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## ABSTRACTS

### THE CLINICAL SPECTRUM OF DYSFERLINOPATHIES

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Mutations in the dysferlin gene (2p13) have been recently identified as responsible for an autosomal recessive form of limb-girdle muscular dystrophy (LGMD), classified as LGMD2B. We describe three patients with dysferlinopathy showing different phenotypes.

Two siblings, aged 24 and 21, had severe weakness and wasting in the muscles of the pelvic girdle and, to a lesser extent, of the shoulder girdle, and waddling gait. A 26-year-old woman had progressive difficulty walking by age 20. Examination showed severe weakness and wasting of the lower limbs with distal predominance, and mild weakness in the upper limbs with pseudohypertrophy of the deltoid muscles. Muscle biopsy showed, in all patients, dystrophic changes. Immunohistochemical labelling for dysferlin on frozen sections and Western blot analysis showed absence of dysferlin band. Genetic studies for the identification of the mutation(s) are in progress. At least 14 LGMD genes have been mapped, including both autosomal dominant and autosomal recessive forms. On the other hand, discordant phenotypes may be seen in patients carrying the same mutation, even among affected siblings. Mutations in the dysferlin gene may manifest with mainly distal involvement (Miyoshi myopathy), or with classical clinical features of LGMD. Future knowledge of gene-protein function and protein interactions, and of modifying genes, will better clarify genotype-phenotype correlations in LGMD.

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### LONG TERM TREATMENT WITH ALBUTEROL IN JUVENILE AND ADULT ACID MALTASE DEFICIENCY PATIENTS

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Acid maltase deficiency (AMD) is a prototypical lysosomal disorder that can present as infantile, juvenile or adult onset. Acid  $\alpha$  glucosidase replacement was so far tried only in infantile cases but is not feasible in adult patients. Since 3 years we performed an open prospective clinical trial with a  $\beta_2$  agonist (albuterol) and interni Heart pulsed chain aminoacids. In our trial we included five adult onset ambulant women (age 56-69 years) and a juvenile onset 56 year old man (disease duration: 30 years), who was wheelchair bound and respirator depend-

ent. The rationale of treatment is that  $\beta_2$  agonists antagonise muscle wasting and increase muscle mass and strength. A protocol was designed to follow these patients by functional tests and spirometry. We followed the patients every 3 months initially and then every 6 months evaluating five muscles by MRC scale. We also timed and monitored the performances of four functions (walking, climbing stairs, Gowers' and rising from a chair).

Our patients tolerated well 6-8 mg oral albuterol for this period and their strength and clinical performances appear stabilized after 3 years.

Our data suggest that in adult glycogen storage type 2 the use of oral  $\beta_2$  agonists might be useful.

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### ULLRICH SCLEROATONIC MUSCULAR DYSTROPHY IS CAUSED BY RECESSIVE MUTATIONS IN COLLAGEN TYPE VI

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Ullrich congenital muscular dystrophy (UCMD) is a recessive CMD affecting connective tissue and muscle. Reverse-transcription PCR amplification of RNA extracted from fibroblasts or muscle of three UCMD patients, followed by heteroduplex analysis, displayed heteroduplexes in one of the three genes coding for collagen type VI (COL6). In patient-A we detected a homozygous insertion of a C leading to a premature termination codon in the triple-helical domain of COL6A2 mRNA. Both consanguineous parents were healthy carriers. In patient-B, and in the affected brother, we found a deletion of 28 nucleotides due to an A->G substitution at nucleotide -2 of intron 17 causing the activation of a cryptic acceptor site inside exon 18. The second mutation was an exon skipping due to a G->A substitution at nucleotide -1 of intron 23. The healthy mother carries the first mutation, while the second mutation is carried by their healthy father. In patient-C we found only one mutation so far, the same deletion of 28 nts found in patient-B, a 'de novo' mutation, absent in her parents. mRNA and protein analysis of patient B showed a very low amount of COL6A2 mRNA and of COL6. Severe defect of COL6 was demonstrated by immunofluorescence in fibroblasts and muscle. Our results demonstrate that UCMD is caused by recessive mutations leading to a severe reduction of COL6.

## NON PROGRESSIVE XP21 BECKER MUSCULAR DYSTROPHY IN THE EIGHTH DECADE

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A 60-year old man was referred to us in 1987 complaining of difficulty walking and climbing stairs developed over the previous 10 years. Clinical examination disclosed waddling gait, Gower's sign, calves hypertrophy and proximal wasting and weakness, prevalent in the lower limbs. Serum creatine kinase levels were increased (200 U/l), EMG showed myopathic alterations. Quadriceps muscle biopsy was performed. Routine staining showed fibre size variability, fibre degeneration and increase of connective tissue. Monoclonal antibodies against dystrophin showed regular, continuous but pale contours. Western blot demonstrated reduced molecular weight of dystrophin (370 Kd). Genetic analysis detected a deletion of the exons 45-53 in the Xp21 gene.

Fourteen years' follow up did not show significant impairment of muscle strength or development of cardiac alterations. At the age of 74, the patient can still walk unassisted.

The case underlines the importance of the study of dystrophin in all the myopathies of uncertain definition also in elderly patients.

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## VERY LATE ONSET AND MILD EXPRESSION IN TYPE II GLYCOGENOSIS

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Type 2 glycogenosis is an autosomal recessive disorder due to deficiency of the lysosomal enzyme acid maltase. The clinical spectrum includes infantile, childhood and adult variants. These latter usually have their onset in the third or fourth decade. Few cases with later onset have been described. The oldest case in the literature is a 65-year-old man.

We observed a 74-year-old woman with a two years' history of easy fatigability, proximal weakness in her lower girdle. Neurological examination showed waddling gait, lumbar hyperlordosis, Gowers' sign, and pelvic girdle weakness with normal strength of the upper limbs. Serum CK was 400 U/l. Echocardiography, Holter-ECG and spirometry were normal. Muscle biopsy disclosed a vacuolar myopathy with intralysosomal glycogen storage. Biochemical analysis showed increased glycogen and marked reduction of acid maltase activity (10% of residual activity). A cerebral spiral CT scan did not demonstrate intracranial aneurysms.

The case is interesting for the mild clinical phenotype, limited to weakness of the pelvic girdle muscles and without involvement of the respiratory muscles, which are typically affected

in the adult form. To our knowledge this is the patient with the latest onset reported in the literature and it emphasizes the importance of considering the possibility of a metabolic myopathy even in elderly patients with neuromuscular disorders.

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## ACUTE QUADRIPLEGIC MYOPATHY (AQM): CLUES FROM AN IN VITRO MODEL

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AQM is often associated with corticosteroid therapy and neuroblocking agents, metabolic impairment, sepsis, intensive care and surgery. The morphological hallmarks of AQM are muscle fibers atrophy and thick filaments loss. We have previously shown that apoptosis plays a role in AQM pathogenesis.

As an in vitro model of AQM, normal aneural primary muscle cultures were exposed to 300 mM mannitol, as a metabolic stressor, and 100 microM dexamethasone and then studied with TUNEL, to detect nuclear DNA fragmentation, and immunocytochemistry for caspase 3, Bax and calpain.

Treatment with mannitol or dexamethasone alone induced none or only minor morphological abnormalities, while combined dexamethasone and mannitol resulted in swelling and fragmentation of myotubes. No TUNEL positive myonuclei were found in control and dexamethasone treated cultures, while only a small proportion was found in mannitol-treated ones. In contrast, combined dexamethasone and mannitol produced a high number of TUNEL positive myonuclei and increased staining for caspase 3, Bax and calpain.

Our results confirm a possible role of apoptosis in the pathogenesis of AQM and suggest that i) dexamethasone highly potentiates metabolic stress-induced apoptosis, possibly by inhibiting the IGF-I anti-apoptotic pathway, and ii) the activation of proteolytic pathways contributes to muscle fiber degeneration.

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## SCREENING OF EXPANDED CTG REPEATS ON CHROMOSOME 19 FOR MYOTONIC DYSTROPHY TYPE 1 (DM1) IN 618 ITALIAN PATIENTS AND THEIR RELATIVES, AND 10 CASES OF PRENATAL DIAGNOSIS

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disease and is the most common form of muscular dystrophy affecting adults. An unstable, untranslated part of the DMPK gene on chromosome 19, composed of CTG repeats, is a genetic marker for DM1. Normal individuals have 5 to 50 CTG repeats, mildly affected or asymptomatic DM patients have 50 to 180 repeats, while fully affected patients

have from over 200 to 2000 repeats (range E1=50-500, E2=500-1000 and E3>1000 CTG).

To detect CTG expansion we used a procedure based on a PCR amplification using XL polymerase followed by Southern blot analysis with a fluorescein-labelled (CTG)<sub>10</sub> probe. We studied 618 Italian patients and their relatives [302 males and 316 females]. We identified CTG expansion in 321 DM patients (51.9%) [among these 54 (16.8%) with range E1, 251 (78.2%) with range E2 and 16 (5%) with range E3] and the normal alleles in 297 subjects (48.1%).

Among 119 analysed families 28 showed the phenomenon of anticipation and 6 showed the intergenerational contraction in PBL, with 100% of maternal transmission and 83.3% of paternal transmission, respectively.

This technique was also used for prenatal diagnosis on genomic DNA extracted from 10 chorionic villi of 7 females. CTG expansions were in range E1 for 1 fetus, E2 for 4 fetuses and E3 for 3 fetuses, while two fetuses were normal even if the mothers had CTG expansion in range E2.

