is fully dedicated to supporting research into rare diseases by facilitating access to quality human biological resources and their associated data from patients with rare diseases. The 16 EBB founding partners represent 8 European countries: Belgium, France, Germany, Hungary, Italy, Malta, Slovenia, and Spain. Italy was represented by Milan - Besta Institute and Policlinico Maggiore, Naples Second University and Padua University. Between 2003 and 2005, while financed by the EC, major milestones were achieved: common quality criteria defined; Standard Operating Procedures and ethical guidelines developed; standards for material transfer and biobanking adopted; a dedicated website developed to offer services to the scientific community (www.eurobiobank.org). A web-based catalogue of samples available was launched, specifically designed to provide easy access to referenced samples. Today, the EuroBioBank Network represents approximately 155,000 documented human biological samples, available for researchers.

The Italian Partners contributed to all workpackages and were leaders for WP3 - Technical and quality issues for tissue - (Angelini), WP4 - Technical and quality issues for cell cultures - (Mora) and WP7 - Communication - (L.Politano).

The EBB Network founding partners intend to consolidate the Network as a self-sustained structure in the long-term and establish EuroBioBank as a permanent and integrated European service Network for research on rare diseases.

EXTENSIVE MUTATION ANALYSIS IN PATIENTS WITH DYSTROPHINOPATHY: A REPORT

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Dystrophin mutations occur in Duchenne and Becker muscular dystrophy.

Mutations are highly heterogeneous and require extensive diagnostic protocols for being identified.

We have available 250 patients with dystrophinopathy under study by using a combined protocol including Multiplex PCR analyses, duplication and deletion detection in females carriers by Real-Time PCR, extensive sequencing of all the 79 exons and RNA analysis of the full transcript in skeletal muscle biopsies.

This approach allowed us to explore 130 cases by multiplex PCR, 85 cases by Real-time, 27 cases by complete sequencing and 14 cases by RNA analysis on biopsy.

This study allowed us the identification of atypical exon deletions, duplication also in symptomatic females and novel small mutations. RNA analysis disclosed complex splicing profiles.

We conclude that our approach is a comprehensive diagnostic strategy in order to identify virtually all mutations type.

RAGE-NF-KB PATHWAY ACTIVATION IN RESPONSE TO OXIDATIVE STRESS IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

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Background - An increased expression of adenine nucleotide translocator (ANT1) in facioscapulohumeral muscular dystrophy (FSHD), is known to lead a decrease in nuclear NF- κ B (NF- κ B) DNA binding, sensitizing muscle cells to oxidative stress. Receptor for advanced glycation end products (RAGE) mediated by NF- κ B activation is involved in proinflammatory pathomechanism and in muscle fiber regeneration in inflammatory myopathies and in limb girdle muscular dystrophy. Oxidative stress can stimulate RAGE- NF- κ B pathway.

Objectives - Our purpose was to verify if oxidative stress may induce RAGE- NF- κ B pathway activation in FSHD, contributing to the pathogenesis of FSHD.

Materials and methods - On muscle samples of eight patients with FSHD and eight normal controls were done the following studies: immunocytochemistry for activated NF- κ B; electrophoretic mobility shift assay (EMSA) of NF- κ B DNA binding activity; western blot of RAGE and ANT1; hydrogen peroxide (HP), peroxidase and glutathione peroxidase (GPx) assays.

Results - An increased RAGE and ANT1 expression in FSHD with moderate increase of NF- κ B DNA binding activity was found together with an increased production of HP and a reduced activity of peroxidase and GPx.

Conclusions - Our data confirm that response to oxidative stress and ANT1 increased activity are early events in FSHD muscle. The study evidences also that RAGE- NF- κ B pathway, induced by oxidative stress, is activated independently of the presence of a clear histochemical evidence of muscle damage in FSHD.

MYOTONIC DYSTROPHY TYPE 1 RE-TESTED AFTER 10 YEARS: DOES SOMATIC INSTABILITY AMPLIFY CTG EXPANSION?

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Background: Myotonic Dystrophy type 1 (DM1) is an autosomal dominant disorder, characterized by multisystemic involvement. Genetic transmission is due to a CTGn polynucleotide expansion on chromosome 19 (19q13.3), which shows in-vitro instability.

Objective: to subject a group of nine patients affected with DM1 to a second DNA analysis after 10 years, in order to compare the new to the previously established CTGn expansion.

Materials and methods: we genetically re-tested, nine unrelated DM1 patients (4 females, 5 males), thus re-quantifying CTGn expansion size. Our patients were subgrouped on their expansion size as follows: E1 (<500 CTG), E2 (500-1000 CTG), E3 (>1000 CTG).

