

## Abstracts

### [1] MITOCHONDRIAL DNA DEPLETION SYNDROME CAUSING SPINAL MUSCLE ATROPHY

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**Objective:** We report four patients with clinical and histopathological features of Spinal Muscle Atrophy (SMA) and primary mitochondrial DNA (mtDNA) depletion.

**Background:** The mtDNA depletion syndrome (MDS) is an autosomal recessive condition characterized by decreased mtDNA copy number. The myopathic form of MDS has been linked to mutations in the thymidine kinase-2 (TK2) gene, and the hepatoencephalopathic form to mutations in the deoxyguanosine kinase (dGK) gene.

**Patients and Methods:** We report four children with SMA phenotype but without mutation in the survival motor neuron (SMN1) gene. Three of them developed progressive motor difficulties between the ages of 12 and 24 months. Presently, at ages 11, 11 and 12 yrs, they are alive, profoundly weak, hypotonic, with no control, and unable to sit unsupported. The fourth patient was normal until the age of 4 yrs, when he developed increasing lumbar lordosis and waddling gait. At the age of 12 years, he is alive but wheelchair-dependent. EMG suggested denervation, both venous lactate and CK levels were elevated, and muscle biopsy histopathology was consistent with SMA. We performed histochemical studies in muscle from three patients and biochemical analyses of respiratory chain complexes in muscle from one patient. Molecular studies included Southern-Blot for mtDNA depletion and direct sequencing of the TK2 gene.

**Results:** The histochemical reaction for succinate dehydrogenase (SDH) showed several fibers with increased oxidative activity but negative for the cytochrome c oxidase (COX) stain. Southern blot analysis showed marked reduction of the mtDNA/nuclear DNA ratio in muscle from all patients (55 to 94%). Respiratory chain activities in the only available muscle revealed increased SDH and citrate synthase activities, but decreased activities of complexes containing mtDNA-encoded subunits. Two patients had TK2 mutations: isoleucine-to-methionine at position 22 (I22M) (with reduced TK2 activity in muscle) in one; aspartate-to-glycine at the position 11 (D11G) in the other. The molecular cause(s) of mtDNA depletion in the other two patients remain unknown.

**Conclusions:** mtDNA depletion can mimic SMA both clinically and histopathologically, and should be considered in all SMA patients without mutations in the SMN gene.

### [2] A NOVEL MITOCHONDRIAL TRNAPHE MUTATION CAUSES MERRF SYNDROME

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**Objective:** to report a novel mutation in the tRNAPhe gene in a patient with typical clinical, histological, and biochemical features of myoclonic epilepsy with ragged red fibers (MERRF).

**Background:** MERRF is one of the most common mitochondrial syndromes and is usually associated with mutations in tRNALys gene. About 80% of patients harbor the A8344G mutation; most of the remaining cases have the T8356C, G8363A and G8361A mutations.

**Patients and Methods:** A 42-yrs old woman developed progressive limb myoclonia, hearing loss, exercise intolerance, weakness, and loss of balance in her 20's. We performed histochemical and biochemical studies of respiratory chain complexes in muscle biopsy specimens, and sequenced the coding regions of all the 22 tRNA genes.

**Results:** Histochemical analysis showed abundant cytochrome c oxidase (COX)-negative ragged-red fibers (RRF), and biochemical analysis showed multiple defects involving respiratory chain complexes containing mtDNA-encoded subunits. We identified a novel G-to-A mutation at nucleotide 611 of the tRNAPhe gene (G611A). The mutation was heteroplasmic (91%) in muscle, but undetectable in accessible tissues from the patient and her maternal relatives. Single-fiber PCR analysis showed that the proportion of mutant genomes was higher in COX-negative RRF than in COX-positive non-RRF.