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#### MYOGLOBINURIA TRIGGERED BY CONVULSIONS IN MCARDLE'S DISEASE

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Human myophosphorylase deficiency (McArdle's disease; Glycogenosis type V) is one of the most common muscle glycogenoses. It is typically a disease of young adults characterized by exercise intolerance, myalgia, cramps, and recurrent myoglobinuria. The diagnosis is confirmed by histochemical and/or biochemical documentation of phosphorylase deficiency in muscle biopsy or by molecular analysis of the myophosphorylase gene (PYGM) of blood cells. Although the clinical phenotype is rather uniform, different clinical variants both in infancy, childhood, and adulthood have been reported. We report a 11-year-old girl in whom, starting age 8 and in several occasions, muscle necrosis with myoglobinuria were preceded by convulsions or exercise. Neurological examination was normal. A muscle biopsy was obtained from the left quadriceps, and histochemical reactions were performed by described methods. Genomic DNA was extracted from white blood cells of the patients and her parents. Histochemical analysis of muscle showed myophosphorylase deficiency and genetic analysis of PYGM gene showed that the patient was homozygous for the most common mutation encountered in McArdle's disease (R49X), while her parents were heterozygous.

Our case further illustrates the atypical clinical presentation of McArdle's disease and confirm the lack of genotype/phenotype correlation in this disease.

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#### SUBCLINICAL INFLAMMATORY MYOPATHY IN CHRONIC HEPATITIS B VIRUS INFECTION

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An inflammatory myopathy may be associated with different viral illness but muscle fibres are reported to be resistant to direct viral infection. Up to now a clinically evident myopathy has been reported in 7 patients with hepatitis B virus (HBV) infection. The immunofluorescence for HBV was performed on muscle biopsy in only one case with negative result.

We report two patients with chronic active HBV hepatitis, persistent hyperckemia (3-10 X normal) and no weakness. Other causes of asymptomatic hyperckemia were excluded. The EMG was myopathic in both patients with mild spontaneous activity in one. Muscle biopsy showed moderate muscle fibres size variability, necrotic fibres, scattered endomysial CD4+, CD68+ and rare CD8+ cells. Deposits of complement membrane attack complex (MAC) were present in blood vessels walls and in some necrotic muscle fibres. Immunoperoxidase showed positivity of the HBV core antigen in some infiltrating cells and, in one patient, in a few necrotic muscle fibres. At follow up (at least one year) both patients had still active hepatitis and increased CK but did not develop weakness.

We deem that these patients have a subclinical inflammatory myopathy associated with HBV infection. Our immunohistochemical findings may suggest a direct role of HBV in associated myositis.

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#### ASYMPTOMATIC HYPERCKEMIA: THE ROLE OF QUANTITATIVE EMG

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With the inclusion of CK determination in the automated blood chemistry profile an increasing number of patients with apparently unexplained raised CK are referred to neuromuscular clinics. The main problem is how much extensive investigations should be performed to rule out a subclinical neuromuscular disorder.

In the four largest (at least 10 patients) series with asymptomatic hyperckemia qualitative EMG was abnormal in percentages variable from 28 to 78 % and the concordance with histopathology extremely variable.

In 18 subjects with asymptomatic hyperckemia we performed quantitative EMG and muscle biopsy in the contralateral muscles. Quantitative EMG showed reduced mean value of MUAP duration in 6 and increased percentage of polyphasics in 9 patients. Muscle biopsy indicated a specific disorder in 5 patients: 2 had inflammatory myopathy, 1 dystrophinopathy, 1 glycogen storage disease type II, 1 mitochondrial myopathy. One patient had myoadenilate deaminase deficiency and five others showed minor non specific myopathic features. Quantitative EMG was highly specific being normal in all patients

with normal biopsy, and highly concordant with histopathological findings being normal only in the patient with asymptomatic acid maltase deficiency.

In conclusion in presence of a normal quantitative EMG it may be wise to refrain from extensive ancillary test as long as the patient do not have complaints or develop neurological abnormalities.

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#### CARDIAC INVOLVEMENT IN AUTOSOMAL DOMINANT MYOTUBULAR/CENTRONUCLEAR MYOPATHY

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We studied a 23-year-old man and his 43-year-old father with proximal muscle weakness, onset in childhood and slowly progressive course. Elevated CK levels and a myogenic EMG pattern were found. Muscle biopsy showed increased variability in fibre size, type 1 fibre predominance and many central nuclei. At the ATPase stains, many of the fibres contained a central zone of non-reactivity, corresponding to the nucleus. These findings suggested a diagnosis Autosomal Dominant Centronuclear/Myotubular Myopathy. The echocardiographic study showed dilated hypokinetic myocardium ranging from severe in the father to mild in the son. Centronuclear/Myotubular Myopathy is an example of genetic heterogeneity: autosomal-dominant with adult onset; autosomal recessive with childhood onset, and the most common X-linked recessive. The autosomal dominant form has a later onset and milder course than the X linked form, and the autosomal recessive form is intermediate in both respects. Cardiac involvement is extremely rare, even in the X-linked recessive form. The occurrence of dilatative cardiomyopathy in our patients, regardless the differences in the age at the diagnosis and the different general conditions such as ventilatory capacity, suggests a primary involvement of the cardiac muscle.

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#### ADENOVIRUS-MEDIATED UTROPHIN GENE TRANSFER MITIGATES THE DYSTROPHIC PHENOTYPE OF CANINE X-LINKED MUSCULAR DYSTROPHY (CXMD)

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We injected tibialis anterior muscles of newborn CXMD dogs with an adenoviral vector containing truncated utrophin (AdVCMV-Utr) and examined utrophin expression by RNA and protein analysis in cyclosporin treated and untreated ani-

mals. We also evaluated extent of fibrosis and expression of dystrophin-associated proteins (DAPs) as measures of the functional efficacy of gene transfer. In cyclosporin-immunosuppressed animals, 10, 30 and 60 days after treatment, intensely utrophin positive (transfected) fibers were found in variable size clusters. The average proportions of positive fibers were 28.3% at 30 days and 30.3% at 60 days. In animals not cyclosporin treated only scattered transfected fibers were found.

The presence of the shortened utrophin was confirmed by immunoblot. mRNA-PCR analysis of injected muscles revealed the expected 136 bp band of the truncated utrophin in cyclosporin-treated and untreated animals. This band was absent from uninjected dystrophic dogs.

DAP expression was greater in transgenic utrophin-positive areas and these areas were characterized by polygonal-shaped fibers and significantly less fibrosis ( $p < 0.0001$ ) than areas not expressing the exogenous protein.

Transgenic utrophin is expressed at the extrajunctional membrane of CXMD muscle fibers after AdV-mediated gene transfer, with stable expression for at least 60 days in immunosuppressed animals and efficiently mitigates the dystrophic phenotype.

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#### A EMG STUDY OF MYOTONIC AND DYSTROPHIC PHENOMENA IN STEINERT DISEASE

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The phenotypic expression of myotonia and dystrophy is variously combined in patients with Steinert disease (MyD). This represents a difficulty when evaluating the extent to which each one contributes to muscle function impairment and when studying the physiopathological processes underlying the disorder.

The aim of this study was to apply the surface EMG technique, using low and high stimulation frequencies and analyzing an amplitude parameter, in order to fully corroborate the contribution of myotonia and/or dystrophy to muscle impairment in MyD subjects.

Methods: a motor point stimulation protocol, at 15 and 35 Hz, was carried out on the *tibialis anterior* (TA) of 25 MyD patients. These were subdivided into 3 subgroups, MyD3 (9), MyD4 (10) and MyD5 (6), on the basis of their TA MRC score. The surface myoelectric signal was recorded and the average rectified value of amplitude (ARV) was evaluated.

Results: each subgroup presented a characteristic ARV trend both at 15 and 35 Hz: increasing in MyD3 (like the controls), slightly decreasing in MyD4 and clearly decreasing in MyD5.

Conclusions: the analysis of the ARV during a stimulated contraction permits the identification and quantification of the sarcolemma excitability alteration and/or the myofiber degeneration contributing to muscle impairment in MyD.

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## IMMUNOLOGICAL DYSFERLIN SCREENING IN A LARGE POPULATION OF MYOPATHIC PATIENTS

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Recently, a novel mammalian gene has been discovered, whose mutations cause two different myopathies: Limb Girdle Muscular Dystrophy (LGMD) type 2B and distal Miyoshi myopathy (MM). The gene product is a 230 kDa sarcolemmal protein called dysferlin, normally expressed at skeletal muscle level and absent in affected patients.

We analysed muscle biopsies from 151 patients with myopathy and/or hyperCKemia, myopathic EMG, normal muscle expression of dystrophin, sarcoglycans and, in tested patients, calpain, merosin, emerin and caveolin.

By immunohistochemistry, complete dysferlin deficiency was found in 4 patients. Western blot (WB) analysis confirmed protein absence in three patients, whereas residual protein amount was present in the fourth one. Clinically, one had limb-girdle myopathy, two distal myopathy and one paucisymptomatic hyperCKemia. Muscle biopsy was dystrophic in the myopathies and mildly unspecific in the hyperCKemia. Perivascular infiltrates were present in one dystrophic biopsy. In another case, partial calpain deficiency was evident at WB. Apoptotic studies in two muscle biopsies showed scattered positive nuclei with TUNEL reaction.

Dysferlin deficiency was found in 2.6% of the screened population. This percentage is much higher (28.6%) if distal myopathy alone is considered, and lower if the restriction regards limb-girdle myopathy (1.7%) or paucisymptomatic hyperCKemia (1.2%).

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## RIBOFLAVIN-RESPONSIVE MYOPATHY WITH MULTIPLE ACYL COENZYME A (COA) DEHYDROGENASE DEFICIENCY (RR-MAD): A CASE REPORT

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Adult onset RR-MAD is a rarely recognized disorder characterized by progressive limb and axial muscles weakness, lipid storage myopathy and responsiveness to riboflavin supplementation. Riboflavin is the precursor for flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), both being prosthetic groups of various mitochondrial enzymes.

A 55-year old man presented with a progressive proximal muscle weakness, severe fatigability, muscle pain, persistent diarrhoea and weight loss. Laboratory findings showed elevated CK, normal thyroid function, no evidence of malabsorption and absence of urinary organic acids. EMG was myopathic with mild neurogenic signs. Muscle morphology showed increased lipids and ragged-red fibers. Reduced activities of medium- (27%) and long-chain acyl CoA dehydrogenases (56%) and of complex II (58%) with secondary carnitine deficiency were found. Muscle FAD and FMN were 46% and 20% of normal. Clinical symptoms did not respond to steroids or carnitine, but resolved promptly with riboflavin 100 mg/daily. Although riboflavin deficiency may result from malabsorption, in our patient diarrhoea resolved after riboflavin supplementation. Hence, we propose that diarrhoea is also due to riboflavin deficiency.

