2nd AiM National Congress

Turin (Italy), June 14 -15, 2002

Palazzo Carignano, Museo del Risorgimento Italiano
Sala delle Conferenze
Sala del Parlamento Italiano
Via Accademia delle Scienze, 5
Turin, Italy

In October 2001 at Camogli the First National Congress of the Italian Association of Myology (*Associazione Italiana di Miologia*, AIM) first summoned most Italian clinicians dealing with adult and pediatric neuromuscular diseases. The meeting had great success. Thanks to the impressive scientific advancements in the field, Myology has become a discipline in itself, closely linked with almost all the other branches of Medicine. Due to the great number of important and interesting contributions and the potentiality of its members, Italian Myology represents a meeting point of physicians and researchers with different backgrounds and origins. This is a brand new idea of a scientific association. The Second National Congress, that will take place in a surrounding with great historical meanings, namely the Hall of the Italian Parliament in Palazzo Carignano, Torino, will see a further step towards multidisciplinarity. In fact the two Magistral Lectures are centered on two different aspects of neuromuscular diseases: basic research, specifically "The Molecular Bases of Muscle Atrophy", and therapy, with two lectures considering the intensivist's and the pneumologist's viewpoints about the most advanced treatments of respiratory insufficiency secondary to muscle impairment. As an invited speaker prof. DiMauro will honour us with a lecture about glycogenoses.

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Program of the 2nd AiM National Conference

June 14-15, 2002, Turin, Italy

Friday, June 14, 2002

8.30-9.00	Conference Openings
9.00-9.30	Lecture (Chairwoman: Laura Palmucci, Torino): **Molecular bases of muscular atrophy** *Prof. Stefano Schiaffino, Padova, Italy*
9.30-10.45	Session I: Pathogenic Mechanisms *Chairmen: Corrado Angelini, Padova, Giuseppe Vita, Messina*
10.45-11.00	Coffee Break
11.00-11.45	Lecture: **Acute and chronic respiratory insufficiency in myopathies** **Intensivist experience**, *Prof. V. Marco Ranieri, Turin, Italy* **Pneumologist experience**, *Dr. Andrea Vianello, Padova, Italy*
9.30-10.45	Session II: Pathogenic Mechanisms *Chairmen: Lucia Morandi, Milano, Carlo Minetti, Genova*
13.00-14.00	Lunch
14-00-15.30	*Poster session 1* *Chairmen: Luisa Politano, Napoli, Gabriele Siciliano, Pisa* *Poster session 2* *Chairmen: Giuseppe Dilorio, Napoli, Carlo P. Trevisan, Padova*
15.30-16.45	Session III: Genotype-phenotype correlation *Chairmen: Laura Palmucci, Torino, Roberto Rigaldetto, Torino*
16.45-17.00	Coffee Break
17.00-18.00	Session IV: Genotype-phenotype correlation *Chairmen: Giovanni Meola, Milano, Carlo Doriguzzi, Torino*
18.00-19.00	Convention of AiM members
20.30	Social Dinner

Saturday, June 15, 2002

8.30-10.00	Muscle Club Session: Discussion of clinical cases *Chairmen: Tonino Uncini, Chieti, Luciano Merlini, Bologna*
10.00-10.30	Invited Lecture (Chairman: Pietro Tonali, Roma): **Glycogenoses** *Prof. Salvatore DiMauro, New York, USA*
10.30-10.45	Coffee Break
10.45-12.00	Session V: Mitochondrial Encephalomyopathies *Chairmen: Serenella Servidei, Roma, Maurizio Moggio, Milano*
12.00-13.00	Session VI: Free communications
13.00	Conclusive remarks

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June 14-15, 2002, Turin, Italy

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G. Azan, G. Miscio, L. Priano, G. Traversa, A. Mauro

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OXIDATIVE STRESS AND APOPTOSIS INDEXES IN MUSCLE SPECIMENS FROM DMD AND SMA PATIENTS

A Berardinelli*, F Blandini §, A Mangiagalli §, F Morello*, A Samuele§, G Lanzi*

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D Biral[1], R Betto[1], D Danieli-Betto[2], S Picunio[2], E Germinario[2], H Chomontowska[3], Jakubiec-Puka[3]

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I Bosone, S Bortolotto, L Chiadò-Piat, I Ugo, C Borghese, L Vercelli, R Tupler*, R Mutani, C Doriguzzi, T Mongini, L Palmucci

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C Bruno, FM Santorelli°, O Sacco*, M Bado, GA Rossi*, C Minetti

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R Cagliani*, A Gallanti^, M Sironi*, P Ciscato^, V Cardin^, S Bonato^, S Galbiati^, L Chiveri^, S Corti^, A Prelle^, M Moggio^, N Bresolin^*, GP Comi^

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R Falsaperla, A Di Giorgio, [#]G Romeo, A Sorge, T Trigilia, P Pavone

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L Pasquali[1,2], P Zhao[1], K Gorni[1], FW Booth[3], B Tseng[4], M Bakay[1], Y Chen[1], EP Hoffman[1]

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PROTON MAGNETIC RESONANCE SPECTROSCOPY IN MITOCHONDRIAL DISEASES: METABOLIC ABNORMALITIES AS PHENOTYPIC APPEARANCE OF BRAIN DISEASE

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THE DYSTROPHIN ROD-DOMAIN IS ALTERNATIVELY SPLICED IN BOTH NORMAL HUMAN TISSUES AND IN DMD/BMD SKELETAL MUSCLE

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PROXIMAL REVERSIBLE UPPER LIMB MYOPATHY WITH LOSS OF MYOSIN FILAMENTS

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We report on a 58 year-old man complaining for eight months of difficulty in rising his arms above the head and proximal upper limbs muscle weakness. Cervical NMR showed only mild spondylosis. CK level was increased four times and EMG recording revealed a myopathic pattern on proximal upper limb muscles. Biopsy of deltoid muscle showed patchy loss of ATPase activity in nearly 15 % of muscle fibers, which were also atrophic; few type 2 fibers were observed. Electron microscopy revealed a selective loss of thick myofilaments. Investigation of muscle proteolytic pathways disclosed an activation of calpain mediated proteolysis in the atrophic fibers. Four years later, on examination, the patient had regained normal upper limbs movements and strength. We discuss the pathological analogies between our case and what reported in acute quadriplegic myopathy.

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FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY: A MULTICENTER STUDY ON CARDIAC INVOLVEMENT AND ITS CORRELATION WITH THE 4q35 DELETION

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PRIMARY MYOPATHY IN A NEW CASE OF THE CAREY-FINEMAN-ZITER SYNDROME

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2nd AiM National Conference
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Abstracts of the 2nd AiM Conference

June 14-15, 2002, Turin, Italy

[1] CENTRONUCLEAR MYOPATHY AND PERIPHERAL NERVE DISORDER: A CLINICO-PATHOLOGICAL STUDY

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Centronuclear myopathy is a congenital muscle disease characterized by clinical and genetic heterogeneity. From a genetic point of view different forms are recognized as being autosomal dominant and X-linked recessive. The former seems clinically to be the least severe, compared to the latter marked by earlier onset and occasional involvement of central and peripheral nervous system. The autosomal recessive mode of inheritance is not acknowledged with clear certainty.

A 50 years old female was referred for evaluation of weakness, which had showed an extremely slow progression since childhood. The patient suffered discomfort in walking and climbing stairs. The neurological examination revealed facial weakness; amyotrophy and weakness of arms and legs evident at proximal and distal level; hyporeflexia; scoliosis, lordosis and pes cavus. Electromyography showed neurogenic and myogenic features; lack of myotonic pattern was evident. Sural nerve biopsy revealed a chronic axonal neuropathy. On biopsy muscle fibres featured centrally placed nuclei whose area was intensely stained by oxidative enzymes and depleted of ATPase stain. An identical clinical picture, except for being less severe in gravity, affected the elder daughter aged 30 years. Neither ophthalmoplegia nor ptosis were present in the mother and the daughter. The occurrence of peripheral nerve involvement in centronuclear myopathy is an instance of the variable expressivity of centronuclear myopathy, whose dealing in depth with the myotubularin related genes would shed light on the pathogenesis of the disease.

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[2] OXIDATIVE STRESS AND APOPTOSIS INDEXES IN MUSCLE SPECIMENS FROM DMD AND SMA PATIENTS

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The pathogenesis of DMD (Duchenne Muscular Dystrophy) and SMA (Spinal Muscular Atrophy) remains unknown, despite many years of active research. Among many theories, it has been hypothesized that the necrosis induced by free radicals may be one possible cofactor in DMD and that an alteration of neuronal apoptosis may play a significant role in SMA.

The most significant studies now available have been carried out on mouse frozen muscle samples and on mouse myoblast cultures. Studies utilizing human muscle are very rare.

In our research we determined oxidative stress and apoptosis indexes in muscle specimens from 9 DMD and 9 SMA patients and in a control population of 11 normal individuals.

We considered the following parameters: superoxide dismutase (SOD) in cytoplasm and in mitochondria, and glutation (GSH) as oxidative stress indexes; Bcl-2 and caspase 3 as apoptosis indexes, looking for a different expression among the selected groups of individuals.

We found significant differences in Bcl-2 and caspase 3 activity in patients and in controls, consistent with presence of apoptosis; moreover, we observed an increase of GSH, which is more difficult to interpret.

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[3] APOPTOSIS-RELATED PROTEINS AND MYOGENIC REGULATORY FACTORS IN SKELETAL MUSCLE DURING DENERVATION ATROPHY AND RECOVERY FOLLOWING SELF-REINNERVATION

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Denervation of skeletal muscle causes progressive atrophy of fibres and impairment of functional properties. Reinnervation, in turn, sees the complete recovery of tissue mass and function. This study investigated whether apoptotic events and/or myogenic regulatory factors are associated to the loss of tissue mass and recovery. Rat soleus muscle was examined after crushing of the sciatic nerve. After 7 days, the highest number of TUNEL-positive nuclei was observed. The expression of apoptosis-related proteins (bcl-2, bax, and caspase-3, -7, and -9) was also noted. In parallel, a significant number of nuclei positive to myogenin was evident. Recovery of soleus muscle took place after self-reinnervation, which occurred at about the 15th day. During recovery, the number of TUNEL-positive nuclei was highly reduced to levels as in controls, while myogenin protein expression was still evident. Consistently, no morphological signs of apoptosis were found in muscle fibres. Surprisingly, the subsarcolemmal region of numerous large fibres revealed strong immunoreactivity of anti- and pro-apoptotic proteins (bcl-2 and bax). However, these regions were completely negative with anti-caspase antibodies (-3, -7 and -9). On the other hand, in the same fibers, H-E staining revealed the presence of basophilic rings in the subsarcolemmal region. Electron microscopic analysis demonstrated in these areas the accumulations of polisomes, mitochondria, endoplasmic reticulum and of newly formed myofibrils, revealing the occurrence of an intensive protein synthesis. Present results indicate that components of the Bcl-2 fam-

ily may act in skeletal muscle both as modulators of the apoptotic process and of cell anabolism.

Supported by TELETHON Italy (grant 1286 to RB) and Nencki Institute of Experimental Biology (Internal grant 206 to AJP).