Results: our data show: increase of CTGn expansion in 5 patients; decrease in 2 patients; one was stable; in one patient the exact CTGn expansion size was unknown. Considered by E-classification: 3 patients shifted to the upper class; 6 patients maintained their original class. Percent variation of CTG size ranged from 130,3% to -23,1% (mean value 35,4; standard deviation 55,4).

Conclusions: nine DM1 patients were genetically re-tested; CTG expansion size was recalculated. Eight patients presented a different CTG expansion size. The calculated mean value of this variation is increased of almost 33%. CTG expansion size appears unstable in time, but further investigation is needed to confirm these data.

MUSCULAR DYSTROPHIES DUE TO GLYCOSYLATION DEFECTS IN A GROUP OF ITALIAN PATIENTS

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In order to provide a systematic classification of defects of a-dystroglycan glycosylation in our patients, we are performing genetic screening for mutations in the FKRP, POMGnT1, POMT1 and POMT2 genes by PCR amplification and sequencing of the genes. We have selected 38 patients with undetectable or greatly reduced a-dystroglycan expression. In these patients we also examined laminin α 2 expression: this varied from normal to slightly reduced in most patients and was greatly reduced in two.

We found six known and four previously undescribed FKRP missense mutations affecting highly conserved aminoacids in seven patients; they were all compound heterozygous for the mutations. Four patients presented a severe or moderate MDC1C phenotype and three had either a LGMD1 phenotype or an asymptomatic hyperCKemia. MRI performed in three patients, was significantly abnormal in one case showing cerebellar cysts.

We also found two undescribed missense mutations in the POMT1 gene in a patient with a congenital muscular dystrophy.

By defining the molecular alteration responsible for a-dystroglycan glycosylation defects, this study contributes to accurate characterization of the different spectra of disease. This is particularly important as new phenotypes are increasingly emerging.

EFFECT ON COLLAGEN VI EXTRA-CELLULAR ASSEMBLY OF COL6A1 AND COL6A2 C-TERMINAL MUTATIONS IN ULLRICH CONGENITAL MUSCULAR DYSTROPHY

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Ullrich Congenital Muscular Dystrophy (UCMD) is a condition characterized by congenital muscle weakness, proximal joint contractures, distal joint hyperextensibility and dystrophic changes in muscle biopsy. It is caused by mutations in the three collagen VI genes COL6A1, COL6A2 and COL6A3. Collagen VI is a major extra-cellular matrix (ECM) protein expressed in several tissues and cell cultures. It consists of three genetically distinct chains (α1, α2 and α3) secreted in the ECM where they form an extended microfilamentous network. The intracellular and extracellular assembly of collagen VI is a complex process which involves specific molecular domains at different steps. Previous studies demonstrated that α1 (VI) and α2 (VI) C-terminal domains are required for intracellular association into dimers and tetramers but their role in extracellular microfibrillar assembly has been poorly investigated. In this study, we examined the consequences of COL6A1 and COL6A2 mutations in C1 and C2 domains, on collagen VI extra-cellular assembly in fibroblast cultures of four UCMD unrelated patients. Immunofluorescence analysis showed that all mutations caused a decreased deposition of collagen VI in the extra-cellular matrix. Electron microscope examination of replicas obtained from rotary shadowed cultured cells revealed that secreted mutant tetramers could form microfilaments and fibrils. However the microfilamentous network appeared altered and the extension of webs was time dependent. Our data confirm that α1 and α2 C-domains are essential for intracellular dimer and tetramer association and suggest an additional critical role in extracellular network assembly.

CORONARY ARTERY DISEASE IN MYOTONIC DYSTROPHY TYPE 2 (DM2)

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Objective: To determine whether cardiac alterations in DM2 are limited to the conduction system or whether these extend to coronary arteries.

Background: Cardiac involvement in DM2 is generally considered less severe than in DM1. Recent reports have however described sudden death in DM2 as a result of ventricular arrhythmia. Moreover, the frequency and severity of coronary artery involvement in DM2 is yet undetermined.

Methods: 36 patients with genetically determined DM2 (mean age 51.6 ± 13.8) and 82 patients with moderately severe age- and sex-matched DM1 (mean age 43.1 ± 14), were subjected to: (i) neuromuscular assessment (MRC scale); (ii) 12-lead ECG; (iii) 2D echocardiograms; (iv) 24-hour ECG-Holter (v) cardiovascular risk factor assessment. A step-like protocol was applied for patients complaining of chest pain: treadmill stress testing; myocardial perfusion imaging; coronary angiography.