Thus, a diagnosis of RR-MAD should be considered in patients affected by a proximal myopathy associated with gastrointestinal symptoms, in view of the possible successful treatment.

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## NONSENSE MUTATION IN THE *LAMA2* GENE RESULTING IN EXON SKIPPING AND MILD MUSCULAR DYSTROPHY

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Exon skipping is a mutational mechanism usually caused by changes in consensus sequences at splice sites or lariat branch-point regions. Nonsense mutations outside the splicing consensus sequence have been reported to cause skipping of the nonsense-containing exon in several human diseases. We describe, for the first time, nonsense-mediated exon skipping in the *LAMA2* gene. Two adult siblings from a consanguineous family had a slight reduction in laminin  $\alpha 2$  chain expression and moderate clinical manifestations. In both, molecular analysis revealed a homozygous nonsense mutation, Arg744Stop, expected to result in a totally non-functional protein and a severe phenotype. Analysis of the transcript revealed skipping of exon 15, containing the mutation, although the consensus sequences for splicing at both ends of the exon and beginning of intron 15 were unaltered. Exon skipping restored the open reading frame of the mutant transcript and resulted in a truncated protein. Our data confirm the importance of mRNA analysis to clarify the effect of mutations on mRNA processing, which may be different to that predicted by genomic analysis, and also point to the necessity of immunochemical screening for expression of the laminin  $\alpha 2$  chain in atypical dystrophic adults as well as children.

## AUTONOMIC NERVOUS SYSTEM FUNCTION IN MYOTONIC DYSTROPHY

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To investigate the Autonomic Nervous System (ANS) in patients with Myotonic Dystrophy (MD). 23 MD patients, without severe heart involvement or diabetes, underwent a battery of six cardiovascular reflex tests, PSA of heart rate, electro-neurography, spirometry (FVC) and heart examination. 20 sex and age-matched healthy subjects were also investigated. All cardiovascular reflex tests were normal in 35% of the patients. 61 % had a borderline ANS dysfunction and 4% had a definite ANS dysfunction with two abnormal tests. The most frequently altered tests were the 30/15 ratio and beat to beat variation during deep breathing (DB) (26% and 26% respectively). The mean value of each test recorded in MD patients was not significant different from controls, except for DB, in which it was significantly lower in MD ( $p < 0.0001$ ). Low frequency power resulted significantly lower in MD ( $p < 0.03$ ). Nerve conduction velocity study was altered only in two consanguineous patients. FVC was impaired in 50 % of the patients, without any correlation to DB. There was no correlation between presence of heart abnormalities (48%) and ANS dysfunction. Cardiovascular tests, PSA, electroneurography and respiratory function were non related to CTG repeat size. According with previous studies autonomic dysfunction is not a major feature in MD, without relationship to heart involvement or presence of a peripheral neuropathy.

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## HEPATITIS C VIRUS (HCV) INFECTION AND MYOSITIS: VIRUS LOCALIZATION AND POSSIBLE IMMUNOPATHOGENESIS

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HCV in addition to the liver, may affect other organs and tissues. Although a direct viral pathogenesis has been hypothesized for liver and kidney, some complications are probably related to the secondary production of autoantibodies and immunocomplexes. An inflammatory myopathy has been rarely reported and poorly documented. We describe a patient with chronic active HCV infection without cryoglobulins who developed an inflammatory myopathy.

Muscle biopsy showed perifascicular atrophy, necrotic and regenerating fibres, perivascular and endomysial infiltrates. Immunofluorescence showed deposits of IgG and fibrinogen in the wall of muscle vessels. Complement membrane attack complex (MAC) deposits were evident in many blood vessels

and on sarcolemma of some muscle fibres. The predominant cell subsets within the infiltrates were CD4<sup>+</sup> and CD68<sup>+</sup>. Reverse transcription in situ polymerase chain reaction showed positive signals in several endomysial cells surrounding muscle fibres. Double staining immunocytochemistry with CD markers and monoclonal ab against HCV core NS3 antigen documented positivity in some CD8<sup>+</sup> cells.

Our findings confirm the known resistance of muscle fibre to viral infection and indicate that HCV is confined to infiltrating cells like in myositis associated with HIV and HTLV-I. The perifascicular atrophy, the deposits of IgG and MAC in the wall of the intramuscular vessel resemble the features usually encountered in dermatomyositis and suggest an antibody-dependent pathogenesis, mainly directed against the capillary endothelium, in HCV myositis.

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## RECURRENT MYOGLOBINURIA AFTER TRICHLOROETHYLENE INHALING

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Trichloroethylene (TCE) is a halogenated hydrocarbon, like chloroform, widely used as dry-cleaning agent and industrial solvent. Depression of central nervous system, deep narcosis, coma, cardiac arrhythmias are the effects of TCE inhalation reported in occasional, acute poisonings; axonal neuropathies are possible consequences of chronic professional exposure.

A 37-year old man with a history of alcoholism, was studied for two episodes of rhabdomyolysis occurring after TCE abuse through vapours inhalation. In the first episode, CK serum levels raised up to 32000 U/l, in the second to 1000 U/l. Neurological examinations, renal and hepatic functions were normal. Muscle biopsy, performed after CK normalization, showed moderate fibre size variability and slight hyperactivity of oxidative enzymes. Biochemical analysis excluded the enzyme defects commonly related to rhabdomyolysis.

The effects of TCE on muscle are poorly known. Some experimental findings have shown an effect on muscle membranes, inducing a higher Ca<sup>++</sup> release and enhancing contraction; its clinical significance is however still unclear. In our case the concurrent alcohol addiction could be relevant, causing a sub-clinical myopathy and enhancing the effects of TCE on muscle.

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## ABSENCE OF APOPTOSIS IN SKELETAL MUSCLE TISSUE OF PEO PATIENTS WITH MUTATIONS IN THE ADENINE NUCLEOTIDE TRANSLOCATOR 1 GENE

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Autosomal dominant progressive external ophthalmoplegia (adPEO) is a human disease associated with multiple mtDNA deletions. Linkage analysis assigned some affected families to the 4q34-35 locus. Recently, mutations have been identified in the nuclear gene encoding the heart/skeletal muscle isoform of the adenine nucleotide translocator 1 (ANT-1), located in the critical region of the 4q locus. Because ANT-1 is also a structural component of the mitochondrial permeability transition pores, and has a role in mitochondrial-mediated apoptosis, it is hypothesized that apoptosis may have a role in the pathogenesis of adPEO caused by ANT-1 mutations.

To verify this hypothesis, we studied muscle biopsies from seven patients from five 4q-linked adPEO families. We used TUNEL reaction as a marker of nuclear DNA fragmentation, and antibodies against pro- (Fas, Bax) or anti- (Bcl-2) apoptotic factors. Also, we performed ultrastructural studies.

In all cases, we found no significant expression of both pro- and anti-apoptosis related proteins, nor did we find TUNEL positivity. This finding is confirmed by lack of morphological evidence of apoptosis in all the fibers examined at ultrastructural level. We conclude that ANT-1 defects cause accumulation of multiple mtDNA deletions and the consequent adPEO phenotype by a mechanism other than the apoptotic one.

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## FURTHER DELINEATION OF DIAGNOSTIC CRITERIA OF CONGENITAL MUSCULAR DYSTROPHY BASED ON 16 PATIENTS

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The CMD are an heterogeneous group of diseases characterized by a marked weakness, generalized hypotonia, joint contractures and a muscle histology suggestive of dystrophic changes. They are divided into pure or classical form and another group including Fukuyama muscular dystrophy, Muscle Eye Brain and Walker-Warburg syndrome (WWS) which are associated with cerebral abnormalities. Following discovery of merosin, a muscular protein, pure CMD was subdivided into merosin positive (normal distribution) and in merosin negative (no immunostaining) types.

We have reviewed the clinical, neurophysiologic, neuroimaging data of 16 patients that were divided in two groups, the first one (Group A) included 14 cases with merosin positive CMD and the second (Group B) 2 patients with WWS.

Regarding the group A 6 out of 14 had a clinical onset in the neonatal time with arthrogyposis, the others had the clinical onset after 6 months of age. The muscle biopsy showed the typical dystrophic pattern in 10 and 3 had the myopathic picture and in only one there was not evidence muscular damage. In the group B the clinical onset was during the first day of life with muscle biopsy typical of muscular dystrophy. These preliminary data show the necessity of a new definition and classification.

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## NEUROPSYCHIATRIC EVALUATION IN BECKER MUSCULAR DYSTROPHY PATIENTS

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Becker muscular dystrophy (BMD) is an allelic disease due to a partial deficiency of a cytoskeletal muscular protein called dystrophin (dys). The dys gene is localised on short arm of X chromosome. BMD is a progressive disorder characterised by progressive degeneration of muscular skeletal tissue without central nervous system (CNS) involvement. The typical BMD represents the benign variant of Duchenne Muscular Dystrophy (DMD) and the symptom of onset is usually proximal weakness. After the dys discovery there are, also, atypical BMD with slight muscular symptoms as cramps, myalgias, fatigability, dilatative cardiomyopathy and in rare cases psychiatric disturbances.

The literature shows evidence of neuropsychiatric onset in BMD patients without muscular symptoms. In the Unit of Pediatric Neurology we have evaluated 20 BMD patients (ranged from 4 to 25 years) diagnosed by muscle biopsy and confirmed by gene deletion.

We have examined the degree of muscular involvement by Vignos Scale and performed neuropsychological tests to correlate the BMD to the psychiatric disorders.

We have noted that in 1/20 patient was made a diagnosis of schizophrenia and in 6/20 were evidences of attention/hyperactivity disorders (ADHD). In conclusion we have to consider that BMD patients could have neuropsychiatric disturbances also as unique clinical sign of the disease.