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[4] FACIO-SCAPULO-HUMERAL MUSCULAR DYSTROPHY: REPORT OF TWO CASES WITH UNUSUAL HISTOPATHOLOGICAL FEATURES

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We present two unrelated cases of facio-scapulo-humeral muscular dystrophy with unusual histopathological findings. The first came to our observation at the age of 52 following progressive difficulty walking. Neurological examination showed stepping gait with marked impairment of the flexion of the foot, facial muscle weakness and wasting of the upper limb girdle. Creatine kinase was 381 U/l. Muscle biopsy showed, beside fiber size variability, a large number of rods and some fibres with mitochondrial disorganization and lipid increase. Genetic analysis demonstrated a fragment of 30 Kb originating from the distal end of chromosome 4. The study of the family showed that the disease was present also in the patient's mother and his two siblings.

The second case was a 58-year-old man who denied family history of neuromuscular diseases and came to the observation for easy fatigability and asymmetry of the shoulders. Neurological examination disclosed facial muscle weakness, wasting of the upper limb girdle with winging of the scapulae especially the right one and severe limitation of the movements of the right shoulder and arm. Muscle biopsy was characterized by prominent lipid increase with normal carnitine. DNA analysis showed a fragment of 24 Kb originating from the distal end of chromosome 4, which was found also in one of the patient's two daughters.

Although it is known that muscle biopsy in FSHD is non-specific and pseudo-inflammatory alterations are often observed, neither lipid storage nor rods have been so far described in genetically confirmed FSHD. These observations may offer new insights in the pathogenesis of the disease.

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[5] MYOPATHY AND SEVERE RESPIRATORY FAILURE ASSOCIATED WITH A NOVEL MUTATION IN THE MITOCHONDRIAL TRNA GLUTAMIC ACID GENE

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We report a novel T14687C mutation in the mitochondrial tRNA glutamic acid gene in a 16-year-old boy with myopathy and respiratory failure. The propositus presented at age 12 with exercise intolerance, fatigue, tachycardia and shortness of breath. In the following years these symptoms had progressed to the point that he could not perform the normal daily activities. He never complained of cramps, myalgia or pigmenturia. Physical examination revealed severe general conditions, respiratory difficulties, cyanosis, and generalized muscle weakness. Lactic acid levels were increased. Thoracic ultrasound showed interstitial pneumopathy. Electromyography (EMG) showed a myopathic pattern. Histochemical analysis of muscle biopsy showed ragged-red COX negative fibers. Biochemical analysis of muscle homogenate revealed partial defect of complex I and complex IV enzyme activities. Large-scale mtDNA rearrangements were excluded by Southern blot analysis and by the PCR screening procedure. By sequencing the 22 tRNAs gene, we identified a T-to-C transition at nucleotide position 14687 in the tRNA glutamic acid. By PCR/RFLP analysis the mutation was nearly homoplasmic in muscle DNA of the proband, but it was absent in his blood and in the blood of his asymptomatic mother, suggesting that it may have been a spontaneous somatic mutation in muscle. By single muscle fiber PCR analysis the mean proportion of the mutant mtDNA in the COX-negative fibers was 95.08±3.08 (n = 20), and that in the COX-positive fibers was 88.90±5.46 (n = 20). This finding was significant (p=0.028, t-test).

This report further enlarges the repertoire of mtDNA mutations and the association of tRNA mutations with respiratory muscle involvement.

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[6] EXERCISE INTOLERANCE RESULTING FROM A NOVEL MUSCLE-RESTRICTED MUTATION IN THE MITOCHONDRIAL CYTOCHROME B GENE

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Exercise intolerance and isolated muscle complex III deficiency due to mutation in the cytochrome b gene of mitochondrial DNA has been recently recognize as a distinct clinical phenotype.

We identified a novel nonsense mitochondrial cytochrome b mutation in a 40-year-old woman with isolated progressive exercise intolerance, muscle cramps and lactic acidosis, and combined deficiency of complexes I and III in muscle.

Neurological examination revealed proximal muscle weakness. Electromyography (EMG) displayed a myopathic pattern. Laboratory investigations showed increased serum concentrations of lactate. Muscle biopsy showed several ragged-red fibers, strongly positive to cytochrome c oxidase staining. Biochemical analysis on muscle homogenate of respiratory chain complexes, revealed a reduction of complexes I and III, normalized to that of citrate synthase.

Direct sequence analysis of the mitochondrial cytochrome b gene revealed the presence of a novel nonsense mutation leading to the replacement of a glutamine with a stop-codon. The mutation was heteroplasmic in the patient's muscle, while it was absent in other examined tissues.

Clinical presentation and laboratory data indicate that this molecular defect is the primary cause of the disease in our patient, enlarging the spectrum of cytochrome b mutations in patients with isolated exercise intolerance and lactic acidosis.

As recently hypothesized by Lamantea et al in a similar clinical case, a primary defect of one of the respiratory chain complexes could interfere on the integrity of the respiratory system, thus explaining the peculiar combined deficiency of complexes I and III.

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[7] A NOVEL SPLICE-SITE MUTATION IN A LGMD-2B FAMILY CAUSING ACTIVATION OF A CRYPTIC SITE AND TOTAL DYSFERLIN ABSENCE

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Mutations in the dysferlin gene cause limb-girdle muscular dystrophy type 2B (LGMD-2B) and Myoshi myopathy. Dysferlin is supposed to be a vesicle-associated membrane protein involved in fusion of large vesicles and membrane docking.

Here we present the case of two sisters, affected by LGMD-2B, carrying a new mutation in the gene. The proband is a 36 year-old woman born from healthy first cousin parents. Her 38 year-old sister is similarly affected. The proband presented progressive lower limb weakness with frequent falls since the age of 20 years. In the following years she also manifested upper limb girdle involvement. The neurological examination showed distal hypotrophy, tibialis anterior, ileopsoas and glutei muscle weakness, absent tendon reflexes, anserine gait with bilateral footdrop. CK was constantly higher than 3,000 U/L. Dystrophic changes were present at muscle biopsy. Dysferlin was absent both by immunohistochemistry and Western blot analysis. The other tested muscle proteins were normal, including calpain 3.

The patients were found to carry a homozygous 3-bp deletion involving exon 45 donor splice-site. Transcript analysis was performed in order to determine the effect of the mutation on mRNA processing: the only detectable product derived from activation of a cryptic splice-site located in exon 45. The aberrant splicing event determines a shift in the reading frame and thus the transcript is predicted to be translated into a truncated dysferlin protein.

This novel dysferlin gene mutation causes a somewhat atypical phenotype in that both limb-girdle and distal muscle compartments are affected.

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[8] A NOVEL AUTOSOMAL DOMINANT CAV3 GENE MUTATION RESULTS IN BOTH RMD AND LGMD IN THE SAME FAMILY

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A remarkable interfamilial phenotypic heterogeneity is observed in association with mutations of the gene encoding the muscle specific caveolin 3 protein (CAV3). Different mutant alleles manifest with several distinct clinical phenotypes: autosomal dominant (AD) limb girdle muscular dystrophy (LGMD) type 1C, idiopathic hyperCKemia, one AD form of Rippling Muscle Disease (RMD) and a variant of distal myopathy. Although this interfamilial variability is mainly accounted for by different CAV3 mutations, three of them (R26Q, A45T and P104L) may cause two different clinical phenotypes in unrelated subjects. Here we present evidence for a new CAV3 mutation, affecting four individuals of a multigenerational family, affected with a range of clinical features that includes all those already reported in other families. The proband is a 51-year-old man, presenting silent muscle contractures after mechanical stimulation of both quadriceps muscles, high CK level (1,120 U/l) and both proximal and distal lower limb weakness. His muscle biopsy showed dystrophic and myopathic features and caveolin 3 immunostaining with monoclonal antibodies was markedly reduced. Sequence analysis of CAV3 gene revealed a heterozygous 3-bp deletion in exon 2. The proband's mother (71 y-o) has high CK level, while a sister, aged 45 years, is affected by LGMD. The proband's 20 year-old son has high CK, toe walking and has been suffering of epileptic seizures. This family summarizes several clinical features of CAV3 mutations and expands our knowledge on the mutational burden of CAV3 gene. The genetic and epigenetic factors that modify the clinical phenotype remain to be investigated.

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[9] DYSFERLIN EXPRESSION DURING THE MYOGENIC DIFFERENTIATION

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Dysferlin is a plasma membrane associated protein that is defective in Miyoshi myophaty and in limb girdle muscular dystrophy type 2B (LGMD2B). Many tissues express dysferlin, in particular skeletal muscle, heart and kidney. Dysferlin co-immunoprecipitates with caveolin-3 in human skeletal muscle, probably trough low-affinity process. Interestingly, mutations in the *CAV3* gene cause the LGMD1C.

We studied dysferlin protein expression in C2C12 muscular cultured cells. Biochemical analysis was done in C2C12 myoblasts, myotubes and in sub-cellular fraction of C2C12 cells after induction of differentiation. Western blotting analysis of proliferating myoblasts showed absence of dysferlin compared to differentiated cells. The time course of dysferlin expression during C2C12 differentiation showed dysferlin after 24 hours and, successively, an increase of protein level after 48 and 72 hours. In the same specimens, caveolin 3 was detectable only after 48 hours of differentiation. To determine the cellular localization of dysferlin by Western blotting analysis, soluble cytosol and total membranes fractions were obtained from C2C12 cells. The specific protein band was present only in the membrane fraction, whereas in the cytoplasmic fraction, dysferlin was absent. In order to investigate the relationship between dysferlin and caveolin-3, a p38 MAP kinase inhibitor, SB203580, which blocks the expression of caveolin-3 and inhibits the myotube formation, was used. While the expression of several specific muscle differentiation markers was unaffected (troponin-T, MHC, β-dystroglycan, dystrophin), dysferlin expression was severely reduced. These data indicate that dysferlin expression is modulated during the myogenic differentiation, probably in association with caveolin 3-related signalling pathways.

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[10] MITOCHONDRIAL CYTOPATHY WITH MULTIPLE SIMMETRIC LIPOMATOSIS: A CASE REPORT

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A 51-year-old man was referred for progressive weakness in the past four years. He had myoclonic epilepsy for 24 years and bilateral neurosensory deafness for 15 years. There was no history of alcohol abuse. The mother of the patient, a 76-year-old lady, was reported to have lipomatosis but no muscle weakness nor seizures.

Examination showed: large confluent lipomas around the neck, shoulders, trunk and proximal limbs; weakness (MRC grade = 4) in proximal muscles of upper limbs and in proximal and distal muscles of lower limbs; myoclonic jerks of fingers and an ataxic gait. Sensations and tendon reflexes were normal. There was neither ophtalmoplegia nor retinitis pigmentosa. CK were 2870 U/L (n.v.<170). Electrophysiological examinations showed myopatic changes, normal sensory and motor conductions and normal visual evoked potentials.

A biopsy of the left biceps brachialis showed marked variability of fiber size, internal nuclei, several necrotic and regenerating fibers, mild fibrosis and many COX negative ragged red fibers.

This patient confirms the occurrence of multiple symmetric lipomatosis in the context of a mitochondrial cytopathy. Besides multiple symmetric lipomatosis, in the variable combinations of systems involved, the presence of myoclonic epilepsy and the absence of peripheral neuropathy have been rarely reported.

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[11] RECOVERY OF FUNCTION BY ELECTRICAL STIMULATION OF LONG-TERM PERMANENT DENERVATED MUSCLE: ROLE OF MYOFIBER REGENERATION

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Satellite cells are small mononucleated skeletal muscle stem cells located between the basal lamina of the muscle and the sarcolemma of myofibers. Satellite cells are mobilized in response to increased loading conditions or after injury to muscle cell.