**Conclusion:** this is the first report of a MERRF not associated with a tRNALys mutation.

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### [3] NON-SYNDROMIC HEARING LOSS CAUSED BY VERY LOW LEVELS OF THE MTDNA A3243G MUTATION

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Here we report a case, the first observed in Italy, of progressive non-syndromic hearing loss (NSHL) with a very low level of A3243G mutation in the mitochondrial DNA (mtDNA). Muscle biopsy showed scattered ragged-red, cytochrome c oxidase negative fibers, whereas the biochemical analysis of the mitochondrial respiratory chain complexes was normal. RFLP analysis showed A3243G mtDNA transition, present at very low in patient's muscle (3%) and in urinary sediments (1%),

and not detectable in blood and buccal mucosa. The patient was submitted to a bilateral cochlear implantation with post-operative excellent hearing and communicative outcomes. Our findings indicate that A3243G mutation may be responsible both for SHL and NSHL, maybe depending on the levels of mutated mtDNA. Patients with hearing loss due to mtDNA mutations should be considered as good candidates for cochlear implantation.

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[4] A CASE OF GENERALIZED MYASTHENIA GRAVIS ASSOCIATED WITH THYMIC HODGKIN'S DISEASE

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Myasthenia Gravis (MG) is a skeletal muscle endplate disorder in which auto-antibodies are directed against the post-synaptic nicotinic acetylcholine receptors (AChR). A part from the relationship with thymoma, MG is not considered a paraneoplastic syndrome, in particular the association with Hodgkin's Disease (HD) is very rare. We report the case of a 52 year-old female affected by generalized MG. The diagnosis was according with clinical, pharmacological and neurophysiological criteria. AChR antibodies were positive and radiological examination of the mediastinum showed a mass of 1 centimetre of diameter suspected for thymoma. Anti-titine and anti-ryanodine antibodies were negative. Anticholinesterase, corticosteroid and immunosuppressive treatment was started and the patient was submitted to thymectomy. Surgery revealed a nodular sclerosing syncizial Hodgkin's lymphoma associated with thymic involution; haematological examinations confirmed a clinically II A staged of HD with mediastinal bulk. After thymectomy chemotherapy followed by mediastinal radiotherapy was started and neurological treatment was completely stopped. The follow-up of the patient during the successive eight years revealed a complete stable remission of both MG and HD with decrease of anti-AChR antibodies serum titre. We conclude that in our case MG was a paraneoplastic syndrome related to HD.

[5] DEFLAZACORT TREATMENT IN DUCHENNE MUSCULAR DYSTROPHY: A REPORT ON TWO PROTOCOLS

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The long-term benefits and side effects of deflazacort using two treatment protocols from Naples (N) and Toronto (T) were compared. Boys with DMD between 6 and 18 years treated for 4 or more years were reviewed: 37 boys were treated with Protocol-N, using deflazacort at a dose of 0.6 mg/kg/day for the first 20 days of the month; 32 with Protocol-T, using deflazacort at a dose of 0.9 mg/kg/day. Treatment started between 6 and 8 years. All boys were monitored every 4-6 months. The results were compared with age-matched controls. For the boys treated with protocol-N, 97% were ambulatory at 9 years (control, 22%), 35% at 12 years (control, 0%), 25% at 15 years (control, 0%). For the boys treated with protocol-T, 100% were ambulatory at 9 years (control, 48%), 83% at 12 years (control, 0%), 77% at 15 years (control, 0%). For protocol-N, no cataracts were observed, while in Protocol-T, 30% of boys had asymptomatic cataracts. Fractures occurred in 20% of boys (control, 15%) on Protocol-N and 17% (control, 14%) of boys on Protocol-T. In boys >13 years, a scoliosis of >20° developed in 30% on protocol-N, 16% on protocol-T and 90% of controls. Beneficial effects on the vital capacity were also observed.