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## ATYPICAL CLINICAL PRESENTATION OF PYRUVATE DEHYDROGENASE DEFICIENCY

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We report on a 2-year-old boy, first child of unrelated healthy parents, who presented at birth with generalized hypotonia. At three months of age he started to present episodes of weakness, with bilateral ptosis, ophthalmoplegia and respiratory distress,

triggered by fever. Laboratory investigations revealed increase levels of lactate and pyruvate in serum and liquor, and massive urinary excretion of lactate. Determination of blood lactate/pyruvate ratio and 3-OH-B/AA moles ratio showed altered values. Between these episodes, neurological examination showed generalized muscle weakness, areflexia, delay of motor milestones, normal intellectual development, and laboratory investigations revealed mild increase of lactate and pyruvate in blood and liquor. EMG showed a neurogenic pattern on the tibialis anterioris muscle and myogenic and neurogenic features on the deltoids. Histochemical analysis of muscle biopsy did not revealed any specific alteration. Brain MRI images showed bilaterally hyperintensity in the globus pallidus. Genetic analysis of the E<sub>1</sub> gene of the pyruvate dehydrogenase complex revealed a previously reported mutation in exon 8, R236G. Substitutive treatment with thiamine was instituted.

The benign clinical evolution together with the mild alterations in CNS collocates our cases in an intermediate form between the severe and lethal neonatal form and the moderate one.

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#### HISTOLOGICAL, HISTOCHEMICAL AND BIOCHEMICAL ANALYSIS IN KEARNS-SAYRE SYNDROME: A CASE REPORT

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Mitochondrial syndromes represent a heterogeneous group of pathologies which frequently involve Central Nervous System and/or Peripheral Nervous System and skeletal muscles. Mutations are found more frequently in mitochondrial DNA (mtDNA) than in nuclear DNA (deletions, duplications, point mutations, etc.).

The Kearns-Sayre Syndrome (KSS) is a progressive multisystem disorder characterized by progressive external ophthalmoplegia associated to multiorgan involvement (mainly heart conduction block, retinal degeneration, neurosensorial deafness, mental retardation, myopathy, etc.). Herein we report the case of a 42 years-old man, who presented a bilateral II-degree ptosis, external ophthalmoplegia, ataxic gait, neurosensorial deafness, diffuse weakness, mental retardation and left conduction block. The symptoms onset was in the second decade of life and progressed very slowly. Serum biochemical findings showed high levels of CK (2020 U/l) and lactic acidosis (3.8 mmol/l at rest; 4.4 mmol/l after 10 minutes of ischemia). His mother suffered from a bilateral neurosensorial deafness and cardiac conduction block. EMG test revealed a myopathic pattern; muscle biopsy demonstrated: marked variation in the size of fibers with large and small fibers being intermingled in a random pattern; deposition of fat within the muscle fibers at lipid staining; more than 30% of fibers lacking glycogen and cytochrome-oxidase; typical ragged-red fibers at the Gomori staining. Genetic analysis evidenced a large deletion in the mtDNA. Primary cell cultures were obtained by muscle and

skin specimens; mtDNA analysis revealed the following mutation percentage: 36% in myoblasts; 34 % in skin fibroblasts; 28 % in muscle fibroblasts, while it was found 86% in skeletal muscle fibers. Considering that patients with KSS may differ widely in the severity of symptoms and disability, we hypothesize that DNA analysis extended to the isolated cell cultures, may represent a key role in the understanding the correlation genotype-phenotype.

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#### THE POSTURAL DRAINING: LONG TERM TREATMENT OF THE RESPIRATORY FAILURE IN DUCHENNE PATIENTS

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Many neuromuscular diseases and especially the primitive myopathies and, more particularly, the Duchenne Muscular Dystrophy, because of the weakness of the thoracic muscles, determine the loss of an efficacious cough. This phenomenon causes a secretion's stagnation, due to the progressive destruction of the "ciliary escalator", for the happen of many infectious episodes.

Though the mechanical ventilation can be a very important method for the treatment of the respiratory failure in Duchenne patients, procrastinating the respiratory insufficiency, the daily postural draining can be useful, especially in some subjects with abundant bronchial secretions; the stagnation of these secretions determine the happen of phlogosis episodes of the respiratory tract.

120 subjects affected by Duchenne muscular dystrophy, were submitted to daily postural draining; all the patients showed a ventilatory failure; 50% of patients had V.C. values = 50% of predicted; 30% of patients had V.C. values = 30% of predicted and 20% of patients had V.C. values < 30% of predicted.

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#### THE PULMONARY SCINTIGRAPHY: A FURTHER EVALUATION PARAMETER OF THE RESPIRATORY FAILURE IN DUCHENNE PATIENTS

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The ventilatory and perfusive pulmonary scintigraphy, not invading medico-nuclear technique, influenced the diagnosis of some diseases and, in particular, of the pulmonary embolism. This method can be used to diagnose many diseases, as well as inflammatory or infiltrative. The neuromuscular diseases are frequently characterized by a severe and increasing ventilatory deficit; the Duchenne muscular dystrophy is the disease that, more typically, shows a precocious and increasing respiratory failure. For an evaluation by images of the pulmonary function, 20 subjects affected by DMD, in various phases of the disease and with a restrictive disventilatory syndrome, were submitted to pulmonary perfusive and ventilatory scintigraphy.

## APOPTOSIS AND EMERIN PROCESSING IN MUSCLE CELLS

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Involvement of apoptosis in muscle disease has been suggested by several authors. However, the role that proteins mutated in muscular dystrophies play in the apoptotic process is still unclear. Emerin is an integral nuclear membrane protein which is lacking or mutated in the Emery-Dreifuss muscular dystrophy. We induced apoptosis in cultured myoblasts to evaluate emerin fate during programmed cell death. Emerin processing was evaluated by Western blot analysis and compared to proteolysis of lamin A/C. Emerin proteolysis occurred in apoptotic myoblast nuclei and emerin staining at the nuclear rim decreased in these cells. Myoblast apoptosis and emerin degradation were associated with morphological changes including chromatin compaction and detachment from the nuclear lamina, as detected by electron microscopy. In vivo inhibition of caspase 6 activity affected emerin proteolysis in apoptotic cells: this finding suggests that emerin cleavage depends on this enzyme. Our results show that the process of programmed cell death leads to emerin proteolysis in myoblasts: this process appears to be related to caspase 6 activation and/or to cleavage of other nuclear lamina proteins, such as LAP2, LBR and lamin A/C that share sequence homologies or functional features with emerin.

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## RESPONSE TO ERGOMETER STRESS TEST IN ATHLETES WITH HYPERCKEMIA

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The increase in CK values obtained after training is caused by a damage of sarcolemma and is closely related to length, intensity and type of exercise. The pattern of muscular isoforms (CK and LDH) in a group of athletes (15; group A) with increased values of CK was evaluated and compared with a control group (20; group B) with normal CK values. All the subjects, age, sex and sport-matched, underwent ergometer stress test with the evaluation of serum values of CK, LDH and related isoforms at resting, at 5 minutes by the effort end, and at 6, 24 and 48 hours. CK values at rest were significantly different in the two groups (185.9 U/L in group A versus 49.3 U/L in group B;  $p = 0.03$ ) and did not vary throughout the entire period of observation. Interestingly, 48 hours after the stress test, 71.4 % of the athletes in group A showed CK values still elevated, compared with 0% of the athletes in group B. The data were statistically evaluated (Student T test for non paired

data, chi square test). We hypothesize that the lack of recovery in the athletes of group A is related to an underlying damage of the muscular membrane and deserves further investigation from a genetic point of view.

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## PHENOTYPIC HETEROGENEITY IN MYOTONIC DYSTROPHY WITH NO CTG REPEAT EXPANSION

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In recent years, a disorder similar to myotonic dystrophy (DM) but lacking the CTG repeat expansion at the DM1 locus has been described. Most of these patients show a peculiar phenotype, defined as proximal myotonic myopathy (PROMM) and characterized by predominant proximal muscle weakness, myalgia, facial muscle sparing and by mild histological changes. However, to date there are no clues as to the pathogenesis of this entity, and ultrastructural studies of muscle are lacking. We report the clinical, histopathological and ultrastructural findings of patients from two families presenting with a multisystem disease including myotonia, myalgia, facial and distal muscle weakness and atrophy, cataracts and white matter abnormalities on brain MRI. However, CTG repeat expansions at the DM1 locus were not found in either of the probands. In family 1, other clinical features were: proximal muscle weakness, mental retardation and possible clinical anticipation. A linkage analysis of this family ruled out the involvement of the DM1 locus. Muscle biopsy in the probands showed severe alterations in family 1, with large group atrophy and scattered necrosis and moderate changes in family 2. In both cases, ring fibers and sarcoplasmic masses, typical of DM, were absent. These findings demonstrate that DM without trinucleotide repeat expansion can present clinical and histopathological features intermediate between DM1 and PROMM.

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## SELECTIVE ANTIBODY NEUTRALIZATION OF IGF-I AND IGF-II CORRELATES WITH DIFFERENT MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAYS IN DMD MYOBLASTS

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The extracellular signal-regulated kinase (ERK) and the c-jun kinase (JNK) are two MAP kinases that could play a role in the DMD myoblast response to IGFs growth factors. Antibody neutralization of IGF-I contained in DMD muscle extract culture media, as well as antibody neutralization of IGF-I receptor, significantly increased ERK phosphorylation of DMD myoblasts and the activity of its downstream substrate, the p90 ribosomal S6 kinase 2 (RSK2), by 1.5-folds, but it had no ef-

fects on JNK activity. In contrast, antibody neutralization of IGF-II had no effect on ERK phosphorylation or RSK2 activity, but it increased JNK activity by twofold, an effect that was inhibited by specific antibodies against JNK. Furthermore, the phosphorylation of both p46 and p55 isoforms of JNK, measured by phosphospecific antibody, was increased several folds. The activity and phosphorylation of MAP kinase kinase (MKK)-4, an upstream regulator of JNK, was unchanged when IGF-II was inhibited. IGF-I and IGF-II have different effects on MAP kinase signaling pathways in DMD myoblasts, which may be one of the underlying mechanisms through which IGFs could control the DMD myoblast growth.