The initial event after satellite cell activation is a proliferative response in which some or all of the activated satellite cells undergo at least one mitotic cycle. After this initial phase, some of the activated cells and/or their progeny differentiate into myoblast-like cells. In regenerating muscle, these myoblasts fuse with each other to form new myofibers or become incorporated into existing myofibers. In the case of the hypertrophy response, satellite cell-derived myoblasts are thought to fuse with existing myofibers, thereby providing additional myonuclei. The mechanisms underlying the recruitment of satellite cells for regenerative or hypertrophic processes have not been established, but interactions through cytokines among resident phagocytes, inflammatory cells (macrophages) and satellite cells/myoblasts seem to be essential.

Myoblasts and myotubes express peculiar isomyosins. Light and heavy chains of embryonic myosins are sensitive indicators of myogenic events in adult muscles. Likewise, MyoD and myogenin are markers of cellular differentiation. Two isoforms of MNF (Myocyte Nuclear Factor, a winged helix transcription factor) are reciprocally regulated as satellite cells withdraw from a quiescent state, proliferate, differentiate into myofibers and re-establish a stem cell pool.

Long-term permanent denervation induces severe atrophy of skeletal muscle accompanied by apoptotic loss of myonuclei. Morphologic characteristics of the long-term denervated muscle suggest that the original fibers are lost and those seen are the results of repeated cycles of cell death and regeneration. Electrical stimulation of permanent denervated muscle increases the mean size of the myofibers, maintains the sarcomeres and pos-

sibly prevents secondary degeneration and apoptosis/necrosis. Since satellite cells activity is required for hypertrophy of overloaded adult muscles, positive regulation of activation, division and fusion of myoblasts could be important to the understanding of the limits of recovery of neurogenic muscle myopathies by functional electrical stimulation (FES).

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[12] STUDY OF EXPRESSION AND DISTRIBUTION OF FIBROBLAST GROWTH FACTORS AND THEIR RECEPTORS IN FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is a common muscular dystrophy characterized by progressive involvement of facial and limb muscles and significant clinical and pathological variability. Its gene maps on chromosome 4q35; no transcribed sequences have been identified and the pathophysiology of the disease remains obscure.

Fibroblast Growth Factors (FGFs) are a large family of proteins participating in a wide variety of biological activities which involve cell growth, differentiation, migration and chemotaxis. Specifically they stimulate the proliferation of fibroblasts and they intervene in the pathogenesis of abnormal fibrosis.

It has been reported that the severe clinical phenotype of FSHD may be associated with overexpression of FGF1, FGF2, and FGFR4, leading to abnormal muscle fibrosis.

The purpose of this study is to detect the presence and to study the distribution of FGF1, FGF2 and FGFR4 in muscle biopsy specimens from 16 facioscapulohumeral muscular dystrophy (FSHD) patients, compared with normal control individuals and with patients affected by other muscle diseases, using immunoistochemistry and Western blotting analysis. Our hypothesis is that the overexpression of FGF1, FGF2 and FGFR4 may be part of the pathological mechanism of FSHD. Preliminary results in 3 cases of FSHD with milder phenotype did not disclose altered expression of FGF1, FGF2 and FGFR4; we are now testing FGF expression in more severe cases with fibrotic alterations in muscle biopsy.

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[13] CONCOMITANT INVOLVEMENT OF CARDIAC AND SKELETAL MUSCLE TISSUES IN HIV SEROPOSITIVE CONVERTED PATIENT

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A 32 years old man was admitted to the emergency room of Niguarda General Hospital because of sudden onset of chest pain, dyspnea, and muscle weakness. The physical examination, instrumental and laboratory tests were compatible with the diagnosis of myocarditis. Serum CK levels were about 40.000 U/L (normal values < 185 U/L). Clinical history was negative for heart and muscle diseases. Other causes of heart damage, namely hypertension, diabetes and hypercolesterolemia, were excluded. The patient underwent cardiac external assisted circulation because of heart failure. A myocardial biopsy showed histological changes typical of myocarditis and appropriate therapy was thus started. Since serum CK levels were persistently higher than normal, a muscle biopsy was also performed, which showed mild myopatic changes. During hospitalization HIV positivity was detected, the admission screening being negative.

Myocarditis is documented in 31-81% of AIDS patients with clinical signs of congestive heart failure and it is almost always a late AIDS manifestation. It is characterized by multifocal or diffuse interstitial inflammatory infiltrates associated with degenerative changes. Muscular weakness due to myopathy may develop at any stage of HIV infection and may be an early, even the presenting manifestation of the infection. HIV-related myopathy may present as polymyositis (or other infectious myopathy), toxic myopathy (zidovudine myopathy), nemaline rod myopathy or rhabdomyolisis.

We describe early histological alterations in both skeletal muscle and myocardial tissues during HIV seropositive conversion.

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[14] GLYCOGEN STORAGE DISEASE TYPE III: GENOTYPE AND PHENOTYPE STUDY ON A COHORT OF MEDITERRANEAN PATIENTS

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Deficiency of amylo-1,6-glucosidase, 4- glucanotransferase enzyme (*AGL* or glycogen debrancher enzyme) is responsible for Glycogen Storage Disease type III, a rare autosomal recessive disorder of glycogen metabolism. The clinical manifestations of GSD III are hepatomegaly, hypoglycemia, hyperlipidemia, short stature, cardiomyopathy and myopathy. To date, we reported 15 new mutations in a group of 22 subjects: six nonsense point-mutations: R34X, S530X, R1218X, W1398X, R864X, W1327X; two microinsertions: 1072insT and 4724insAA; one bp deletion: 676 G; two missense: C234R and R675W; four splice-site base changes: IVS6^{+3} A/G, IVS4^{+2} T/A, IVS21^{+1} G/A and IVS26^{+1} G/C (Hadjigeorgiou et al., 1999; Lucchiari et al., 2002a; Lucchiari et al., 2002b). So far, we identified seven subjects carrying the IVS21^{+1} G/A; indeed, this sample of patients shows that this one is the most spread muta-

tion, accounting for 25% of 40 independent alleles. All the mutations detected are disease causative since they impair the protein synthesis or give trunk, unstable protein products. Due to the remarkable extension of the mRNA (35 isoforms, 7kb), the haplotype analysis on genomic DNA was performed by heteroduplex analysis of the shorter PCR products, direct sequencing and PCR-RFLP procedure. We attempted to establish a genotype-phenotype correlation in 11 patients in whom both mutated alleles were detected: our data confirm the extreme genetic heterogeneity of this disease, which precludes any kind of screening of recurrent common mutations. The pathogenic sequence changes we found, together with the ones described in related published papers, may indirectly provide information on the poorly known structure of the AGL protein.

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[15] PENTOXIFYLLINE INHIBITS MUSCULAR DYSTROPHY FIBROBLAST GROWTH: POSSIBLE ANTIFIBROTIC TREATMENT

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Abnormal proliferation of connective tissue within muscle is a striking feature of muscular dystrophies leading to irreversible derangement of muscle and hindering myofiber trophism. In DMD the extent of fibrosis correlates significantly with the expression of TGF- 1, a key cytokine in fibroblast proliferation and collagen synthesis. Since muscle fibrosis may represent a major obstacle to the efficacy of gene therapy in muscular dystrophies, a therapeutic anti-fibrotic protocol should be included. Pentoxifylline (PTX), a methylxanthine-derivative widely used in peripheral vascular disease, inhibits the proliferation of fibroblasts isolated from normal human skin, keloid skin, hypertrophic scars and from skin of patients with pretibial myxoedema. Since increased fibroblast proliferation is a crucial event in ECM proliferation, we tested the *in vitro* effects of PTX on TGF- 1- and PDGF-driven proliferation of fibroblasts isolated from DMD and control muscles. In both DMD and control fibroblasts, TGF- 1 and PDGF induced significant increases in cell growth, 4-fold and 2-fold increase of [^{3}H]-thymidine incorporation, respectively. This confirms previously reported data on hepatic/dermal fibroblasts, and the known fibrogenic effect of these cytokines on cells. PTX decreased significantly both TGF- 1 and PDGF-driven fibroblast proliferation in a dose-dependent manner, even at the lowest dose tested (0.001 mg/ml; P = 0.008 and 0.03, respectively), and at 1 mg/ml PTX totally blocked the proliferative effect of both cytokines. Our findings suggest the utility of extending the studies on this compound to elucidate its mechanism of action on collagen production and also its possible effect *in vivo* as anti-fibrotic treatment in DMD animals models.

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[16] THE FIRST MATERNALLY-INHERITED MUTATION OF MITOCHONDRIAL T-RNA^{HIS} GENE RESULTS IN RETINITIS PIGMENTOSA AND NEURO-SENSORIAL HYPOACUSIA

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We describe a new heteroplasmic mutation at mitochondrial DNA (mtDNA) nucleotide 12,183 - tRNA Histidine (His) gene – detected in three differently-affected related subjects.

The proband is a 30-year-old male patient born after uncomplicated pregnancy and delivery to healthy nonconsanguineous parents. He progressively developed bilateral visual loss, cataract and hypoacusia, dysarthria, retinitis pigmentosa (RP), muscle weakness, hypotonia, muscle hypotrophy, short stature and hypogonadism. Cognitive functions were normal. His mother had RP and bilateral cataract. An older sister presented neurosensorial hypoacusia since childhood and RP. One brother had died immediately after birth.

Proband’s laboratory tests showed lactic acidosis, normal CK levels, reduced testosterone and normal Luteinizing and Follicle-Stimulating Hormone. The EMG was mildly myopathic. The EEG was dysrithmic with irritative features. Brain MRI showed bilateral paramagnetic signals in the caudate and thalamic nuclei and mild vermis atrophy.

Muscle biopsy revealed numerous COX-negative fibers. Both Southern blot analysis and the most common mtDNA point mutations were negative. MtDNA sequence analysis showed a G-to-A transition at position 12,183 in the tRNA His: In the proband, this substitution was almost homoplasmic in skeletal muscle and heteroplasmic in blood (47.3%), whereas different degrees of heteroplasmy were evident in blood DNA from both sister (24.8%) and mother (13.2%). The mutation was absent in 41 healthy controls.

The mutated site is conserved among higher and lower species during evolution and is located in the tRNA variable Loop, therefore leading to incorrect tRNA structure formation. This is the first pathogenic, maternally-inherited mutation involving the mitochondrial tRNA His gene.

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[17] PRIMARY COENZYME Q_{10} (COQ_{10}) DEFICIENCY MANIFESTING AS SLOWLY PROGRESSIVE CONGENITAL ENCEPHALOPATHY: EXPANDING THE CLINICAL PHENOTYPE

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Primary CoQ_{10} deficiency is a rare, treatable disorder with different phenotypes ranging from myopathy to encephalopathy with predominant cerebellar involvement. CoQ_{10} transfers electrons from complex I and II to complex III of mitochondrial respiratory chain; it has antioxidant and membrane stabilizer properties.