Results: 7 patients with DM2 (19.4%) and 6 patients with DM1 (7.3%) complained of atypical chest pain, but with normal ECG and laboratory biomarkers. Coronary artery disease was demonstrated in 3 patients with DM2 (8%) and 2 with DM1 (2.4%).

Conclusions: Although originally considered more benign than DM1, there may be severe cardiac involvement, including coronary artery disease, in a cohort of patients with DM2. Further studies on a larger number of patients will confirm/refute a higher prevalence of coronary disease in DM2 compared to the general population.

INCREASED EXON EXCLUSION CAUSED BY A NOVEL MUTATION AFFECTING A SPLICING ENHANCER (ESE) BINDING MOTIF RESULTS IN THE SANDHOFF 'MOTOR NEURON PHENOTYPE

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We describe a novel mutation in the HEXB gene associated with a familial adult-onset Sandhoff's disease manifesting as a lower motor neuron disease.

Disruption of exonic splicing enhancers (ESEs) by point mutations affecting the correct pre-mRNA splicing has been proposed as novel pathogenic mechanism for human diseases. Such mechanism has never been described in association with Sandhoff's type GM2 gangliosidosis.

Biochemical studies on leukocytes from two family's probands documented a significant reduction of total hexosaminidase, with absence of the B isoform activity. HEXB gene sequencing revealed a novel homozygous A1556G transition in exon 12, producing a change from aspartic acid to glycine at position 494 (D494G). The mutation was ruled out in 60 controls. RT-PCR analysis of a cDNA fragment encompassing exons 10-14 showed the expected 538 bp fragment, but also an abnormally spliced 447 bp fragment in which exon 12 skipping has occurred. "ESEfinder" analysis revealed that the A1556G mutation involved an ESE sequence disrupting a binding motif for the human SR proteins SC35 and SRp55. Our data confirm that point mutations located within coding regions may exert their pathogenic role not only producing an amino acid change but also affecting the correct pattern of pre-mRNA splicing.

Finally, biochemical data in our family highlights that HEX activity is crucial for the integrity of spinal motor neurons.

BONE METABOLISM ALTERATIONS IN CHILDREN WITH DUCHENNE MUSCULAR DYSTROPHY: RESPONSE TO A FIRST-LINE TREATMENT

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In children affected by Duchenne muscular dystrophy (DMD) very little if any attention is paid to bone mass and bone metabolism. The detrimental effects of long-term steroids on bone, even at low doses, are well known. We followed 32 DMD children for 3 years: 1 year of observation and 2 years of a first-line treatment, i.e. adjusted calcium in the diet plus calcifediol. All the children were treated with steroids for at least 6 months before the study. 9 children were non-ambulant at baseline, all the others maintained the possibility to walk during the entire study. We evaluated bone mineral density (BMD) with DXA, parathyroid hormone (PTH), 25-OH vitamin D, osteocalcin and N-terminal telopeptide (NTx). At baseline and after 1 year of observation, NTx was increased and osteocalcin was at the upper limit; 25-OH-D levels were significantly lower and PTH was at upper levels. Moreover hypocalciuria was present. Bone mass progressively reduced. After 2 years of treatment, all these parameters returned to normal range, or close to it. Bone mass increased in 64% of patients, remaining stable in 36%.

We suggest that bone mass and metabolism should be assessed before starting prednisone therapy as well as during follow-up, in order to assess bone mass accrual, and take corrective action when necessary.

HOMOPLASMIC POINT MUTATIONS IN MITOCHONDRIAL tRNA GENES IN PATIENTS WITH SEVERE ENCEPHALOPATHY

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Mitochondrial disorders are an heterogeneous group of diseases that impair mitochondrial ATP production and are often associated with multisystemic expression.

Heteroplasmy has been traditionally considered important evidence for the pathogenicity of a mtDNA mutation. Conversely, there are pathogenic mtDNA mutations that are homoplasmic and considered relatively mild.

We report two families with two different homoplasmic mutations in tRNA genes associated to severe phenotypes. In particular, a patient with profound mental retardation, epilepsy, tetraplegia, cerebellar and extrapyramidal signs, muscle atrophy presented the A5814G homoplasmic mutation in the tRNA gene for cysteine; the second patient, instead, presented severe mental retardation, mitochondrial myopathy,

mioclonic status epilepticus associated to a novel T7484C homoplasmic mutation that affects the anticodon region of tRNA^{Ser} (UCN). In both cases, the homoplasmic mutation was found in other members of the family that presented milder phenotypes. Our results support the concept that homoplasmic mutations in tRNA genes can be responsible for mitochondrial disorders, with variable penetrance.