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#### RIGID SPINE CONGENITAL MUSCULAR DYSTROPHY (RSMD1): CLINICAL AND MUSCLE IMAGING DATA IN 5 PATIENTS

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Rigid Spine congenital Muscular Dystrophy (RSMD1) is an autosomal recessive condition characterised by mild weakness of the girdle and limb muscles, early and diffuse contractures, severe progressive shortening and weakness of the trunk muscles leading to fixation and loss of movement of the spine and the thoracic cage, resulting in rigid spine, scoliosis, and early restrictive respiratory failure. The gene has been mapped on chromosome 1p35-36. We have studied 5 patients in 3 families affected by RSMD1. Onset of symptoms was at birth in one, in the first year of life in 3, and at the age of 4 in one. First steps were before the age of 18 months. Facial and neck weakness were present in all. Mouth opening was particularly limited in 3. All had nasal voice. They were thin with diffuse muscle wasting. Muscle weakness was usually 4/5 in the shoulder girdle and proximal limb muscles. Finger extensors were particularly weak (2-3/5). Hip girdle muscles were 2-3/5. The contractures were more prominent in the proximal joints and in the spine. Scoliosis had an early onset (5 to 10 years of age) and at least in 2 cases a rapid characteristic progression with rigid lordoscoliosis, lateral bending and flexion of the trunk. Cardiac evaluation was normal. Vital capacity was severely reduced. The 4 older were mechanically ventilated with Bipap at night. CPK was mildly elevated in two (1.5-3 times normal). Muscle CT showed a peculiar consistent pattern. Selective 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.

#### ROLE OF CAVEOLINS IN MUSCULAR DYSTROPHIES

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Mutations in the caveolin-3 gene (*CAV3*) cause a severe deficiency of caveolin-3 protein expression in muscle fibers and are associated with a specific form of autosomal dominant limb girdle muscular dystrophy (LGMD1C) (Minetti et al, 1998). We recently described a novel mutation in *CAV3* gene in two unrelated children with partial caveolin-3 deficiency and isolated hyperCKemia, without any clinical symptom of myopathy. This is the first demonstration of a new phenotype associated to *CAV3* mutations and indicates that a partial caveolin-3 deficiency should be considered in the differential diagnosis of idiopathic hyperCKemia.

In DMD muscle, we found an increased number of caveolae at the sarcolemma that corresponds to an over-expression of caveolin-3. These data suggest a possible role for caveolae and caveolin-3 in the pathogenesis of DMD. To test this hypothesis, wild-type caveolin-3 was overexpressed as a transgene in mice. Transgenic mice over-expressing caveolin-3 show a dystrophic pattern and virtually undetectable levels of dystrophin. To understand a possible mechanism to explain this phenotype we demonstrated that caveolin-3 directly interacts with beta-dystroglycan and that this interaction may competitively regulate the recruitment of dystrophin to the sarcolemma. Taken together, this findings may open new perspectives in elucidating the pathogenesis of muscular dystrophy.

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#### EPIDEMIOLOGY OF DUCHENNE MUSCULAR DYSTROPHY IN THE PROVINCE OF TURIN: RESULTS AFTER TWENTY YEARS OF COUNSELLING

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In 1980 our epidemiological survey of DMD in the province of Turin found an incidence of  $24.23 \times 10^{-5}$ , meaning 1 case in 4127 live male births during the years 1955-1974. The prevalence rate was  $2.15 \times 10^{-5}$ . These results were comparable with other data in the world literature at that time.

After the identification of dystrophin, a new approach to genetic counselling for the disease allowed accurate carrier identification and prenatal diagnosis, with reduction of new cases in informative families. On the other hand, the great advances of mechanical ventilation in the last decade allowed a prolonged survival of DMD boys, now reaching the fourth decade.

To verify the efficacy of counselling in our Province, we performed a second survey, considering the years 1992 -1999. A

total of 35 prenatal diagnoses for DMD were performed, resulting in 18 female, 15 normal male, and 2 DMD affected male fetuses. No new familial case was observed. According to preliminary data, six DMD boys were born in this period, corresponding to an incidence rate of  $7.5 \times 10^{-5}$ . On the contrary, prevalence did not significantly differ from 1974, also due to new cases among immigrated population. The median age of DMD patients was increased.

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#### NUCLEAR FACTOR KAPPA B EXPRESSION IN INFLAMMATORY MYOPATHIES

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Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is an ubiquitous rapid response transcription factor in cells involved in immune and inflammatory reactions, and exerts its effect by expressing cytokines, chemokines, cell adhesion molecules, growth factors, and immunoreceptors. NF- $\kappa$ B contributes to immunologically mediated diseases such as allograft rejection, rheumatoid arthritis, and bronchial asthma. It is also thought to play an important role in the expression of genes expressed in response to ischemia/reperfusion injury. We studied expression of NF- $\kappa$ B by immunocytochemistry and western blot in skeletal muscle specimens from 5 patients with polymyositis and 5 patients with dermatomyositis. Immunohistochemistry for macrophages, B, CD4 and CD8 cell subsets, and major histocompatibility complex class I and II antigens was also investigated. NF- $\kappa$ B expression was found in some vessel walls, in several atrophic myofibers especially those with a perifascicular distribution, and in a few infiltrating cells. Results suggest that NF- $\kappa$ B plays little pathogenic role in the advanced phases of inflammatory myopathies.

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#### COENZYME Q10 DEFICIENCY IN PREVIOUSLY UNCLASSIFIED CEREBELLAR ATAXIAS

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A mitochondrial encephalomyopathy with Coenzyme Q10 deficiency has been reported in the last few years in an increasing number of patients. A central nervous system involvement with epilepsy, or ataxic syndrome often occurred. In a series of 35 muscle biopsies from patients with cerebellar syndrome in whom cerebellar ataxia could not be attributed to specific metabolic or genetic causes, we measured mitochondrial enzymes activities and Coenzyme Q10 levels. Decreased CoQ10 levels in muscle were found in six patients, with residual amounts varying from 26% to 35% of controls. Muscle biopsies showed minimal morphological changes with no specific mitochondrial markers. Biochemical assays of mitochondrial respiratory chain enzymes evidenced in 2 pa-

tients reduced activity of complex III. MRI of the brain showed severe cerebellar atrophy. All six patients were treated with oral CoQ10 therapy (600-1200 mg/die) for one year. The patients were evaluated before and after one year of replacement therapy. Clinical improvement was assessed with the "International Cooperative ataxia rating scale".

Our results suggest that a Coenzyme Q10 deficiency should be considered in patients with unclassified cerebellar ataxia especially considering the benefits of a specific metabolic treatment.

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#### ASSOCIATION OF MITOCHONDRIAL ENCEPHALOMYOPATHY WITH COELIAC DISEASE: IMPROVEMENT OF CLINICAL AND NEURORADIOLOGIC FEATURES AFTER INTRODUCTION OF GLUTEN-FREE DIET

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Malabsorption due to a metabolic mitochondrial dysfunction is a cardinal feature in MNGIE and in other infantile mitochondrial disorders with villous atrophy. We report a sporadic mitochondrial disorder in whom malabsorption was instead due to the concurrence of a celiac disease. A 19 year-old boy had developed diabetes at age 7. Three years later he received a diagnosis of celiac disease confirmed by intestinal biopsy. At age 19 he started to complain of gait disturbances, tremor and headache. Neurological examination showed ptosis, ophthalmoparesis, truncal ataxia, dysarthria, intentional tremor and pyramidal signs. Brain MRI showed a diffuse leukoencephalopathy and muscle biopsy showed a mitochondrial myopathy associated with dimers of a 8kb deletion of mtDNA. An extensive metabolic screening and CSF examination gave normal results, while vitamin E and folate were below normal. Based on these findings, we prescribed a strict gluten-free diet. Dietetic treatment produced a marked amelioration of ataxia and tremor and also brain MRI showed a mild but significative improvement of white matter abnormalities.

This report underlines the importance of performing accurate studies for malabsorption associated with mitochondrial disorders in order to assess the most accurate treatment and to prevent any progression of the disease due to misdiagnosis.

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#### MITOCHONDRIAL ENCEPHALOMYOPATHY IN ONE OF TWO MONOZYGOUS TWINS

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Mitochondrial diseases include a large variety of clinical expressions often with multisystem involvement. The attainment of the central nervous system rarely features an extrapyramidal syndrome: parkinsonism was described associated with a

4bp deletion of cytochrome b gene and dystonia has been found both associated with Leber optic atrophy and with myopathy, in the presence of different point mutations of mitochondrial DNA. We observed a 48 years old man with a healthy identical twin. He has been complaining of impaired gait for the past two years. Neurological examination showed a dystonic attitude appearing on attempted walking compelling the patient to walk on his toes, increased muscle tone in the lower limbs, distal hypotrophy of the lower limbs and diffuse proximal muscle weakness, limitation of the flexo-extension of the feet, slight bilateral dysmetria. Serum CK was 449 U/l, EMG showed myopathic features. ECG was normal. Brain MRI disclosed atrophy of the vermal portion of the cerebellum and hyperintense areas in the nuclei pallidi. Muscle biopsy demonstrated a mitochondrial myopathy with partial cytochrome c activity. Mitochondrial DNA analysis is in progress. The interest of the case lays on the unusual clinical expression, involving the extrapyramidal system, and in the appearance of the disorder in only one of two identical twins, suggesting an extreme degree of heteroplasmy.