A 33-year old woman was diagnosed of having a cerebral palsy in the early months of life. At 18 months severe spastic tetraparesis and cognitive impairment were certified. Neurological symptoms remained stable and at the age of 31 years she developed psychiatric disturbances well controlled by therapy. When examined, she was wheelchair bound and presented trunk and limb ataxia, nystagmus, disarthria, Babinski sign and mild mental retardation. CK, lactate, folate, sialotransferrin, alfa-fetoprotein, vitamin E, lysosomal enzymes and urinary organic acid were all normal. Genetic analysis excluded SCA. EMG was myopatic with low-amplitude sensory action potentials. A sural nerve biopsy evidenced axonal neuropathy. Brain MRI showed cerebellar atrophy and hyperintensity of white matter. A reduction of N-acetil-aspartate and increase of choline were found in brain white matter by MR Spectroscopy. Muscle morphology and respiratory chain enzymes were normal except for severe decrease of muscle CoQ_{10} (9.2 μg/g, n.v. 25±3). Serum CoQ_{10} was 0.50 mg/l (n.v. 0.60-1). Compared to previously reported cases our patient shows distinctive clinical features as predominant spastic tetraparesis, extremely slow progression, axonal neuropathy and brain white matter abnormalities. In view of the good response to supplementation therapy, CoQ_{10} deficiency should be considered in the differential diagnosis of apparently non-progressive "cerebral palsies" when cerebellar atrophy is present.

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[18] MACROPHAGIC MYOFASCIITIS: AN ITALIAN PAEDIATRIC CASE

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Macrophagic myofasciitis (MMF) is a treatable, inflammatory myopathy recently described prevalently in adults and in France. Myalgias, arthralgias, asthenia, muscle weakness, increased CK, and a typical muscular infiltration of non-epithelioid histiocytic cells characterize it. MMF has been considered an autoimmune disorder triggered by intramuscular injection of vaccines containing aluminium-hydroxide.

We report a female born at term after an uneventful pregnancy and without a familial history of neuromuscular disorders. She did not have perinatal problems and showed a regular growth in the first months of life. At 2, 4 and 10 months she underwent to vaccinations against Diphtheria, Pertussis, Clostridium tetani and Hepatitis B by intramuscular injections. The girl was referred at 7 months because of a long lasting general illness state. She was unable to seat unsupported and showed a mild hypotonia of the lower limbs, with normal deep tendon reflexes. Serum CK were elevated and progressively increased during the following months (4-11 x normal). At 12 months she could not stand. Thyroid function, EEG and cerebral MRI were normal. Muscle biopsy of the right quadriceps showed large infiltrates of macrophages (CD68+) with abundant cytoplasm containing PAS positive granules in the perimisium and penetrating in the peripheral endomisium without evident damage of muscle fibers. $CD4^+$ and $CD8^+$ were rare and sparse. Steroid therapy (1 mg/kg/day of prednisone) induced a progressive clinical and serological improvement. At 19 months her muscular tone is greatly improved and she is able to walk unaided for few steps.

This patient emphasizes that MMF, a treatable disorder, should be considered as another cause of delayed motor development in children.

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[19] CONGENITAL MUSCULAR DYSTROPHY WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT: CLINICAL REVIEW

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Congenital muscular dystrophies (CMD) are an heterogeneous group of muscular diseases, with onset within 6 months, characterized by marked hypotonia, joint contractures and muscle biopsy suggestive of dystrophic pattern.

A group of CMD with a variable central nervous system (CNS) involvement is subdivided into Fukuyama CMD, Muscle-Eye-Brain (MEB) disease and Walker-Warburg Syndrome. In these group of diseases there is a different degree of ocular abnormality. Recently has been identified the gene responsible of MEB on chromosome 1p32-34 that causes a mutations in the protein O-linked beta 1,2-Nacetylglucosaminyltransferase (POMGnT1). We have observed 4 patients with CMD with CNS involvement and only one of them is still alive. We have considered the following criteria: current age or age death, brain pattern included mental retardation, speech (no words or easy sentence), IQ or DQ, type II lyssencephaly, white matter lucency, cerebellar vermis abnormality, hydrocephalus, ventricular dilatation and septum pellucidum agenesys. The eye abnormalities considered are: nystagmus, myopia, retinal dysplasia, optic nerve atrophy, microphthalmia, anterior chamber malformation, cataracts, glaucoma and high

VEP. We have evaluated the CK level, the hystology of muscle biopsy and the distribution of muscular proteins (alfa dystroglycan included). One patient out 4 has POMGnT1 mutations with classical MEB phenotype. This patient is 12 years old female and the only one out of 4 alive.

The other 3 patients are died at 2 months of age and 2 of them were brothers.

We have reviewed the clinical and neuroimaging data to differentiate MEB from WWS. The unique test useful for differentiating the MEB from WWS is the mutation of POMGnT1 that can reveal in advance the disease course.

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[20] CENTRAL CORE MYOPATHY WITH DIFFERENT PHENOTYPE: CASE REPORTS

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Central core disease (CCD) is a congenital myopathy with usually onset within 6 months of life characterized by a marked hypotonia and proximal weakness. The diagnosis is based on the presence to the muscle biopsy of cores. Missense mutations in the skeletal muscle ryanodine receptor gene (RYR1) have been identified in some families with CCD. A specific RYR1 mutation is linked not only to a severe form of CCD but to the malignant hyperthermia phenotype (MHS).

We present 2 cases diagnosed to different age, one in the newborn period and the other during childhood age. For both patients the diagnosis was made to the muscle biopsy that showed a peculiar morphological abnormalities, “cores”, with focal losses of oxidative enzyme activities in type I fibres.

We have evaluated the clinical data and the motor abilities during 10 years of follow-up. We report on the physical examination, the routine test, the electrophysiological examination and the muscle biopsy.

Also we have compared the motor performances of both patients to see if there are differences of the CCD with early clinical presentation and the CCD with childhood onset. For identifying the different CCD is very important to settle the course of the disease.

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[21] CARDIOMYOPATHY IN β-SARCOGLYCANOPATHIES: A NEW PATHOGENETIC HYPOTHESIS

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Mutations in the genes encoding for the sarcoglycan (SG) complex glycoproteins cause 4 limb-girdle muscular dystrophy (LGMD) types called sarcoglycanopathies. Only a few sarcoglycanopathy cases so far had documented cardiomyopathy, despite the SGs are expressed both in skeletal and cardiac muscle.

We studied 4 patients with LGMD due to mutations of the β-SG gene. Two patients (50%) had a severe cardiac involvement: one patient had severe LGMD and dilated cardiomyopathy, and died of cardiac failure at age 14 years; the second patient had moderate LGMD and moderate reduction of ejection fraction, diffuse ventricular hypokinesia and life-threatening arrhythmias. We investigated by immunohistochemistry and western blot analysis the SG expression in the skeletal muscle biopsies in 3 patients: the entire SG complex was lost in 2 patients, and markedly reduced in 1.

We found high levels of a SG protein homologue (presumed ε-SG) in control cardiac tissue; ε-SG is largely expressed also in smooth muscle. While α- and γ-SG are expressed almost exclusively in striated muscle, β- and δ-SG are expressed additionally in smooth muscle and coronary vessels; this suggests that in β- and δ-sarcoglycanopathies a dysfunction of vascular function may be involved in the pathogenesis of the disease. β-SG gene mutations severely affect the heart causing plasmalemma disruption in cardiomyocytes, which might be more susceptible to intermittent ischemia related to a microvascular abnormality.

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[22] FACIO-SCAPULO-HUMERAL MUSCULAR DYSTROPHY: GENOTYPE-PHENOTYPE CORRELATION IN CAMPANIA PATIENTS

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Facio-scapulo-humeral muscular dystrophy is described to be associated to neurosensorial deafness and retinal vasculopathy similar to Coats’ syndrome.

The aim of our study was to verify the incidence of extramuscular involvement and to assess the existence of a genotype-phenotype correlation in our population of genetically confirmed FSH patients.

The 69 FSH patients were clinically evaluated by means of two clinical scores (T score and R score). The following exams were performed: Visual Evoked Potentials (VEPs) (44 patients), Retinal Fluorangiography (RF) (27 patients), Oscillatory Potentials (OP) (7 patients), Audiometric Examination (35 patients) and Brainstem Auditory Evoked Potentials (BAEP) (44 patients).

Results: in 14 patients (51%) we found a pigmentary epithelial atrophy by RF, while only one patient (3.7%) had significant alterations of the retinal vessels. The VEPs showed in 8 patients (18%) a delay of the P100 wave with normal amplitude. The OP was normal in all the patients. Neurosensorial deafness was present in 7 patients (20%) and BAEPs were abnormal in 14 patients (32%). We found a significant statistic correlation between T and R scores and age at onset (p 0.004 and p 0.003 respectively), disease duration (both $p<0.0001$) and the size of the EcoRI fragment (both $p<0.05$). Molecular data do not correlate with any other clinical or instrumental findings.

Conclusions: Our results confirm the presence of a correlation between genetic findings and clinical severity. We did not find significant alterations of the retinal vessels. On the other hand, we found a visual pathway impairment in 8 patients.

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[23] EVALUATION OF CK-MB ISOENZYME AFTER MUSCULAR EXERTION

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The hyperckemia is an abnormal condition, but we can't ever say that it is really a pathology. The aim of the study is to evaluate the response to strenuous effort in a group of subjects with hyperckemia, comparing it with a control group. The C control group composed of 25 males (mean age 31 ± 10) (36% sedentary subject; 64% athletes) with normal CK at rest (< 82 U/l). The A group included 32 males (mean age 25 ± 10) (53.1% sedentary subjects; 46.9% athletes) with CK values at rest higher than normal (> 82 U/l). Both groups were submitted to a blood test in the morning after 72 hours of resting period and after stress test stopped at muscular exhaustion. At the maximal exertion group C has reached the 95% of maximal heart rate, and group A 92%. We took a blood sample for evaluation of CK and its isoenzymatic forms at the time: 5 minutes, 6 hours, 24 hours, 48 hours after stress test. Both groups showed significant differences in CK level: rest = C 48.25 vs A 175.36; post stress = C 60.30 vs A 189.96; 6 hours = C 53.20 vs A 175.9; 24 hours = C 59.95 vs A 179.96; 48 hours = C 50.77 vs A 208.92.Moreover, while the CK values of the C group were made only of CK-MM, the CK obtained in 36.40% of group A (21.20% of sedentary subjects and 15.20% of athletes) was made of CK-MB. The CK-MB isoenzyme seems to be always correlated with a condition of membrane's lability, cause we have never found it in the C group: the correlation of this enzyme with the myocardium needs more investigation.

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[24] EVALUATION OF RESPONCE TO STRESS TEST IN ATHLETS WITH HYPERCKEMIA

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The muscular fiber CK release is related with many variables, like the adaptation to training.

The aim of our study is to evaluate the response to strenuous effort in a group of athletes with hyperckemia at rest comparing it with a group of sedentary subjects with the same CK values. We have studied two groups of subjects with CK at rest > 80 U/l.: group A of 15 athletes (CK values at rest between 89 and 630 U/l.); group S of 15 sedentary subjects (CK values at rest between 82 and 450 U/l.). Both groups underwent a stress test till muscular exhaustion achieved at 90% of max. HR and for both we took a blood sample for the detection of serum CK and its isoenzymatic isoforms before and after stress test at the following times: post stress (5 min. after the end of performance), 6 hours, 24 hours and 48 hours. It seems to be interesting the different release of total CK and its isoenzymes (expressed in percentage to the resting value) in all the blood samples and specially 24 hours after the stress test: at this time (even if we don't have statistical significance for the small number of samples and the big range of CK values) the athletes' group showed the lower CK release (88.9% ± 35.2), while the sedentary group had the higher values (124% ± 85.5). The CK-MB isoenzymatic form has been detected in 40% of sedentary subject and in 33.3% of athletes in different percentage at the different time samples, and it seems to have the same course of total CK, because 24 hours after stress we found the lowest percentage in athletes and the highest percentage in the sedentary group. In athletes with hyperckemia at rest, the muscular adaptation to exercise could interfere with the CK serum release after exertion.