A MICROARRAY STUDY OF MUSCLE TRANSCRIPTOME IN PATIENTS WITH NEUROGENIC ATROPHY

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Background: the molecular mechanisms underlying muscle atrophy caused by denervation in humans are poorly understood. Aim of this study is to focus on both degenerative and protective mechanisms activated in patients with neurogenic atrophy. Methods: we performed a microarray analysis on muscle biopsies from 29 patients with different types of neurogenic atrophy (ALS, SMA, MMN, Kennedy disease, spine injury) and 7 controls. Results: we obtained a list of more than 600 differentially expressed genes that we clustered into functional categories. Genes encoding neuromuscular junction components (nAChR subunits, ERBB3) and developmental isoforms of myofibrillar proteins were induced, as well as those involved in extracellular matrix remodeling (collagen type I, III and VI, TIMP1, MMP2, TGFB1 and 3) and cellular atrophy (including FOXO1A, cathepsin D, K and O). We also detected increased transcription of genes related to protein synthesis (IGF1, EIF4A1 and a large set of ribosomal proteins). Accordingly, 4EBP1 expression was reduced. Conclusions: transcriptional programs activated in muscle in response to different types of neurogenic lesion share common aspects, including the induction of anabolic pathways. Different degrees of deregulation correlate with the severity of muscle involvement. Since histological examination showed in our biopsies coexistence of atrophic and hypertrophic fibers, selective approaches may result more suitable for assigning specific molecular alterations to different fiber phenotypes.

DOMINANT AND RECESSIVE INHERITANCE IN CAV3 DEFICIENCY

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We report a clinical and molecular genetic study on two patients with novel caveolin-3 (CAV3) mutations. Patient 1, a 40-year-old man, had an isolated hyperCKemia without any signs or symptoms of myopathy. Patient 2, a 58-year-old woman, showed dilated cardiomyopathy, proximal muscle weakness and wasting, hyperCKemia and diabetes.

CAV3 genetic analysis showed in both patients two different nucleotide changes in the same amino acid, located in the highly conserved transmembrane domain, in codon 233. In patient 1 a heterozygous mutation, converting threonine to lysine, correlated with an autosomal dominant transmission and in patient 2 a homozygous mutation, converting threonine to methionine and correlated with an autosomal recessive hereditary.

In conclusion, our data remark that two missense mutations affecting the same amino acid, correlate with traits differing for pattern of transmission and clinical features in unrelated patients, and confirm the critical role of the transmembrane domain in caveolin function.

A NOVEL CYSTEINE-TO-TYROSIN MUTATION AT THE CYSTEINE-RICH DOMAIN OF DYSTROPHIN IS ASSOCIATED WITH DMD

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Molecular diagnosis of DMD/BMD is particularly challenging when large deletions or duplications of the dystrophin gene are absent. In such cases, time-consuming screenings for small mutations are required. An additional complication is when nonsense or frame-shift mutations are also excluded. Since a large variety of amino acid substitutions are also found in normal subjects, it becomes difficult to recognize true causative mutations.

We studied 126 DMD/BMD assigned on the basis of the onset/development of the disease, X-linked inheritance, absence/reduction of dystrophin and absence of deletion/duplications. All DNA samples were assembled in pools and PCR reactions were performed to screen the full exon and flanking intron set. Amplicons were screened by DHPLC and heteroduplex fragments fully sequenced. In addition to 45 clear-cut causative small mutations; a number of other cases remained unresolved. However, we detected a single novel missense mutation C3337Y (c.10010 G>A) in a DMD patient. This mutations replace a cysteine in the region of dystrophin involved in the beta-dystroglycan binding. Mutation of the following cysteine was already observed in another DMD case. These two patients constitute a valuable tool to get in-depth insight into the role of dystrophin-dystroglycan binding for muscle function.