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#### LATE ONSET MULTISYSTEM DISORDER WITH MUSCLE MITOCHONDRIAL DNA DEPLETION: A CASE REPORT

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Quantitative mitochondrial DNA (mtDNA) defects have been associated with mitochondrial disorders, usually characterised by infantile or childhood onset of progressive myopathy, and/or hepatopathy, encephalopathy and renal involvement. Individuals exhibit variable levels of mtDNA depletion (up to 98%) in affected tissues. In addition, different tissues may be involved in related patients. The primary pathogenetic mechanism underlying mtDNA depletion is unknown.

We now describe a 26 year-old female patient born from healthy, non-consanguineous parents, affected with a late onset mitochondrial disorder. She attended school till the age of 13 and developed normally until the age of 14 years, when a primary hypothalamic amenorrhoea was found. At age 24 she began to complain fatigue after a mild muscular exercise with proximal muscle weakness. Serum CK and rest lactate levels were elevated (CK: 1691 U/l ; n.v.: <200; lactate 28,8 mg% ; v.n.: <19,8 mg%). Neuropsychological investigations revealed a mild mental impairment (MMSE: 26/30; WAIS: 71; n.v.: >69). Brain CT and MRI showed two lesional areas in the left fronto-mesial and right thalamic associated with hyperintense bilateral signal alterations in the hemispheric white matter. CSF and EEG were normal. Fundus oculi revealed a dystrophic maculopathy. EMG demonstrated chronic mild myopathy in proximal muscles without active denervation and associated mild neuropathy. Muscle biopsy revealed marked fiber size variability, many central nuclei, some necrotic fibers with a macrophagic infiltration. A high percent-

age of fibers showed strong SDH activity and COX deficiency. Immunohistochemistry revealed the presence of CD4, CD8 positive cells and a milder CD19 positivity. Quantitative Southern blot analysis revealed the presence of 20 % residual muscle mtDNA. This patient, sixth report of a late-onset of mtDNA depletion, is peculiar for the age, the dramatic dystrophic-like and inflammatory aspects of the muscle biopsy, and the nature of central nervous system involvement.

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#### DIAGNOSTIC ROLE OF MUSCLE BIOPSY AND DNA ANALYSIS IN THE INCIDENTAL HYPER-CK-EMIA IN CHILDREN

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Diagnostic role of muscle biopsy and DNA analysis in a cohort of pediatric patients investigated for incidental hyperCKemia. Clinical and laboratoristic findings including muscle biopsy of 75 patients, aged 11 months-12 years at the time of biopsy ( follow-up 6 months-11 years ), presented with an incidental, asymptomatic, persistent serum creatine kinase ( CK ) increase were reviewed. Muscle biopsies were studied by light microscope and, when indicated, biochemical and/or ultrastructural examinations were performed. In cases with normal or myopathic-dystrophic histological picture the immunohistochemical evaluation of dystrophin was made. Patients with dystrophinopathy were investigated by DNA analysis for dystrophin gene deletions and/or by further immunohistochemical tests. In 37 patients out of 75 a preclinical diagnosis of a specific muscle disorder was obtained. In 38 cases the cause of hyperCKemia remained unknown. Pediatric patients presented with incidental asymptomatic hyperCKemia must be studied with muscle biopsy and appropriate DNA analysis which may discover a preclinical myopathy in a high percentage of cases. The chapter of Idiopathic iperCKemia must be revisited by the light of new etiopathogenetic knowledges and techniques. A diagnostic protocol is proposed. The approach to the diagnosis by the investigation of many muscle-membrane proteins as first step is discussed.

## CLINICAL, GENETIC AND EPIDEMIOLOGICAL ASPECTS OF LIMB-GIRDLE MUSCULAR DYSTROPHIES IN SOUTHERN ITALY

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Clinical, genetic and epidemiological aspects have been evaluated in 140 patients, followed at the Cardiomyology and Medical Genetics of 2<sup>nd</sup> Naples University, affected by limb-girdle muscular dystrophies and in 328 relatives, seeking genetic advice, in order to study the occurrence of the autosomal recessive muscular dystrophies in Southern Italy, to correlate the clinical features with the wide spectrum of genetic variability, to detect the gene carriers, to allow a prenatal diagnosis. All the patients underwent clinical examination, standard and dynamic eeg, ecocolor Doppler cardiography, spirometric evaluation, DNA analysis and/or muscle biopsy. So far, 24 patients with sarcoglycanopathy, 47 with calpainopathies, and 3 with mutations in the telethonin gene have been identified. The clinical features of patients were related to the type of mutation. A prenatal diagnosis was made in 2 families, respectively with gamma and delta sarcoglycanopathy and resulted in 3 not affected fetuses. Calpainopathies seem to be the most diffuse form of limb-girdle muscular dystrophy, reaching the 25% of all cases. In respect of sarcoglycanopathies, calpainopathies show a less severe clinical course, a greater incidence of heart conduction defects and of respiratory involvement.

A careful estimate of carriers in the general population will consent a more effective genetic counselling and an accurate prenatal diagnosis.

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## DETECTION OF CARRIERS OF DUCHENNE/BECKER MUSCULAR DYSTROPHIES

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During the last 7 years 1153 females sought advice to the genetic counselling of Cardiomyology and Medical Genetics of 2<sup>nd</sup> Naples University. This large group included females closely related to Duchenne/Becker patients, often dead before the DNA analysis era, and females with increased serum CK values and a negative family history for dystrophinopathy. The first step of this process was to identify the causative mutation in each family and then to test females at risk to detect mutations. In families with dystrophin gene deletions or duplications, the carrier status was ascertained by quantitative multiple PCR amplification, using 72 exons. In females without deletion and/or duplication we used a novel fully automated instrument (DHPLC, Transgenomic Wave System) specifically designed to rapidly separate single- and double-stranded DNA fragments and identify the presence of point

mutations. Mutations of dystrophin gene were identified in 275 (73%) families, 214 Duchenne and 65 Becker. A carrier status was ascertained in 39.7% of consultants, and was excluded in 60%. Forty-three percent of mothers did not show the mutation detected in their children; 5 cases of germ-line mosaicism were documented.

Mutations in the dystrophin gene were detected in 40 families in which the affected male was not available and in 12 females with isolated hyperCKemia.

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## INCLUSION BODY MYOPATHY: AN ITALIAN FAMILY WITH AUTOSOMAL DOMINANT INHERITANCE

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Familial or hereditary inclusion-body myopathy (h-IBM) constitutes a heterogeneous group of debilitating disorders, histologically characterized by presence of "rimmed vacuoles" and filamentous inclusions positive for various alien proteins. Autosomal recessive inheritance (AR-IBM) has been described in quadriceps-sparing h-IBM, linked to chromosome 9p1-q1. Autosomal dominant inheritance has been rarely reported, and no chromosomal linkage has been presented to date. We describe an Italian kindred manifesting clinical features and pathologic changes of D-IBM. In this family six members were affected in four generations with a direct male-to-male and female-to-female transmission. Onset of disturbances ranged from 22 to 28 years of age. In 3 patients the onset of the disease was mainly characterized by a distal weakness at lower limbs; progressive wasting and weakness of proximal muscles were then evident in all affected members. Two also had dysphonia. CK levels were increased (~ 1500 IU/l). To our knowledge this is the first report of D-IBM in our country. Linkage DNA analysis may be helpful to better classify such a disease.

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## COGNITIVE AND BEHAVIORAL ASSESSMENT IN CHILDREN WITH MEROSIN POSITIVE NON PROGRESSIVE CONGENITAL MYOPATHIES

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Children with non-progressive congenital myopathies are usually very well studied for muscle and cardiac function, and figures on extent and frequency of muscle weakness and cardiopathy are reported in the literature. Cognitive, linguistic, social abilities of these children are much less investigated. Nevertheless, these variables are of great importance in determining functional outcome and quality of life. We reviewed and assessed cognitive, social, behavioral, and learning abilities in 8 children (6 males and 2 females) with biopsy proven merosin positive non-progressive congenital myopathy. Pres-

ent mean age was 8.8 years (range 2-18). Severity of muscle involvement was moderate (6) to severe (2), moderate to severe respiratory problems were reported in 3. No cardiomyopathy was reported in the group. The sample was studied with formal cognitive (WAIS/WPPSI) and behavioral (CBCL/MOS SF36) testing, academic performance was scored according to teacher's reports. Mild cognitive impairment was found in 2 patients, in one of whose linguistic abilities and academic performances were compromised. The pathological diagnosis in this case was fiber type disproportion. Behavioral-social problems were reported in one boy with normal I.Q. Family adaptation to the children disabilities was satisfactory in all cases. Children with merosine-positive congenital myopathy may show cognitive and behavioral problems which, albeit milder than those described in DMD patients, need to be addressed while planning a long term rehabilitation project for these subjects.

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#### MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY WITH ADULT ONSET

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A 49 year-old man was hospitalised for recurrent episodes of muscle pain, marked fatigability, nausea and vomiting associated with progressive and diffuse muscle weakness during the last two years. He complained also of sporadic and mild attacks of cramps and fatigue in his legs from age 12. Neurological examination showed severe muscle wasting (he lost 15 kg body weight in ten months) and weakness, prevailing proximally. CK was 2746 U/l, LDH 3123 U/l, AST 299 U/l and ALT 277 U/l. Electromyography was myopathic. Muscle biopsy showed a vacuolar myopathy with lipid storage. Carnitine determination performed in muscle showed a severe reduction of free and total fractions with elevation of long-chain esters. GC/MS of plasma total fatty acids revealed the presence of tetradecenoic acid (C-14:1  $\omega=9$ ). A deficiency of long-chain fatty acid oxidation was suspected. Biochemical studies demonstrated a multiple acylCoA dehydrogenase deficiency in the muscle. Carnitine (4 g/day), riboflavin (100 mg/day) and prednisone (37.5 mg/day) were administered. The patient's clinical condition ameliorated within a month of this therapy. His muscle strength improved, his body weight increased from 63 to 66 kg and CK, LDH, AST and ALT returned to normal levels. At five months follow-up the general condition improved progressively.