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[25] A CASE OF FAMILIAL MULTIPLE SYMMETRICAL LIPOMATOSIS WITH STOKE ONSET

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We describe a familial case of multiple symmetrical lipomatosis (MSL) with stroke onset. The propositus, a 62-year-old non-alcoholic man, affected by multiple lipomatosis, neuro-sensorial hearing loss and hyperuricemia, had left ponto-mesencephalic ischemic stroke with severe right hemiparesis. Five years later he developed a progressive increase of hemiparesis with bilateral cramps of calves. The sovraortic and transcranic echo-flow and echocardiographic study were normal. The electrophysiological nerve studies demonstrated a diffuse axonal polineuropathy; the muscle biopsy revealed "ragged –red fibers" and COX activity was absent or deficient in about 15% of fibers. This case supports the pathogenic role of mitochondrial disjunction in MSL and amplifies the clinical spectrum of MSL with unusual CNS involvement. The molecular and genetic studies are in progress.

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[26] A8381G MUTATION ON ATPASE8 GENE ASSOCIATED WITH DEAFNESS AND LACTIC ACIDOSIS

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Background: Mitochondrial DNA (mtDNA) homoplasmic mutations have previously been recognized as causes of diseases such as blindness and sensorineural hearing loss (SNHL).

Methods and results: here we report on a 59-years-old man who presented with a progressive hearing loss since his 40s and lactic acidosis. The neurological examination was negative, as well as MRI and proton spectroscopy of brain. Routine histologic and histochemical studies performed at muscle biopsy showed non-specific myopathic signs. Molecular analysis of mtDNA by direct sequencing revealed a change that is not recognized polymorphism (from MITOMAP), a A8381G point mutation resulting in a Threonin to Alanine at residue 6 of the subunit 8 of ATPase. The patient was homoplasmic for the mutation in muscle, whereas it was not detected in the patient's blood and urine and in 90 healthy controls. A marked reduction of ATPase activity (0.065 vs 0.15 uM/min/mg protein), with a decreased oligomycin sensibility (0.028 vs 0.036 uM/min/mg protein) was found in the patient's muscular tissue, data suggesting a structural abnormality of the F1-F0-ATPase complex.

Conclusions: Clinical presentation and laboratory findings in our patient support the hypothesis that this mutation could play a role in the etiology of the disease, reinforcing the hypothesis that homoplasmic mtDNA mutations, which pathogenic role is not a simple task to confirm, may be more common than previously thought.

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[27] ASSESSMENT OF ENDOGENOUS DNA OXIDATIVE STRESS IN MITOCHONDRIAL ENCEPHALOMYOPATHIES

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Recent evidences indicate that endogenous oxidative stress can play an important role in the onset and/or in the progression of mitochondrial encephalomyopathies (MEM), resulting in a widespread genotoxic damage, both at mitochondrial and nuclear DNA levels. Aims of this study has been to investigate nuclear DNA (nDNA) alterations in basal conditions and their modifications after a 2-week therapy with coenzyme Q_{10} (CoQ_{10}) in circulating lymphocytes of patients with MEM.

We assessed nDNA alterations, in terms of chromosome breakage and chromosome loss, by FISH analysis and single cell gel electrophoresis (comet assay) to quantify primary and oxidative nDNA damage. Higher chromosome damage, expressed as frequency of micronucleated lymphocytes, was found in patients than in healthy individuals of corresponding sex, age and smoking habits (27.0±10.9‰ vs. 8.0±6.0‰, $p<0.001$). CoQ_{10} treated patients showed a statistically significant reduction in the frequency of micronucleated cells (27.6±11.4‰ before therapy vs. 17.6±10.1‰ after therapy). A slight decrease was observed in the levels of nDNA damage in patients after CoQ_{10} administration.

Our results indicate that the occurrence of oxidative damage in mitochondrial diseases is marked by a genotoxic damage in nDNA of somatic cells and that a significant reduction of cytogenetic alterations can be obtained after antioxidant therapy in MEM.

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[28] PHENOTYPE MODULATORS IN MCARDLE'S DISEASE

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Myophosphorylase deficiency is characterized by exercise intolerance, cramps, and recurrent myoglobinuria. Some patients are severely affected, and others are minimally symptomatic or asymptomatic altogether. The molecular basis of the disease has been elucidated, but there is no explanation for the observed clinical variability. In a large cohort of patients with myophosphorylase deficiency we tested the hypothesis that genetic polymorphic variants in myoadenylate deaminase (MADA) or angiotensin converting enzyme (ACE) could act as modulators of phenotype expression.

Forty-seven patients with myophosphorylase deficiency were clinically evaluated according to a severity scale of four grades. MADA activity was studied by histochemistry and biochemistry, and the mutation in *AMPD1* was investigated molecularly. Insertion/deletion (I/D) polymorphism in the *ACE* gene was identified by PCR. Complete MADA defect, confirmed molecularly, was detected in one patient presenting a severe phenotype. Eleven patients were heterozygous for the Q12X mutation. There was no association between clinical grading and MADA status. There was a highly significant ($p<0.01$) association between *ACE* genotype and clinical severity, with strong correlation between severe phenotype and number of D alleles.

Our study demonstrates that *ACE* I/D polymorphism could play a significant role as phenotype modulator in McArdle's disease.

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[29] SUBCELLULAR LOCALIZATION OF THE MYOTONIC DYSTROPHY TYPE 2 PROTEIN ZNF9 IN SKELETAL MUSCLE

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Myotonic dystrophy type 2 (DM2) is usually expressed with the proximal myotonic myopathy (PROMM) phenotype. The genetic defect underlying DM2 is a CCTG expansion located in intron 1 of the zinc finger protein 9 (ZNF9) gene in chromosome 3q21. Since, as in DM1, this mutation involves an untranslated region of the gene, the pathogenic mechanisms that produce such a peculiar, and yet so similar to DM1, phenotype are not known. ZNF9 is a small protein of 19kDa which is highly conserved in several species and expressed in a variety of tissues, but its cellular localization and function are still unclear. We have therefore used a previously characterized polyclonal antibody to detect by immunofluorescence (IF) the subcellular localization of ZNF9 in human and rat skeletal muscle. In longitudinally sectioned myofibers of both species, IF reactivity for ZNF9 showed a regular transverse banding pattern throughout the fiber width. The transverse bands were about 1-1.2 m thick, corresponding to the width of sarcomeric I-bands, and showed in some instances a beaded appearance. In double IF experiments observed by confocal microscopy, ZNF9 and the sarcoplasmic reticulum (SR) Ca/Mg ATPase (SERCA1) localized to the same transverse elements, but the two signals did not show a superimposition in merged images. These data indicate that, in skeletal myofibers, ZNF9 localizes to I-band associated elements, other than the SR terminal cisternae. Such a distribution does not match exactly the one observed for DMPK, the protein product of the DM1 gene, therefore suggesting different functions for these two proteins.

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[30] SEVERE AND MILD PHENOTYPE OF ULLRICH CONGENITAL MUSCULAR DYSTROPHY: REPORT OF TWO CASES

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We report two consanguineous families, one with the classical severe form of UCMD, and the other with a milder phenotype. The first case an 11-year-old boy had a severe presentation with generalized hypotonia at birth, marked contractures and striking distal joint laxity. Muscle biopsy showed a myopathic pattern with normal expression of laminin alpha 2 chain and complete absence of collagen type VI. Immunofluorescence analysis of collagen type VI on cultured fibroblasts revealed absence of secreted protein in the extracellular matrix and positive staining inside the cytoplasm. Electron microscopy of shadowed whole mounted samples confirmed the absence of secreted protein. The patient was homozygous for microsatellites, D21S171, D21S2058, D21S156, and intragenic microsatellites, HCOL6A-1INT and COL6A-2INT suggesting a mutation in one of these two genes is responsible for the disease. The second case was a 16-year-old girl who was floppy at birth, slow in walking from the beginning and never able to run. Muscle weakness was severe in the axial and distal segments and mild in the girdles and proximal limbs. In the fingers she had a combination of finger flexor contractures and hyperlaxity. Muscle biopsy showed dystrophic pattern, normal laminin alpha 2 chain labeling and reduced expression of collagen type VI at the basal lamina. Collagen VI was also reduced in the extracellular matrix of cultured fibroblasts. Electron microscopy study showed that secreted tetramers of collagen VI could assemble in microfilaments with a reduced ability to develop a complex network interconnecting cells and other extracellular matrix proteins. Mutation analysis demonstrated a nonsense mutation in the N-terminal domain of *COL6A3*, which induced an aberrant splicing of exon 5 with an in-frame deletion. These results suggest clinical heterogeneity in UCMD, and a possible correlation between clinical phenotype with the expression of collagen type VI in muscle and cultured fibroblasts.

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[31] CT MUSCLE IMAGING IN MUSCULAR DYSTROPHIES WITH RIGID SPINE AND CONTRACTURES

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A number of muscular dystrophies presents with rigidity of the spine and limb contractures as the main features. Onset may be congenital or later up to adult-onset. In addition there is a large overlapping of clinical presentation in genetically distinct diseases, which complicates the genetic search particularly in sporadic cases. This is true for rigid spine muscular dystrophy 1 linked to 1p (RSMD1), Emery-Dreifuss muscular dystrophy (X liked and autosomal dominant forms: XL-EDMD, AD-EDMD), Bethlem myopathy (BM), and Ullrich congenital muscular dystrophy (UCMD) due to collagen type VI mutations. We collected CT muscle scans in patients with mutations in these 5 diseases. CT study includes scans at neck, shoulder, lumbar, hip, mid-thigh, and leg. Scans were assessed for normal or abnormal (atrophy/hypertrophy) muscle bulk and for normal and abnormal signal intensity within the different muscles. Collagen type VI disorders present a “diffuse” muscle involvement with a peculiar pattern of *peripheral degeneration with central sparing* in each muscle. EDMD patients showed a “selective” muscle involvement characterized by early abnormalities of vasti and hamstrings with long-lasting preservation of sartorious, gracilis, and particularly of rectus femoris. Comparing the XL-EDMD and the AD-EDMD cases no evident difference was seen at the lumbar, thigh and leg levels. RSMD1 cases showed a different selective pattern with involvement of the paravertebral, sartorius, adductors,

biceps femoris and gastrocnemius muscles at an early stage; more diffuse involvement in older patients; long-lasting sparing of the rectus femoris and gracilis muscles.