SERCA1 AND CALSEQUESTRIN STORAGE MYOPATHY: A NEW SURPLUS PROTEIN MYOPATHY

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We report on four patients, from four different families, affected by a mild myopathy or asymptomatic elevated serum creatine kinase levels. The histological striking features of our cases were the presence of many inclusions within the muscle fibers of which we distinguished two types according to their morphology in resin-embedded samples stained with toluidine blue: type 1 inclusions were quadrangular and stained deep blue while type 2 inclusions were round and pale-blue. They were not detectable on hematoxylin and eosin and modified Gomori trichrome stained sections where single/multiple clear vacuoles of different size and shape were evident within the muscle fibers. The sarcoplasmic or endoplasmic reticulum calcium 1 (SERCA1) ATPase and/or calsequestrin reactivity of inclusions by immunohistochemistry and the SERCA1 and calsequestrin increased expression by immunoblot suggested that inclusions were constituted by an excess of resident proteins in the terminal cisternae of sarcoplasmic reticulum. Our cases, both sporadic and familial, represent a new type of surplus protein myopathy.

NOVEL MUTATIONS IN LAMIN A/C GENE

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Maintenance of nuclear integrity is critical for the survival of highly mechanical-stressed tissues such as muscle. Lamins A and C play the major role as structural elements, organizing the nuclear lamina underneath the inner nuclear membrane. Mutations of the lamin A/C gene cause different human pathologies affecting various tissues, from heart (CMD1A) and muscle (LGMD1B, EDMD2/3) to adipose tissues (FPLD), but they are also responsible for more generalized diseases, such as progeria and progeroid syndromes.

We screened the lamin A/C gene in a population of sixty patients. These show a variable clinical phenotype, including LGMD without heart involvement, typical Emery-Dreifuss phenotype with or without heart involvement, and congenital myopathy.

We identified ten heterozygous putative mutations (16.7%). Among these, six mutations were novel: four missense mutations (N39S, R89C, R249W and R527C) affecting exons 1, 4 and 9 respectively, and two deletions [103_105 delCTG (L35del) and 367_369delAAG (K123del)] affecting exons 1

and 2. Lamin A/C gene test should be included in the protocol of analysis in all unresolved cases of genetic myopathies.

SELECTIVE HYPOTROPHY OF PECTORALIS MUSCLES REVEALS AN ATYPICAL CASE OF FSHD

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We describe the case of a 35-year-old man who noticed isolated hypotrophy of his pectoralis muscles during exercise training. He was working in a fitness center as a body-builder and six months before our observation he observed progressive wasting of his right pectoralis muscle later extending to his left side. Family history was negative for neuromuscular disorders. Laboratory analyses revealed moderate hyperCKemia (240 U/L). Clinical examination showed conspicuous muscle development except for hypotrophy of pectoralis major muscles, normal muscle strength in all the district; no facial weakness or scapular winging was observed. Thyroid function was normal, radiography of the cervical spine, cervical magnetic resonance (MRI) were not significant. Muscle ultrasound examination and MRI showed non-homogeneous aspect of the right pectoralis major muscle. Electromyography disclosed myopathic signs only in the pectoralis major muscles. Open brachialis muscle biopsy demonstrated moderate fibre size variability and many internal nuclei. Immunohistochemical analysis and immunoblotting excluded alterations of structural proteins. Molecular analysis with Southern blot of the EcoRI-digested genomic DNA, using probe p13E-11, detected a fragment of 31 Kb, diagnostic of facioscapulohumeral muscular dystrophy (FSHD). Isolated involvement of pectoralis muscles without other signs has never been reported in FSHD and could have been triggered by repeated localized muscle injuries effected by body-building.

VARIABLE ALTERATION OF ECM COMPONENTS IN DIFFERENT MUSCULAR DYSTROPHIES

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To better understand the role of decorin and biglycan in muscle fibrosis, we investigated their transcript expression by competitive PCR and real time PCR and protein expression by immunohistochemistry and immunoblot, in skeletal muscle of several forms of muscular dystrophy.

Biglycan mRNA levels varied in DMD, MDC1A and LGMD2A, but were greatly increased in adult BMD, sarcoglycanopathies and dysferlinopathy. In DMD and MDC1A, decorin mRNA was significantly downregulated whereas TGF- β 1 was significantly upregulated. Decorin mRNA was normal in paediatric BMD, but upregulated in adult BMD, sarcoglycanopathies and dysferlinopathy, in these patients

TGF- β 1 was normal or moderately increased. In LGMD2A patients both decorin and TGF- β 1 transcripts were downregulated. By immunohistochemistry, decorin and biglycan were mainly localized in muscle connective tissue; their presence increased in relation to increased fibrosis in all dystrophic muscle. The intensity of decorin bands on immunoblot, quantitated against vimentin and normalized against sarcomeric actin, was significantly lower in DMD and MDC1A than in age-matched controls. Variations in the transcript and protein levels of these proteoglycans in different muscular dystrophies probably reflect the variable disruption of extracellular matrix organization that occurs in these diseases. The significantly lowered decorin levels in DMD and MDC1A may be related to the increased TGF- β 1 levels, suggesting a therapeutic role of decorin in these severe dystrophies.