#### MEXILETINE IN MYOTONIC DISORDERS: ASSESSMENT OF STRENGTH AND MYOTONIA BY QUANTITATIVE MUSCLE ASSESSMENT (QMA)

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To quantify the effects of mexiletine on muscle strength and on grip myotonia in dystrophic and non-dystrophic myotonias. Previous trials on a limited number of patients suggest that class 1 anti-arrhythmic agents may improve myotonia. A one-month cross over trial of 200mg tid of mexiletine vs placebo was performed in 20 patients with moderately-severe myotonic dystrophy type 1 (DM1), 10 patients with proximal myotonic myopathy (PROMM), and 10 patients with myotonia congenita (MC). Muscle strength was assessed by quantitative muscle assessment (QMA) considering maximum voluntary contraction (MVC) expressed in Kg as well as by manual muscle strength testing using the 5-point MRC scale. Myotonia was assessed by an arbitrary 4-point scale of subjective evaluation of severity; by timed functional tests and by half relaxation time after MVC. All tests were performed at baseline, after the first month and at the end of the trial. Mexiletine improves muscle strength in DM1 and especially in MC. In PROMM patients it rather improves muscle pain. It reduces half relaxation time in DM1 but especially in MC.

Mexiletine is a well-tolerated drug in the treatment of both muscle weakness and myotonia in the myotonic disorders, especially in MC.

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#### HYPERORNITHINEMIA, HYPERAMMONEMIA AND HOMOCITRULLINURIA (HHH) SYNDROME IN ITALY: A CLINICAL AND MOLECULAR CHARACTERIZATION

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The hereditary spastic paraplegias (HSP) are genetically heterogeneous disorders characterized by degeneration of the pyramidal tract. It is known that a subgroup of HSP patients have features typical of oxidative phosphorylation (OXPHOS) defects because of mutations in the SPG7 gene. Hyperornithinemia, hyperammonemia and homocitrullinuria (HHH) syndrome is a recessive disease characterized by impaired ornithine transport across the inner mitochondrial membrane. Affected patients usually present with spastic gait, pyramidal tract signs, and ataxia. Furthermore, the abnormal intramitochondrial ornithine transport causes a functional impairment of the urea cycle responsible for recurrent hyperammonemia with loss of consciousness, lethargy and coma. Mutations in the ORNT1 gene have been associated with HHH. We investigated eight unrelated HHH patients followed by Italian referring centers for inborn metabolic diseases. In three patients, we obtained deltoid muscle biopsies to look for OXPHOS defects. All patients invariably presented evidence of pyramidal tract dysfunction. Worsening of neurological signs did not re-

late to relapses of hyperammonemia that were observed only in patients with a delayed diagnosis. Molecular studies demonstrate nine different ORNT1 mutations, seven of which are new. Mutations mostly resulted in shorter gene product, via premature translation termination. There was no correlation between residual size of the predicted ORNT1 gene product and clinical and metabolic severity. Interestingly, a R179X nonsense mutation led to exon 4 skipping. Histochemical and biochemical studies in muscle showed moderate mitochondrial proliferation with abnormally shaped, enlarged mitochondria by electron microscopy. The identification of ORNT1 mutations adds a novel mitochondrial determinant to the broad group of hereditary spastic paraparesis.

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#### IDENTIFICATION OF TWO NEW MUTATIONS IN THE MYOPHOSPHORYLASE GENE IN ITALIAN McA RDLE'S PATIENTS

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Genetic defects of muscle-specific isozyme of glycogen phosphorylase (myophosphorylase) cause a metabolic myopathy (McArdle's disease) characterized by exercise intolerance, myalgia, cramps, and episodic myoglobinuria. Molecular genetic studies of the myophosphorylase gene (PYGM) have thus far identified around 25 different mutations in patients with McArdle's disease from different countries. The most common genetic defect among north-European and American patients is a nonsense mutation at codon 49 in exon 1 (R49X), which seems to be less frequent in Mediterranean populations. Here, we report two new mutations in the PYGM gene in two unrelated Italian McArdle patients with documented histochemical and biochemical phosphorylase deficiency in their muscle biopsies. Screening by PCR/RFLP confirmed the presence of the R49X in both patients. By sequencing the entire coding region and intron/exon boundaries of the PYGM gene of the patients, we identified: i) in patient 1, a novel heterozygous C-to-T mutation, changing CGA (arginine) to TGA (stop codon) at codon 269 in exon 7 (R269X); ii) in patient 2, a novel heterozygous G-to-C mutation, changing GCT (alanine) to CCT (proline) at codon 686 (A686P). Several lines of evidence suggested the pathogenicity of both mutations. Our data further expand the genetic heterogeneity in patients with McArdle's disease.

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#### FAMILIAL T8993C MUTATION CAUSING BOTH THE NARP AND THE MILS PHENOTYPE IN THE SAME GENERATION: A MORPHOLOGIC, GENETIC, AND SPECTROSCOPIC STUDY

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Neurogenic muscle weakness, Ataxia and Retinitis Pigmentosa (NARP) and infantile subacute necrotizing encephalomyopathy (Maternally Inherited Leigh Syndrome, MILS) are the most severe clinical patterns caused by a maternally inherited mitochondrial DNA (mtDNA) point mutation at nucleotide position 8993 (ATPase6 gene). We describe a 27 y.o. woman (proband) with a 20-year history of slowly progressive cerebellar ataxia, fatigability, hearing loss, retinitis pigmentosa, epileptic seizures and learning difficulties. Her only sister had died of undetermined encephalopathy at age 3 years. The mother presented short stature and distal limb paresthesia. Proband's left biceps muscle biopsy and Southern blot analysis on skeletal muscle DNA were normal. Restriction Fragment Length Polymorphism (RFLP) revealed the presence of a mtDNA heteroplasmic T to C base substitution at nucleotide 8993 (88.4% mutated mtDNA). Blood DNA from the mother revealed the same mutation (13.2% mutated mtDNA). Proton magnetic resonance spectroscopy (1H-MRS) showed lactate (Lac) increase in the occipital lobe. So far, few families presenting the T8993C mutation have been reported, usually with a milder phenotype than the more typical T8993G-related cases. In our family, MILS and NARP coexist in the same generation, the mother being mildly symptomatic. 1H-MRS shows metabolic changes even in clinically unaffected areas.

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#### METABOLIC MUSCLE ADAPTATION TO AEROBIC TRAINING IN PATIENTS AFFECTED BY MITOCHONDRIAL MYOPATHIES

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Mutations of mitochondrial DNA at the skeletal muscle level are responsible for insufficient ATP production and deranged metabolism, a main effect of which is represented by abnormal production of lactate. Aim of this study was to evaluate in 10 patients affected by chronic progressive external ophthalmoplegia (CPEO) and large-scale mtDNA rearrangements functional adaptation of skeletal muscle to supervised constant workload 10-week aerobic training, by assessing modifications of anaerobic lactate threshold and relating it to muscle biopsies parameters. A significant decrement (- 36.5%, p<0.01) of exercise lactate levels after training was observed. The training-related decrement in exercise peak lactate correlated with cytochrome c oxidase (COX) enzyme activity (r = -

0.84,  $p < 0.05$ ), the number of COX- (  $r = 0.75$ ,  $p < 0.05$ ) and ragged red fibers ( $r = 0.68$ ,  $p = 0.05$ ). On the contrary, no relation was found with the amount of deleted mtDNA in muscle biopsy. These results indicate that aerobic training can be beneficial also in those CPEO patients more severely affected by mitochondrial dysfunction. The level of COX activity in muscle biopsy rather than the amount of mutated mtDNA seems to be a useful predictor for the effectiveness of aerobic training program, suggesting some gene expression mechanisms in mediating muscle adaptation to training itself in these patients.

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#### INTELLECTUAL IMPAIRMENT IN DUCHENNE MUSCULAR DYSTROPHY. PART II

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In a previous study, investigating the frequency of the mental retardation in Duchenne patients, we found no correlation with nor the genetic defects nor the severity of the disease. In contrast with previous reports, a verbal intelligence quotient (VIQ) higher than the performance intelligence quotient (PIQ) was observed in 58.8% of the cases. Aims of the study were to support this result enlarging the sample size and investigate the distribution of patients on the normality-mental retardation axis. IQ was evaluated using the WISC-R and the intellectual impairment diagnosed according to the DSMIV. The results confirmed that VIQ was higher than PIQ in 58.1% (mean difference 14.1); on the contrary,  $PIQ > VIQ$  was observed in 34.9% (mean difference 8.6, not significant according to the WISC). Concerning the distribution on the normality axis, a higher percentage of "normal patients" was observed evaluating VIQ, compared with PIQ. These data confirm that a major role in the diagnosis of mental retardation of Duchenne patients could be played by the involvement of the fine hand movements (influencing the time of the performance tests and then the final IQ).

Obviously, a common strategy of analysis of the patients and tests will provide definitive conclusions on this aspect of Duchenne Dystrophy.

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#### FACIO-SCAPULO-HUMERAL DYSTROPHY IN NORTH-WEST TUSCANY: A MOLECULAR GENETICS-BASED EPIDEMIOLOGICAL AND GENOTYPE-PHENOTYPE STUDY

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Facio-scapulo-humeral muscular dystrophy (FSHD) is an autosomal-dominant, inherited disorder, with almost complete penetrance, characterized by a slowly progressive course. However, clinical appearance can be quite variable, with re-

spect to age at onset, severity and pattern of muscle involvement, both between and within families and asymptomatic cases are found in about 30% of the patients. For this reason diagnosis of FSHD can be sometimes difficult and molecular diagnosis is therefore necessary. The latter is at present time based on the detection of large deletions of variable size of *kpnI* repeat units on chromosome 4q35. A molecular genetics-based epidemiological investigation was carried out in 2000 to calculate prevalence rate of FSHD in the territory of North-West Tuscany, central Italy, an area covering 6597 km<sup>2</sup>, with a population nearly of 1300000 inhabitants. Results have been compared to those of a previous study conducted in the same area in 1981, in the pre-molecular era.