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[32] BASAMENT MEMBRANE IN WALKER-WARBURG SYNDROME

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Walker-Warburg syndrome (WWS) is an autosomal recessive disorder characterized by congenital muscular dystrophy, structural eye abnormalities, and severe brain malformations. We report further analysis on a gypsy patient who exhibited severe hypotonia, ocular malformation, and hydrochephalus at birth. MRI showed huge ventricular dilatation, lyssenchephaly type II with agyria, vermis hypoplasia, and agenesis of corpus callosum. Ocular examination showed buphthalmus, retinal dysplasia and lens opacity. CK was 87 times normal on day two, and 18 times normal at one month of age. The course was marked by absence of psychomotor development and occurrence of infantile spasms. He died at the age of 2 and half. A third degree cousin, who presented with marked hypotonia, hydrochephalus, and buphthalmus at birth, died at the age of 40 days. Genetic analysis in this family showed absence of linkage with the known loci of CMD. The previously reported immunohistochemical study of muscle biopsy from this patient showed dystrophic changes and deficiency of laminin alpha 2, laminin beta 2 and gamma sarcoglycan. In this study we investigate the expression of several components of the extracellular matrix, by immunofluorescence analysis, and the organization of basement membrane, by electron microscopy. We found the absence of alpha dystroglycan both in muscle fibers and in intramuscular peripheral nerves using an antibody against the glycosylated epitope. Perlecan was reduced in some muscle fibers while other components of the extracellular matrix as collagen type VI, collagen type IV and nidogen were normally expressed. Electron microscopy study showed focal loss and detachments of basal lamina in several non-necrotic muscle fibers. Rare sarcolemma discontinuities were detected in areas corresponding to basal lamina alterations. We report evidence of substantial alterations of basal lamina, consisting in alpha dystroglycan deficiency and structural alterations of basal lamina of muscle fibers in a patient with WWS. These results represent an interesting parallel with alterations in Fukuyama congenital muscular dystrophy and suggest a common pathway of muscle-brain involvement in these disorders.

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[33] DYSFERLIN SHOWS INTERNAL LOCALIZATION IN CAVEOLIN-3 DEFICIENT MUSCLE

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Caveolin-3, a muscle specific caveolin related protein, is the principal structural protein of caveolar membrane domains in skeletal muscle, Different mutations in the caveolin-3 gene *(CAV3)* have been associated with various clinical phenotypes: a specific form of autosomal dominant limb girdle muscular dystrophy (LGMD1C), isolated hyperCKemia, rippling muscle disease and distal myopathy.

Dysferlin is a surface membrane protein in muscle whose deficiency causes limb girdle muscular dystrophy type 2B (LGMD2B) and distal myopathy (Miyoshi myopathy). It was recently shown that dysferlin co-immunoprecipitates with caveolin-3 in normal human muscle.

We studied the localization of dysferlin by immunogold crioultramicroscopy in normal and in caveolin-3 deficient muscle, and the expression of dysferlin by immunochemical techniques in muscle samples from LGMD1C patients, caveolin-3 deficiency and isolated hyperCKemia patients and in transgenic caveolin-3 deficient mice.

We found that caveolin-3 co-localizes only partially with dysferlin at the cell surface in normal muscle, while in caveolin-3 deficient muscle dysferlin reveals a severe deficiency at the membrane level and shows an abnormal internal localization.

Taken together these data confirm the in-vivo interaction between caveolin-3 and dysferlin and open new perspectives in the understanding of the role of caveolin-3 and dysferlin in muscle fiber.

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[34] IMPAIRMENT OF CAVEOLAE FORMATION AND T-SYSTEM DISORGANIZATION IN LGMD1C MUSCLE FIBERS

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Caveolin-3, a muscle specific caveolin-related protein, is the principal structural protein of caveolar membranes. We have recently identified an autosomal dominant form of limb girdle muscular dystrophy (LGMD-1C) that is due to caveolin-3 deficiency and caveolin-3 gene mutations. Here, we studied by electron microscopy, including freeze-fracture and lanthanum staining, the distribution of caveolae and the organization of the T-tubule system in caveolin-3 deficient human muscle fibers. The mean density of caveolae in normal muscle plasma membrane was 9.68 (SD ± 2.05) per square micron. In sharp contrast, in LGMD-1C muscle plasma membrane we observed very few caveolae in both P and E faces of the membrane. This severe impairment of caveolae formation at the muscle

cell surface demonstrates that caveolin-3 is essential for the formation and organization of caveolae in muscle fibers.

We also detected a striking disorganization of the T-system openings at the sub-sarcolemmal level in LGMD-1C muscle fibers. We observed beneath the plasma membrane several large vacuolated structures, which were labeled by electron-dense lanthanum. In addition, some of the large vacuolated structures showed continuity with the plasma membrane, possibly representing abnormal caveolae-like membrane invaginations or disrupted T-tubule openings. Some of the vacuolated structures were also associated with lanthanum positive honeycomb structures, a known indicator of abnormal proliferation of the T-tubule system.

These observations provide new perspectives in our understanding of the role of caveolin-3 in muscle and of the pathogenesis of muscle weakness in caveolin-3 deficient muscle.

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[35] ANALYSIS OF CLINICAL AND GENETIC FINDINGS IN A LARGE COHORT OF ITALIAN DM1 PATIENTS

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We reviewed 207 DM1 patients followed-up by multidisciplinary approach to assess a) spectrum of expression of the disease and b) genotype-phenotype correlations. The mean age of onset was 25 yrs; 10% manifested a congenital form. Onset was characterized by myotonia (73%), distal weakness (20%), bulbar involvement (4%), anesthaesiologic complications (1.5%), cataract (1.5%). Generally muscle weakness had a typical distribution involving facial, axial and distal compartments. Cataract developed in 21%, diabetes in 5%, dysthyroidism in 15%, bowel dysmotility in 10%, male infertility in 3%. A-V conduction defects and atrial or ventricular arrhythmias developed in 51% and 20% of patients respectively: 2% implanted a pacemaker. One showed a cardiomyopathy. A reduced respiratory capacity occurred in 50%; 1.4% required mechanical ventilation. Premature death, either related to cardiac or respiratory complications, occurred in 6% of patients. nCTG significantly correlated with age of onset, degree of muscle involvement, % FVC ($p<0.0001$) and occurrence of frequent and/or repetitive ventricular arrhythmias ($p< 0.05$). In conclusion, clinical spectrum appeared uniform in our patients, although we registered a lower incidence of diabetes and infertility; interestingly, we also noticed an intrafamilial bias to develop cataract and cardiac arrhythmias. The persistence of significant mortality rates despite updated specialized follows-up underlines the importance of identifying specific diagnostic and therapeutic strategies: to this regard, our results suggests that genetic data may be useful for prognostic evaluation of cardiac and respiratory involvement.

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[36] UTILITY OF TROPONIN TO DETECT CARDIOMYOPATHY IN DYSTROPHIC PATIENTS

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Troponin I is a 28 kDa myofibrillar protein present on thin filaments; there are cardiac and skeletal muscle isoforms, detectable by immunocytochemical analysis. The cardiac isoform of troponin I (cTnI) is widely used for the early detection of cardiac ischemic accident and for the prognosis of cardiovascular disease.

We quantified cTnI immunoenzymatically in serum from 37 dystrophic patients after finding high levels in one. Troponin was elevated in two: a 14 year-old boy with limb girdle muscular dystrophy (LGMD), and an 8 year-old boy with Duchenne muscular dystrophy (DMD). cTnI was 0.34µg/l and 0.17µg/l respectively (>0.10µg/l indicates cardiac damage); both patients later developed severe progressive cardiomyopathy, but neither the treating physician nor the technician knew they were cardiopathic at evaluation. In the LGMD patient, ECG and cardiac function were normal at blood sampling (ejection fraction 25%); subsequently dilatational cardiomyopathy developed and worsened rapidly. In the DMD patient, cardiomyopathy became evident two years later. In the other 35 patients cardiac function was normal at the time of blood sampling. These findings suggest that routine screening of dystrophic may be useful to detect early cardiomyopathy controllable by adequate medication.

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[37] A NEUROPHYSIOLOGICAL PROTOCOL FOR EVALUATION AND FOLLOW-UP OF MYOTONIC PATIENTS

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Myotonia is a hallmark of myotonic syndromes. In individuals severity may vary on a daily basis or over a longer timescale. Because of progressive muscle weakness and wasting the myotonic phenomena usually reduce over time. The lack of clinical studies on the natural history and progression of myotonia, using validated and reliable measures, makes it difficult to assess the efficacy of treatment for myotonia. We propose a protocol to evaluate and follow myotonic phenomena during disease progression and to relate their intensity to reduction in hand strength. The protocol comprises the following: median nerve conduction evaluation to detect a possible neuropathy; assessment of maximal isometric contraction with a dynamometer; determination of EMG relaxation time after maximal voluntary effort, assessing thenar eminence muscle. During this test to ensure constant effort, isometric contraction is measured. Determination of myotonic discharges elicited by two supramaximal 100 Hz stimuli at the wrist. During follow-up of each patient, we correlate variation

in cMAP amplitude and isometric contraction with changes in EMG relaxation time. Data obtained are compared with clinical evaluation and imaging studies. We shall present preliminary data on myotonic patients using this procedure.

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[38] CARNITINE PALMITOYL TRANSFERASE II DEFICIENCY AND TUBULAR AGGREGATES

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Messina

Abnormalities of sarcotubular system presenting as tubular aggregates (TA) have been observed in a variety of skeletal muscle disorders. We describe a 60-year-old patient who experienced a severe episode characterized by diffuse myalgias, stiffness, pigmenturia and massively elevated serum creatine kinase (CK) levels. Neurological examination revealed pain on muscle palpation, proximal mild weakness at lower limbs and hyporeflexia. Serum CK was markedly increased (48,000 U/L). Electrophysiological studies showed no alterations. Mild elevation of serum lactate was observed after exercise. Muscle biopsy revealed some atrophic fibers, several central nuclei. Basophilic material, bright red with the Gomori trichrome stain, located especially in subsarcolemmal regions, had an intense reaction for NADH-TR and negative for SDH. At ultrastructural examination, these abnormal areas contained tubules, 60 to 80 nm in diameter, having a parallel, double-wall arrangement and packing in hexagonal arrays. Biochemical investigations showed an isolate defect of CPT II activity (30 % of normal value). Glycolitic and mitochondrial enzymes were normal. Presence of TA has never been reported in CPT II deficiency but it is known that they may represent a possible response to dysfunction of energy metabolism.

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[39] MYOPATHY IN A PATIENT WITH MUSCLE CARNITINE DEFICIENCY AND LOW SERUM TESTOSTERONE

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Messina

Secondary carnitine deficiency has been described in several pathological conditions such as organic aciduria, respiratory chain defects, chronic valproate therapy, and hemodialysis but the mechanism leading to the carnitine depletion is not always well understood.

We describe an 18-year-old boy whom experienced since childhood several episodes of diffuse myalgias, cramps and weakness at lower limbs trigged sometimes by high fever or fasting.

Physical examination showed a presence of gynecomastia and increased adiposity. Neurological examination showed proximal muscle weakness, hypotonia and areflexia. CK was normal. Lactate and piruvate after exercise and ischemic conditions were normal. Muscle biopsy showed lipid storage and type 2 fibers atrophy. Biochemical investigations on muscle homogenates revealed low levels of total and free carnitine (9.5 nmol/min/ mg protein-n.v. 18.6±4.6; 9 nmol/min/mg protein, n.v. 14.6±4). Free serum testosterone was 8.9 pg/ml (n.v. 15-40) and total testosterone was 2.1 ng/ml (n.v. 3-10). Patient was treated with riboflavin 200 mg/die with remarkable clinical improvement.

The association of low testosterone and low carnitine has never been reported so far. We postulate that muscle carnitine deficiency is related to testosterone deficiency, which is known to cause decrease of fat oxidation and muscle strength.