Author Index

- Aguenouz M, 48, 52, 53, 57
 Ali G, 50, 56
 Amati A, 53
 Amini M, 55
 Angelini C, 43, 45, 46, 54, 57, 58, 62
 Angelozzi C, 43, 51
 Armani M, 54
 Assereto S, 60
 Aurino S, 48
 Badiali L, 45
 Baggi F, 42
 Battini R, 43, 51
 Belicchi M, 42
 Benchouir R, 42
 Berardinelli A, 43, 45, 51, 56
 Bernasconi P, 42, 62
 Bertini E, 43, 45, 51, 55, 57, 59
 Biancheri R, 45, 60
 Bianchi M.L, 60
 Bitto A, 52
 Boffi P, 43, 51
 Boito C, 45
 Bonifazi E, 58
 Bonneman C, 59
 Bordoni A, 50
 Borgione E, 43, 60
 Borsato C, 43, 46
 Bottinelli R, 42
 Brahe C, 43, 51
 Brancalion B, 47
 Bresolin N, 42, 46, 49, 50
 Broccolini A, 44, 49, 60
 Bruno C, 43, 45, 51, 53, 55, 59
 Buemi M, 46
 Cagliani R, 49
 Calabrese G, 43
 Calzolari E, 57
 Cannistraci E, 57
 Capasso M, 44
 Cappelletti C, 42, 62
 Cappellini A, 50
 Cardani R, 51
 Carlesi C, 44, 50, 56
 Carrozzo R, 55
 Cassandrini D, 53
 Castello F, 43, 60
 Catteruccia M, 44, 47, 50
 Cenacchi G, 45
 Chiatamone S, 44
 Cini C, 43, 51
 Ciranni A, 53
 Colantoni L, 49
 Colitto F, 43, 51
 Comi G.P, 42, 46, 49, 50
 Comi L.I, 54
 Confalonieri P, 42
 Cossu G, 42
 Crugnola V, 50
 D'Amico A, 43, 45, 45, 51, 55
 D'Amico F, 61
 D'Angelo M.G, 42, 46, 49
 D'Ascenzo C, 46
 Dal Borgo R, 43
 D'Antona G, 42
 Dassi P, 60
 De Ambroggi L, 59
 De Giorgi, 45
 De Luca G, 57
 Del Bo R, 46, 49
 Del Corona A, 56
 Del Vecchio W, 50
 DellaMarca G, 44
 Di Blasi C, 58
 Di Filippo T, 53, 54
 Di Giacomo R, 44, 47, 50
 Di Giorgio R.M, 57
 Di Lella G M, 49
 Di Leo R, 46
 Di Muzio A, 44
 Di Tomo P, 44
 Donati M.A, 60
 Dubini A, 60
 Elia M, 43, 60
 Evoli A, 47
 Fagiolari G, 42
 Falace A, 45
 Falorni M, 56
 Falsaperla R, 47, 48
 Fanin M, 43
 Farina L, 58
 Ferlini A, 56, 57
 Filippini M, 56
 Filosto M, 48
 Fiorillo C, 48, 50
 Fontanini G, 56
 Fortunato F, 49
 Franzini M, 44, 56
 Fruguglietti E, 50
 Frulio R, 60
 Frusciante R, 49
 Galluzzi F, 44
 Galluzzi G, 47, 49, 50, 54
 Gandossini S, 59
 Garcia L, 42
 Garelli F, 54
 Gazzero E, 60
 Giacca M, 52
 Gidaro T, 44, 49
 Giusti B, 55, 59
 Gliubizzi C, 44, 49
 Gorni K, 56
 Gozzo F, 47
 Ghezzi S, 46
 Grigoli L, 61
 Grisanti S, 53
 Gualandi F, 56, 57
 Guglieri M, 42, 46, 49
 Iodice V, 50
 L'Erario R, 61
 Lamperti C, 42, 49, 50
 Lanzillo R, 48, 50
 Lisanti MP, 60
 Lucarini L, 55, 59
 Lucchiari S, 49, 50
 Macaione V, 57
 Magri F, 46, 49
 Mancinelli E, 51
 Mancuso M, 44, 48, 50
 Manganelli F, 50
 Mantegazza R, 42
 Maraldi N.M, 59
 Marchesi C, 42
 Mariani E, 42
 Marini M, 61
 Martinelli G.N, 45
 Maciullo M, 52
 Mattioli E, 55, 59
 Mazzeo A, 57
 Mela J, 47, 49, 50
 Meola G, 51, 59
 Mercuri E, 43, 45, 51, 55, 57
 Merlini L, 52, 57, 59
 Messina S, 43, 51, 52
 Miceli C, 47, 48
 Migliorato A, 52
 Minetti C, 51, 55, 57, 60
 Minetti M, 43
 Minicuci G, 47
 Mirabella M, 42, 44, 49, 55
 Modoni A, 52, 59
 Moggio M, 42, 46, 49, 50, 57
 Mollica C, 50
 Mongini T, 43, 51, 57, 61, 62
 Mon-Li Chu, 55
 Mora M, 42, 46, 49, 56, 57, 58, 62
 Morandi L, 42, 43, 49, 51, 52, 58, 60, 62
 Moroni I, 58
 Morosetti R, 44, 49
 Mostacciolo M.L, 54
 Musumeci O, 46, 53
 Musumeci S.A, 43, 60
 Mutani R, 62
 Napoli L, 46
 Nascimbeni A, 46
 Neri G, 43, 51
 Nigro V, 48, 57, 61
 Novelli G, 58
 Nuti S, 56
 Olivero N, 62
 Orcesi S, 43, 51
 Pace M, 44
 Palladino A, 54
 Palmucci L, 61, 62
 Pandolfi A, 44
 Pane M, 43, 45, 45, 51
 Pantaleoni C, 58
 Panzeri M, 59
 Paolicchi A, 44, 56
 Parisi L, 53, 54
 Passamano L, 54