We found a prevalence of 3,16 x 10000 inhabitants, a value two times as higher than that found in our previous study. Genotype-phenotype relationships were assessed in order to characterize mildly and severely affected patients. This results confirm the usefulness of routine adoption of the molecular genetics-based screening to be extensively applied to make correct diagnosis of FSHD.

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#### STRUCTURAL ALTERATIONS OF COLLAGEN TYPE VI IN SKIN FIBROBLAST CULTURES FROM PATIENTS AFFECTED BY ULLRICH SYNDROME.

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Ullrich congenital muscular dystrophy is caused by recessive mutations in collagen VI gene (Camacho et al. PNAS 2001, in press). Collagen VI is widely expressed in the extracellular-matrix of skin cultured fibroblasts: monomers are assembled inside the cytoplasm in tetramers and secreted to form fibrillar networks connecting the extracellular-matrix and membrane receptors. We evaluated by immunofluorescence and immunoelectron microscopy the amount and organization of secreted collagen VI of cultured skin fibroblasts from 6 patients carrying mutations on COL6 genes.

Immunofluorescence showed absence of collagen VI in 4 patients, while in the other 2 appeared strongly reduced. Immunoelectron microscopy in the latter 2 cases revealed inability to constitute fibrils or well developed networks. The interactions with the other proteins were also affected and some isolated collagen VI deposits showed a rolled shape. To evaluate if reduced expression or secreted mutated protein may affect the collagen VI organization, we analyzed fibroblast cultures from a Bethlem patient with haploinsufficiency. Immunofluorescence analysis of collagen VI showed a reduced expression of secreted protein comparable with the Ullrich patients while

the ultrastructural organization appeared normal. Our findings suggest that immunofluorescence analysis may help to identify quantitative defects, while electron microscopy may give insight about the fate of the mutated protein.

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#### A NEW TRNA<sup>HIS</sup> MUTATION IN THE MTDNA RESULTS IN MITOCHONDRIAL ENCEPHALOMYOPATHY

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Mitochondrial encephalomyopathies are heterogeneous disorders associated with mutations in the nuclear or in the mitochondrial DNA (mtDNA). We identified a family in which a 20-years-old man developed a severe disorder resembling MELAS (mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes). His mother had died of a stroke-like attack in young age. In muscle biopsy of our patient, we identified high percentages of a novel tRNA<sup>HIS</sup> mutation (T12417G). The mutation fulfilled accepted criteria for pathogenicity. The mutation was less abundant in the proband's peripheral blood and it was not detected in hairy roots or blood from seven maternal relatives who complained of either migraine without aura or tension headache. In microdissected skeletal muscle fibers from the proband a lower rate of ATP production was shown, respect to control cell lines. Reporting the first mutation in the tRNA<sup>HIS</sup> gene associated with mitochondrial encephalomyopathy, our data expand the genetic variants associated with MELAS.

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#### A CASE OF DISTAL MYOPATHY WITH PREMINENT INVOLVEMENT OF GASTROCNEMIOUS MUSCLE AND RIMMED VACUOLES

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Among the early adult onset distal myopathies of lower limbs, a form with preferential involvement of anterior tibial muscle and rimmed vacuoles in muscle biopsies (Nonaka type distal myopathy) and a form with predominant involvement of gastrocnemious muscle and dystrophic change in muscle biopsy (Miyoshi type distal muscle dystrophy) were described. We report a sporadic case affected by early adult onset distal myopathy with preminent involvement of gastrocnemious and consequent impossibility in standing of the toes. S-CK was increased more than 10 times the upper normal limit. On EMG a neurogenic pattern was recorded. A first muscle biopsy obtained from vastus lateralis muscle showed small group of

atrophic angulated fibers suggesting a neurogenic atrophy. In a second biopsy, obtained from the gastrocnemious, peculiar myopathic change were found: rimmed vacuoles in many fibers and numerous structural abnormalities i.e. core-target formation; excessive variation in fiber size, necrotic fibers and increased endomysial connective tissue were present too. The authors discuss the clinical and pathological findings in relation to other cases with lower limb distal myopathy.

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#### SKELETAL MUSCLE LIPID STORAGE AND MITOCHONDRIAL BETA-OXIDATION

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Inborn errors of the mitochondrial beta-oxidation of long chain fatty acids represent an evolving field of inherited metabolic diseases. The most frequent defects of the beta-oxidation affect the activities of short-chain acyl coenzyme A dehydrogenase (SCAD), medium chain acyl CoA dehydrogenase (MCAD), and long chain acyl CoA dehydrogenase (LCAD). The clinical phenotype of fatty acid oxidation disorders include disease affecting one or more of the fatty acid-metabolizing tissues and can vary considerably.

We have reviewed the clinical and biochemical data of 23 patients, admitted to our Center for Neuromuscular Disorders in the last 5 years, that showed a skeletal muscle lipid storage and defects of beta-oxidation. The clinical presentation greatly varied: myalgias and cramps, iperckemia, diffuse myopathy, encephalomyopathy or neuropathy. Among them we have found: 4 pts with multiple acyl coenzyme A dehydrogenases deficiency (MAD), 4 pts with a combined defect of LCAD and MCAD, 2 pts with LCAD and SCAD deficiency, 1 pt with MCAD and SCAD defect and 12 other pts with an isolated defect with prevalence of MCAD deficiency. Administration of oral riboflavin to pts with MAD resulted in a prompt clinical recovery, but also 5 pts with different combination of enzyme deficiencies manifested a striking clinical improvement.

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#### COENZYME Q10, EXERCISE LACTATE AND CTG TRINUCLEOTIDE EXPANSION IN MYOTONIC DYSTROPHY

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Although causative mutation of Steinert's Myotonic Dystrophy (DM) is recognized as a CTG trinucleotide expansion on 19q.13.3, pathogenic mechanisms of multisystem involvement of DM are still under debate. It has been suggested that mitochondrial abnormalities can occur in this disease and deficiency of coenzyme Q 10 (CoQ10) has been considered one possible cause for this. Aim of this investigation was to evaluate CoQ10 blood levels in DM patients and relate them to the degree of

CTG expansion as well as to the amount of lactate production in exercising muscle as indicator of mitochondrial dysfunction. Plasma total CoQ10 levels were determined by RP-HPLC-UV in 35 DM patients. In selected patients blood lactate kinetics during incremental exercise performed on an electrically braked pedal-rate bicycle ergometer. CoQ10 concentrations appeared significantly reduced with respect to normal controls:  $0.84 \pm 0.25$  vs  $1.58 \pm 0.28$   $\mu\text{g/ml}$  ( $p < 0.05$ ). Blood lactate was significantly higher than controls ( $p < 0,05$ ) both in resting conditions ( $2.9 \pm 0.55$  vs  $1.44 \pm 1.1$  mmol/l) and at its exercise peak ( $6.87 \pm 1.74$  vs  $4.9 \pm 0.6$  mmol/l), while lactate threshold was anticipated (30-50% vs 60-70%,  $p < 0.05$ ). CoQ10 levels significantly inversely correlated with CTG expansion degree and lactate values at threshold level ( $p < 0.05$ ). Our data indicate the occurrence of reduced CoQ10 levels in DM, possibly related to disease pathogenic mechanisms associated to abnormal CTG trinucleotide amplification.

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#### CARDIAC INVOLVEMENT IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY: A CORRELATION WITH THE 4Q35 DELETION IN 54 PATIENTS

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Facioscapulohumeral Muscular Dystrophy (FSHD) is usually associated with deletion of a DNA fragment (q35) on chromosome 4. Extensive studies on the natural history of 4q35 FSHD are lacking and little information is available about the involvement of the extra-muscular tissues, in particular of heart. We studied 54 FSHD patients (31 males and 23 females, with a mean age of 44 years) diagnosed according to the criteria of the European Consortium of FSHD. For all of them, we evaluated the clinical history and the muscular deficit (MRC scale and four functional tests); moreover, possible heart involvement was assessed by clinical examination, surface ECG, 24-hour ECG and echocardiography. The size of the 4q35 deletion was evaluated by Southern blot analysis of genomic DNA digested by EcoRI e BlnI and by the p13E-11 probe. Overt cardiac involvement was evident in 3 of our patients (ischemic alterations, cardiac failure and paroxysmal tachycardia), while 24-hour ECG detected subclinical conduction defects or arrhythmia in 11 of

the 54 patients. The size of the deleted 4q35 fragment ranged between 15 to 27 Kb. The size of this fragment was not correlated with the parameters of the heart involvement.

On the whole, our clinical study showed that symptomatic heart disease is an unusual feature of FSHD, while subclinical cardiac arrhythmia may be detected in 20% of patients; no correlation was evident with the size of the deleted 4q35 fragment.

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#### FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHMD) PRESENTING WITH LOWER LIMB MONOMELIC ATROPHY

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FSHD has a distinctive distribution but may be markedly asymmetric and extremely variable in extent of involvement and severity. A 28-year-old woman presented with left leg atrophy. Examination showed marked wasting of left triceps surae and impossibility to walk on left toes. There was no weakness of facial and all other muscles nor scapular winging. Deep tendon reflexes were hypoactive except absent left ankle jerk. CK was 424 U/L (normal <260 U/L). EMG of left gastrocnemii showed electrical silence in some areas, spontaneous activity and few low amplitude motor units at maximal effort in other areas. EMG of left tibialis anterior was myopathic. Muscular CT scan showed confluent areas of decreased density in left gastrocnemii and soleus and right gastrocnemius medialis muscle. Shoulder, mid-arm, pelvic and mid-thigh scans were normal. Muscle biopsy (right gastrocnemius lateralis) showed increased fiber size variability, numerous small, grouped, angulated fibers, and type 2 fiber prevalence. Familiar history was negative but the 60-year-old father showed slight weakness of orbicularis oculi and some difficulty to walk on heels. CK was normal and EMG myopathic. The 30-year-old brother was normal at examination but had increased CK (700 U/L). DNA analysis showed the 4q35 deletion in all three family members.

This family confirms the wide intrafamilial variability of FSHD and the proband broadens the phenotypic spectrum of FSHD presentation.

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