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[40] DEFINING DISEASE PATHOGENESIS BY COMPARATIVE CROSS-SPECIES PROFILING IN HUMAN AND MOUSE KNOCK-OUTS: THE DOWNSTREAM CONSEQUENCES OF DYSTROPHIN DEFICIENCY

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The absence of dystrophin protein leads to different phenotypes in different species, despite complete loss of dystrophin in all muscles. Human DMD patients show chronic degeneration/regeneration with progressive muscle wasting and an early death. The *mdx* mouse initially shows normal muscle, with widespread skeletal muscle necrosis at the age of 3-4 weeks, followed by "successful" muscle fiber regeneration. We hypothesized that comparison of 60,000-gene profiles in human DMD muscle (U95 GeneChip series and custom Affymetrix MuscleChip), and 36,000-gene profiles in *mdx* and experimental muscle regeneration (U74 GeneChip series) would identify differentially expressed genes as potential candidates for conferring protection to murine dystrophin deficient muscle. These same genes would be targets for modulation of the progressive pathology of the human disease. We used as a starting point a "late muscle regeneration cluster" gleaned from a 27 time-point murine regeneration series. We then compared the expression of these genes in dystrophin-deficient *mdx* mouse muscle and human DMD muscle. We then generated gene lists of those genes upregulated in both mouse models, but not upregulated in DMD. Our true genome-wide cross-species comparative candidate gene analysis showed inhibitors of negative regulators of skeletal muscle mass, specific cell division and differentiation genes, and specific connective tissue modulatory genes as candidates for the species-specific response to dystrophin-deficiency.

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[41] CONGENITAL BENIGN MYOPATHY, ALFA7 INTEGRIN DEFICIENCY AND NO MUTATIONS IN THE ALFA7 INTEGRIN GENE. REPORT ON 2 PATIENTS

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Congenital myopathy with integrin alfa7 deficiency is a rare condition. We describe the clinical and morphological findings in 2 patients with a severe alfa7-integrin deficiency in muscle.

The first patient had normal milestones, with no muscle symptoms at puberty. At age 14 years he had a mild elevation of CK (x4 normal), mild facial weakness (unable to bury eyelashes), he was not able to walk on heels, and had a mild scoliosis similar to the idiopathic type. Now at the age of 27 he presents the same clinical pattern. The other patient was first observed at the age of 1 year. He was the first child of non-consanguineous parents. He was normal at birth and a left hip dysplasia was disclosed during the clinical screening at age 3 months. The child was submitted to tenotomy of the adductor magnus and ileo-psoas tendons at age 5 months. CK was increased (x10). Motor milestones were normal in the first year of age, and he was able to walk with aids at age 1 year showing a mild scoliosis. He had again tenotomy on the right hip at age 18 months. Now he wears brackets and is able to walk with aids. In both patients no mutations were found in the alfa7integrin gene. Morphological analysis of muscle biopsies from patients showed mild myopathic changes. Immunofluorescence showed a marked reduction of alfa7beta1 integrin-complex in 1 patient and only alfa7A in the youngest. Many other analyzed proteins of the sarcolemma, basal lamina and extracellular matrix were normal.

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[42] HEART INVOLVEMENT IN EMERY-DREIFUSS MUSCULAR DYSTROPHIES: NEW PARAMETERS TO DETECT EARLY ATRIAL FIBROSIS AND VULNERABILITY

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Emery-Dreifuss muscular dystrophy is characterized by early contractures of the elbows, Achilles tendon and spine, slowly progressive muscle wasting and weakness with a predominant humeroperoneal distribution and cardiomyopathy, usually presented as heart block (OMIM 310300).

Two main modes of inheritance, X-linked (X-EDMD) and autosomal dominant (AD-EDMD) have been described; however a rare autosomal recessive mode of inheritance has been reported.

The gene responsible for the X-linked EDMD maps on the long arm of the X chromosome (Xq28); in 1998 it was reported that the locus for the AD-EDMD was on 1q11-q23; the year after, the nuclear lamin A/C (*LMNA*) at this locus was found to be responsible for AD-EDMD. Since this first report, five disorders have now been known to be caused by mutations in the lamin A/C gene: AD-EDMD, dilated cardiomyopathy with conduction defects, autosomal dominant limb-girdle muscular dystrophy with Atrio-ventricular conduction disturbances (LGMD1B), Dunnigan-type familial partial lipodystrophy (FPLD) and very recently the Charcot-Marie-Tooth disorder type 2 (CMT2).

Among the hundreds of patients with different types of muscular dystrophies followed at the Cardiomiology and Medical Genetics Section of the Second Naples University, 23 - 13 familial and 10 sporadic - were identified, with a phenotype typical of Emery-Dreifuss muscular dystrophy. The diagnosis was confirmed by molecular analysis for both emerin and *LMNA* gene and/or by immuno-histochemistry analysis on muscle biopsies in 5 patients. The remainders are still undiagnosed by both methods. All the patients underwent an extensive cardiologic study that included clinical examination, standard 12 leads and dynamic ECG (Holter monitoring), mono and 2-D echocardiography, acoustic densitometry. Three patients have required prophylactic ventricular pacing. An electro-physiologic study (EPS) was performed in 5 patients, after informed consent. We investigated the usefulness of the different electro-cardio-graphic and echo-cardio-graphic parameters in detecting the typical features (atrial fibrosis and vulnerability to develop at risk hyper-kinetic arrhythmias) of heart involvement in EDMD. Finally we try for establishing preliminary correlations between the cardiac involvement and the genetic findings.

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[43] FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHD): NEED FOR ADDITIONAL MOLECULAR MARKERS

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FSHD is an autosomal dominant myopathy with early involvement of facial and scapular muscles with possible spreading to pelvic and lower limb muscles. A high degree of clinical variability is observed both between and within families. FSHD gene has been mapped to locus D4Z4 in 4q35 region. Probe p13E-11, detects EcoRI, BlnI-resistant, polymorphic fragments of 35-300 kb in normal subjects and smaller fragments, 10-35 kb, both in sporadic and familial cases of FSHD. The size varia-

tion of the 4q35 short fragments is due to the deletion of a variable number of KpnI repeat units. No transcripts from the D4Z4 locus were identified so far. Genotype-phenotype correlations showed that EcoRI fragment size is a major factor in determining the severity of the clinical phenotype and might have an influence on the disease penetrance, since the number of non penetrant gene carriers progressively increases in families with fragment > 20 kb. However, prognosis can not be based only on the fragment size, except for very short fragments (10-13 kb), invariably associated with severe phenotypes. In our experience with about 300 FSHD patients and families, analyzed by standard gel electrophoresis and PFGE, we found affected subjects, showing a high variety of clinical manifestations, with p13E-11 fragments of 10-40 kb and a significant proportion (7%) of non penetrant gene carriers, with a fragment > 21 kb. In particular, we found 12 families with a fragment ranging 30 to 40 kb, carried by both affected and unaffected subjects. It is worth pointing out that the overlap between normal and FSHD individuals carrying such large fragments causes diagnostic uncertainty, since the occurrence of non penetrant gene carrier condition might not allow to correlate clinical and the molecular features.

Our observations in clinical and molecular analyses of FSHD show the need for additional molecular markers in order to refine the diagnostic protocols and to improve the prognosis and the genetic counselling of the disease.

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[44] FOCAL MYOSITIS OR FOCAL MUSCLE INVOLVEMENT IN POLIMYOSITIS? A REPORT OF THREE CASES

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Messina

Focal myositis is a rare condition with a wide clinical spectrum. Affected patients may rarely develop a polymyositis. Response to steroid is dramatic. We describe three patients: an 18-year-old man with a focal inflammatory involvement of calf muscles, a 25-year-old woman with an orbital myositis and a 52-year-old woman with an isolated impairment of cervical paraspinal musculature. Standard laboratory tests, EMG, MRI were performed in all patients. MRI displayed diffuse increased signal involving muscles clinically affected. Two patients underwent muscle biopsy (in one of them, a clinically unaffected muscle was examined), with immunocytochemistry of inflammatory markers, which evidenced mononucleate cells infiltration, necrosis and myophagia. In all cases there was a favorable response to steroids. MRI findings well correlated with severity of both clinical and histological manifestations, providing a helpful noninvasive test for diagnosis and follow-up. Histological evidence of inflammation at clinically spared muscles suggests that focal myositis may represent a clinical manifestation of a diffuse polymyositis.

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[45] QUANTIFY MUSCLE DAMAGE DUE TO SPONTANEOUS MUSCLE ACTIVITY IN NORMAL AND DISEASED RODENTS

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To determine minimal spontaneous physical activity able to induce muscle damage in normal and *mdx* mice, we investigate contribution of apoptosis to exercise-induced death of myofibers in a time-course study. The runner mice are housed in cages provided of a rotating wheel, and allowed to spontaneously run either 2 hours during late afternoon, or the full night (about 16 hours). The day after, Tibialis Anterior is removed from both legs. Activity of mice is quantified as covered distance (Km) and percent time of wheel rotation (%WR). Apoptosis is assessed by TUNEL and ultrastructural features, and expressed as apoptotic nuclei per mm^3 of muscle tissue. Table shows that normal mice continue to run all over the night up to 6 Km (about 0.8 Km /hr), and *mdx* run 0.8 Km/hr during the first two hrs, and then a distance shorter than normal mice (at total mean 0.3 Km/hr). Peak velocity is similar in both groups. The16-hr *mdx* runners are significantly less active than runner normal mice. Apoptotic nuclei increase in all the runners. Surprisingly in normal and *mdx* mice the 16-hour groups contain less (normal mice), or slightly less (*mdx*) total muscle nuclei in comparison to 2-hr runners. When myonuclei are counted, both by TUNEL and electron microscopy, apoptosis is shown to occur in both fibers and satellite cell. Besides confirming increase of apoptotic myonuclei after exercise, results show that apoptosis of interstitial cells (inflammatory cells, endothelia and fibroblasts) is higher after a burst of exercise followed by rest than after a full-time exercise This could be related to post-exercise hyperemia.

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[46] COPPER SUPPLEMENTATION RESTORES CYTOCHROME C OXIDASE ACTIVITY IN CULTURED CELLS FROM PATIENTS WITH SCO2 MUTATIONS

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Human SCO2 is a nuclear-encoded copper-binding protein presumably responsible for the insertion of copper into the mitochondrial cytochrome *c* oxidase (COX) holoenzyme. Mutations in *SCO2* are associated with a disease characterized by hypertrophic cardiomyopathy, encephalopathy, myopathy and COX deficiency. This condition is rapidly fatal and no treatment is currently available. Studies in yeast and bacteria have shown that copper supplementation can restore COX activity in cells harboring mutations in genes involving copper transport. We therefore investigated whether copper supplementation could restore COX activity in cultured cells from patients with *SCO2* mutations.

Copper chloride was added to the culture media of fibroblasts, myoblasts, and myotubes of patients with *SCO2* mutations, of a

normal control, and of a patient with mutations in SURF1, another COX-assembly gene not involved in copper metabolism. No effect on COX activity was seen in the control cells or in the *SURF1* mutants, but we observed a dose-dependent increase in COX activity in the *SCO2*-deficient cells. COX was restored to normal levels by 200 µM copper chloride. Interestingly the restoration of normal COX activity by copper was a gradual process that required several days to become evident.

Our data suggest that copper supplementation may represent an alternative to gene-transfer strategies in the treatment of this fatal condition. However further studies on animal models of *SCO2* mutations are needed to understand the mechanism of this effect and to evaluate the toxic effects of copper supplementation.