Pastorello E, 54	Romeo V, 58	Tarantino L, 45
Patti A, 57	Rossi A, 45	Tasca G, 55, 60
Pedemonte M, 45, 60	Rotondo G, 51	Tessa A, 45, 45
Pegoraro E, 43, 45, 46, 58	Ruggieri A, 58	Testa D, 53, 54
Pelle A, 50	Russo M.A, 55	Tiziano F.D, 43, 51
Pelliccioni M, 43, 51	Sabatelli M, 59, 60	Tomelleri G, 48, 54, 61
Pennisi E.M, 54	Sabatelli P, 55, 59	Tonali P, 52, 59
Pepe G, 55, 59	Salani S, 49, 50	Tonali P.A, 44, 49, 55, 60
Perrini S, 48	Sale P, 55	Tonin P, 48, 54, 61
Pescatori M, 44, 55, 60	Salerno A, 55	Tonoli E, 60
Petrini S, 45, 55, 59	Salvatore M, 50	Torella A, 61
Piantadosi C, 55	Salvi A, 55	Torrente Y, 42
Piazza S, 44, 50, 56	Sansone V, 51, 59	Toscano A, 46, 53
Piemonte F, 59	Santorelli F.M, 45	Trabanelli, 57
Piluso G, 61	Santarelli L, 54	Traverso M, 60
Pini A, 43, 51, 56	Santoro L, 48, 50	Trevisan C.P, 54
Piro F.R, 55	Santoro M, 52, 59	Trimarco A, 57, 61
Pistilli N, 55	Saraifoger S, 60	Tupler R, 62
Pistoiesi S, 50, 56	Saredi S, 42, 58	Tupler R.G, 54
Polidori L, 54	Scapolan S, 60	Uncini A, 44
Politano L, 54, 57, 61	Scarpelli M, 48, 55	Uziel G, 58
Pompella A, 56	Scoto M, 47, 48	Vattemi G, 48, 61
Prelle A, 46, 49, 62	Scuderi C, 43, 60	Vazza G, 54
Prinster A, 50	Sergi A, 46	Ventriglia V.M, 61
Priori A, 42	Serlenga L, 53	Venturoli A, 57
Quarantelli M, 50	Servidei S, 44, 47, 49, 50	Verardo M, 55
Ragno M, 44	Siciliano G, 44, 50, 56	Verardo R, 49, 50
Ravaioli S, 59	Silvestri G, 52, 59, 60	Vercelli L, 62
Ricci E, 44, 45, 49, 55, 60	Perrini S, 48	Villanova M, 43, 51
Ricci G, 44	Sinicropi S, 46	Vita G, 43, 46, 51, 52, 53, 57
Rimessi P, 57	Solari A, 51	Vitello A, 60
Rizzato N, 48, 61	Soscia E, 48	Vittonatto E, 62
Rizzo G, 43	Sotgia F, 60	Volpi L, 44, 50, 56
Roccella M, 53, 54	Squadrito F, 52	Zanotti S, 62
Rodolico C, 46, 53, 57	Squarzoni S, 59	Zara F, 60
Romano C, 48	Stramare R, 43	Zecca C, 49
Romeo G, 48	Stringara S, 60	Zoccolella S, 53