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[47] CASPASE 3 EXPRESSION IN DUCHENNE AND FACIOSCAPULO HUMAN MUSCULAR DYSTROPHY

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Apoptosis was detected in different muscular diseases, including severe dystrophin deficiency, but apoptotic mechanisms are not completely described in adult skeletal muscle. Studying patients affected by Duchenne Muscular Dystrophy (DMD) and by facio-scapulo-humeral dystrophy (FSHD) we showed an increase of apoptotic myonuclei, bax and bcl-2 positive myofibers. Positive correlation was detected between apoptotic nuclei and bax expression ($p<0.01$). A number of mechanistic pathways to apoptosis have been defined. A common end point for these pathways is the activation of a series of cysteine proteases collectively known as caspases (3-5). Expression of caspases was analyzed by Range protection. Capsize transcript was not detected in normal skeletal muscles. DMD muscles expressed caspase 8, 3, 5, 2, 7 and Granzyme B mRNAs. Low levels of caspase 6, 3 and Granzyme B transcripts were detected in FSHD patients. Tissue levels of caspase 3 protein significantly correlated with apoptotic myonuclei ($p<0.05$) and with bax expression ($p<0.01$). In all DMD cases the activity of caspase 3 was increased, while the FSHD samples were heterogeneous. These data indicate that human skeletal muscle fibers, during the dystrophic process, modulate the expression of caspases and that caspase 3 is involved in myofiber cell death, opening new perspective in the pharmacological treatments of muscular dystrophies, such as the use of caspase inhibitors.

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[48] AVOIDANT BEHAVIOUR CORRELATES WITH SPECIFIC FRONTAL LOBE DYSFUNCTION IN MYOTONIC DYSTROPHIES

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Background: Previous studies in myotonic dystrophy type 1 (DM1) and type 2 (PROMM/DM2) have demonstrated a visual-spatial impairment on neuropsychological tests. This has been correlated with reduced cerebral blood flow in the frontal and temporal lobes.
Objective: To verify whether there is a specific pattern of cognitive and behavioural abnormalities in DM2 and DM1.
Methods: 20 patients from 5 unrelated families, with genetically confirmed PROMM/DM2, 20 patients with moderately severe myotonic dystrophy type 1 (DM1-E2) and 20 age-, sex-, and education-matched controls patients were subjected to: (i) frontal lobe cognitive test measures: Computerized Attentional Assessment, TEA, Winsconsin Card Sorting Test, WCST, Stroop Test, ST, Trail Making Test A and B, TMT, Tower of London Test, TLT (computerized version); (ii) behavioural test measures: SCID-II personality scale, self-administered anxiety and depression scales, neuropsychiatric interview.
Results: Basic attentional and alertness functions (TEA, TMT) were normal in both PROMM/DM2 and DM1 patients. Cognitive strategies and visual-spatial decisions (WCST, TLT) were significantly impaired in patients with PROMM/DM2 and DM1 ($p < 0.001$). None of our patients fulfilled DSM-IV criteria for axis I and II disorders. Both patients with PROMM/DM2 and DM1 showed significant avoidant behavioural trait clustering ($p < 0.05$).
Conclusions: Patients with DM1 and PROMM/DM2 have a characteristic cognitive and behavioural trait clustering characterized by a frontal dysexecutive syndrome and avoidant behavior. Myotonic dystrophies may also be considered as a brain disorder. This suggests that specific parameters of cognitive and behavioral abnormalities in DM1 and PROMM/DM2 may help in the screening .

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[49] A HETEROPLASMIC A13084T MUTATION IN ND5 MTDNA GENE CAUSES EARLY-ONSET PROGRESSIVE MENTAL RETARDATION AND GAIT ATAXIA

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We describe a 16 years old boy affected with a slowly progressive decline of cognitive functions since age 7 years. He later developed gait ataxia, clonic seizures and bilateral ptosis. Both serum and CSF lactate were mildly elevated, serum CK being normal. EKG showed defective anterior repolarization and EEG was characterized by both slow and parossistic multifocal activity. EMG was normal. His mother, now aged 37 years, recently started to complain of headache and visual problems. She has been diagnosed migraine and bilateral optic nerve subatrophy. Her brain MRI is normal, but the EEG presents bitemporal nonspecific abnormalities. The 6 years old sister is apparently healthy. Diabetes mellitus and cardiac abnormalities are described in some proband's relatives on the maternal side.

The proband underwent two skeletal muscle biopsies, which were normal. Biochemical assay of respiratory chain enzymes, however, showed slightly reduced complex I activity. Mitochondrial DNA (mtDNA) Southern blot analysis and search for known structural gene point mutations were normal. Entire mtDNA screening revealed the presence of an A-to-T heteroplasmic point mutation at nt 13,084 in the ND5 gene. The mutation affects a highly conserved site (position 250 in human mitochondrial genome) and causes a Ser-to-Cys aminoacid change. The mutational load is 86.8% in skeletal muscle and 71.6% in blood from the proband, the percentage being lower (31.7%) in his mother's blood. Evaluation of the younger sister is underway.

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[50] PROTON MAGNETIC RESONANCE SPECTROSCOPY IN MITOCHONDRIAL DISEASES: METABOLIC ABNORMALITIES AS PHENOTYPIC APPEARANCE OF BRAIN DISEASE

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In this study integrated Magnetic Resonance Imaging (MRI) and Proton MR spectroscopy (^{1}H MRS) studies of the brain were carried out on 15 patients with different types of primary mitochondrial diseases (MD). Localized spectra were performed at short echo-time in MRI normal-appearing areas. Additional spectra of basal ganglia and a focal cortical lesion with signal abnormalities on conventional MRI were obtained in three cases. A significant choline (Cho) reduction ($p=0.03$ in cerebral cortex and white matter; $p=0.04$ in cerebellum) and N-acetyl-aspartate (NAA) reduction ($p=0.03$ in cerebellum; $p=0.01$ in cerebral cortex) was found in normal MRI areas. Lactate (Lac) was never found in MRI normal-appearing areas, a part from the three infantile cases in which it was found both in MRI altered areas and in normal appearing-areas. An additional abnormal signal at 0.9ppm was found in a consistent number of studies. We concluded that ^{1}H MRS proved to be a useful investigation tool for MD, as it could detect metabolic abnormalities even in normal MRI brain.

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[51] THE DYSTROPHIN ROD-DOMAIN IS ALTERNATIVELY SPLICED IN BOTH NORMAL HUMAN TISSUES AND IN DMD/BMD SKELETAL MUSCLE

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The DMD gene, located on Xp21, codes for dystrophin and, when mutated, is responsible for either Duchenne or Becker muscular dystrophy (DMD and BMD). A major hot spot for DMD/BMD deletions has been identified around exons 45-55. We analyzed splicing patterns in the gene region encompassing exons 17 through 58. Human skeletal muscle, heart and brain tissues from healthy subjects were analyzed. A total of 16 alternative transcripts were identified, the majority of them being present in the three tissues. Tissue-specific splice variants were also detected and brain displayed the widest range of different dystrophin gene products. Transcript analysis was extended to 14 muscle biopsies of DMD/BMD deleted patients. When possible, two or three patients carrying the same deletion were analyzed. Surprisingly, in some instances, short deletions were found to abolish plicing variants that longer and overlapping deletions did not. These data suggest that secondary structure formation on dystrophin pre-mRNAs plays no or little role in directing alternative splicing events. In two cases patients carrying the same exonic deletions displayed different splicing behaviors with respect to the loss or preservation of alternative transcripts. Most interestingly, we carried out transcript analysis on autoptic tissues from a DMD patient with a 45-52 exon deletion: a different pattern of alternative transcript was detectable in muscle, heart and brain.

Our data indicate that alternative splicing events are differently regulated in different organs and that equal deletions can determine diverse splicing behaviors in different patient or even in different tissues of the same individual.

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[52] PROXIMAL REVERSIBLE UPPER LIMB MYOPATHY WITH LOSS OF MYOSIN FILAMENTS

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We report on a 58 year-old man complaining for eight months of difficulty in rising his arms above the head and proximal upper limbs muscle weakness. Cervical NMR showed only mild spondylosis. CK level was increased four times and EMG recording revealed a myopathic pattern on proximal upper limb muscles. Biopsy of deltoid muscle showed patchy loss of ATPase activity in nearly 15 % of muscle fibers, which were also atrophic; few type 2 fibers were observed. Electron microscopy revealed a selective loss of thick myofilaments. Investigation of muscle proteolytic pathways disclosed an activation of calpain mediated proteolysis in the atrophic fibers. Four years later, on examination, the patient had regained normal upper limbs movements and strength. We discuss the pathological analogies between our case and what reported in acute quadriplegic myopathy.

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[53] PRIMARY MYOPATHY IN A NEW CASE OF THE CAREY-FINEMAN-ZITER SYNDROME

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Carey, Fineman and Ziter in 1982 first described a sib pair with congenital hypotonia, VI and VII cranial nerve palsy (Moebius sequence), mandibular hypoplasia–glossoptosis-cleft palate (Robin sequence), facial and limb anomalies, delayed motor milestones, and failure to thrive (CFZ syndrome). Four other children have since been reported as having the CFZ syndrome. In all patients the neuro-muscular impairment represented by generalized muscle mass hypoplasia, hypotonia and proximal weakness is evident. However, electrophysiological and morphological findings were reported to be unspecific or normal, depending on the cases examined.

Recently, we observed a six-month-old boy, first child born at term to healthy non-consanguineous parents, with all CFZ syndrome hallmarks, including neuromuscular impairment. Generalized seizure occurred at age 1 month. Serum laboratories show moderate increased CK and LDH. EEG and MRI of the brain were normal. The electrophysiological examination shows a myopathic pattern. Muscle biopsy (vastus lateralis) discloses myopathic changes (mild fibres size variability, numerous opaque fibres, discrete increase of connective tissue and presence of some ragged red fibres). These findings offer evidence that the neuromuscular involvement in our patient may be caused by a primary myopathy and that primary myopathy is an important manifestation of the CFZ syndrome.

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[54] FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY: A MULTICENTER STUDY ON CARDIAC INVOLVEMENT AND ITS CORRELATION WITH THE 4Q35 DELETION

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The clinical features and the natural history of Facioscapulohumeral Muscular Dystrophy (FSHD), one of the most frequent hereditary myopathies in Western Countries, are currently being evaluated by a multicenter study, concerning 96 FSHD patients with the characteristic 4q35 deletion. The clinical investigation is mainly focused on the occurrence and on the definition of cardiac alterations in the disease, since the heart involvement has been poorly studied in FSHD. In our patients, the cardiac functions have been evaluated by clinical examination, surface ECG, 24-hour ECG and echocardiography. The preliminary results, concerning the cardiac study of 57 out of 96 patients included in the multicenter investigation, are presented. The FSHD cases are 30 males and 27 females, with a mean age of 44 years (range 16 to 75 years), in which the characteristic 4q35 deletion spanned 10 to 38 Kb (mean 21). Overt cardiac involvement was evident in 7 patients (12%) and was mainly represented by symptoms due to conduction delay or arrhythmia; subclinical signs of the same type of heart abnormalities were detected by the 24-hour ECG in other 8 cases. Altogether, 15 patients showed clinical or subclinical alteration of the cardiac rhythm or conduction (26%). In these cases, the heart abnormalities appeared not correlated with the size of the 4q35 fragment.

On the whole, even thought symptomatic heart disease appear an unusual feature of FSHD, our clinical study clearly showed that subclinical cardiac involvement may be detected in a meaningful percentage of patients.