PROGRAM OF MDR 2006

Palazzo Bò- University of Padova

30 June - 01 July 2006

Friday 30 June 2006

MITOCHONDRIAL AND METABOLIC DISORDERS: DIAGNOSTIC CLUES AND NEW TRENDS IN THERAPY

- 9.00 a.m. INTRODUCTION
9.30 a.m. Di Mauro S. (New York, USA): MITOCHONDRIAL DISORDERS: AN UPDATE
10.30 a.m. Zeviani M. (Milano, Italy): MITOCHONDRIAL MYOPATHIES OF NUCLEAR ORIGIN
11.00 a.m. *Coffee break*
11.30 a.m. Pichiecchio A. (Pavia, Italy): MRI IN THE MANAGEMENT OF GLICOGENOSIS TYPE 2
12.00 a.m. Florence J. (St.Louis, USA): THERAPY IN POMPE'S DISEASE
12.30 p.m. Hilton-Jones D. (Oxford,UK): MANAGEMENT OF MYOTONIC DYSTROPHY
13.00 *Lunch*
13.00-15.00 p.m. POSTERS DISCUSSION

LIMB GIRDLE DYSTROPHIES : GENOTYPE/PHENOTYPE CORRELATIONS

- 15.00 p.m. Angelini C. (Padova, Italy): CLINICAL HETEROGENEITY OF LGMD
15.30 p.m. Schoser B. (München, Germany): LGMD 2H OR SARCOTUBULAR MYOPATHY
16.00 p.m. Walter MC (München, Germany): PHENOTYPIC VARIABILITY IN LGMD2I
16.30 p.m. *Coffee break*
17.00 p.m. Nigro V. (Napoli, Italy): MOLECULAR DIAGNOSIS OF LGMDs
17.30 p.m. Vita G. (Messina, Italy): NF- κ B INHIBITORS AND GROWTH FACTORS IN MDX MICE
18.00 p.m. Mishra S.K. (Los Angeles, USA): DISTAL MYOPATHIES

Saturday 1st July 2006

MOLECULAR DIAGNOSIS AND PATHOGENESIS OF CONGENITAL MUSCULAR DYSTROPHIES AND CARDIOMYOPATHIES

- 9.00 a.m. Muntoni F. (London, UK): MUSCULAR DYSTROPHIES SECONDARY TO ALPHA- GLYCOSYLATION DISORDER?
10.00 a.m. Mercuri E.– Pegoraro E.: ITALIAN NETWORK ON CMD
11.00 a.m. Danieli G.A. (Padova, Italy): GENETIC CARDIOMYOPATHIES: AN UPDATE ON NEW LOCI
11.30 a.m. Nigro G. (Napoli, Italy): TREATMENT OF GENETIC CARDIOMYOPATHIES
12.00 a.m. Bresolin N. (Milano, Italy): THERAPY IN MUSCULAR DISEASES WITH STEM CELLS

MDR 2006

Palazzo Bò- University of Padova

30 June-01 July 2006

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LECTURES

S.Di Mauro (New York, USA) – METABOLIC MYOPATHIES

D.Hilton-Jones (Oxford, UK) – MANAGEMENT OF MYOTONIC DYSTROPHY

G.A. Danieli (Padova, Italy) – GENETIC CARDIOMYOPATHIES

LOCAL ORGANIZING COMMITTEE

M.Cassol, E.Pegoraro, L. Verganie - mail: lab.neuromuscolare@unipd.it

GENERAL INFORMATION

This is the sixth meeting always trying to integrate clinical practice and experimental research. We expect in a two day meeting to have several outstanding lectures and contributions on the following topics:

Molecular biology and diagnosis in LGMDs

New molecular techniques (microarray/proteomics).

Advances in treatment of DMD, metabolic myopathies, myositis, myotonic dystrophy and in other muscle diseases. This satellite meeting of XIth International Congress on Neuromuscular Diseases, Istanbul Turkey is addressed toward advances and new trends in Muscular Dystrophy 30/06-01/07/2006.

CALL FOR PAPERS

Submission of abstract for posters on neuromuscular diseases will be done at: lab.neuromuscolare@unipd.it

Deadline for abstract submission: 31 may 2006Registration fee 50 euro.