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[6] ABNORMALLY INCREASED RESPIRATORY CHAIN ENZYME ACTIVITIES IN A PATIENT WITH RECURRENT RHABDOMYOLYSIS

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A 28-year-old male patient presented three episodes of exercise-induced myoglobinuria at the age of 22, 26 and 27 years. Until age 27 years, he performed constant non-agonistic physical activity, adding routinely several vitamins and cofactors to the diet. The episodes were characterized by lower limb myalgia and transitory weakness. Blood exams in acute phases showed increased CK (> 8.000 IU), myoglobin (257 ug/l) and lactate (3 mmol/l). These values normalised after few weeks. EMG showed mild myogenic signs at proximal muscles. At the muscle biopsy examination, Cytochrome c Oxidase (COX) and Succinate Dehydrogenase (SDH) staining were markedly elevated in almost all fibers. Electron microscopy revealed a high

density of mitochondria with increased size. CPT activity was normal. Thyroid function was normal. Respiratory chain (RC) enzyme activities were increased up to three times the mean control values: COX: 78.2 (50.6 + 8.9), NADH-UQ1 DH: 66.6 (22.3 + 5.2) SDH: 30.9 (12.7 + 3.0); Succinate-Cytochrome c Reductase: 40.0 (16.3 + 4.0); Citrate synthase 432.0 nmol/min/mg prot (129 + 17.8). Mitochondrial DNA deletions were absent, Cytochrome b gene was normal. A relatively benign metabolic phenotype seems to be associated with increased RC activity. Whether this last data reflects merely a consequence or a true efficient compensatory reaction remains to be investigated.

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[7] FOLLOW-UP IN A LARGE POPULATION OF ASYMPTOMATIC/OLIGOSYMPTOMATIC HYPERCKEMIC PATIENTS

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In 1998 we performed a retrospective study of 114 patients presenting asymptomatic/oligosymptomatic hyperckemia, a diagnosis being made in 21 of them (Prella et al, 2002). We present our results of a long-term follow-up in 49 (52.7%) of the still undiagnosed 93 patients. Nobody has developed any specific neuromuscular disorder. However, a diagnosis of dystrophinopathy carrier has been indirectly made in a female patient, and, in another case, a condition of type I SMA carrier is being investigated. Almost all subjects still have hyperckemia, but the mean CK value is lower than before. CK levels have become normal in 6 subjects without any modification of working/physical activity. Over half of the subjects have remained asymptomatic, 5 previously oligosymptomatic patients now referring partial or complete improvement. One patient has died of a throat carcinoma, and three have developed non-neuromuscular disorders (monoclonal gammopathy, nephropathy, non-alcoholic steatosis). Though the association between hyperckemia and cancer is known, no correlation has been thus far reported with the other diseases. Also, we noted no follow-up differences between patients with pathologic EMG and/or muscle biopsy and those with normal results at first examination, which seems to be against the hypothesis that only subjects with normal exams are indeed affected with idiopathic hyperckemia.

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[8] BRAIN MALFORMATION IN A CASE OF CONGENITAL MUSCULAR DYSTROPHY: ROLE OF ALPHA DYSTROGLYCAN GLYCOSYLATION

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Congenital muscular dystrophies are a heterogeneous group of autosomic recessive disorders presenting in infancies and characterized by hypotonia, contractures and dystrophic changes at muscle biopsies. Major CNS alterations, secondary to neuronal migrations defect, are often present. All the genes involved in these diseases codify for glycosyltransferase or putative glycosyltransferase. Alpha dystroglycan (DG) is a highly glycosylated protein present in muscle, nerve and CNS. It is a constituent of the basal lamina, playing a central role in brain development through neuronal migration control. During CNS development cell proliferation occurs in the ventricular zone, where alpha dystroglycan is highly expressed. Animal models showed brain alpha dystroglycans glycosylation defects in these diseases. We have studied brain alpha dystroglycan glycosylation in one patient affected with congenital muscular dystrophy with lack of muscle alpha dystroglycan, lissencephalia, pachigiria and heterotopia. One aged matched control baby and one normal adult human brain were also studied. Preliminary results show the expression of alpha dystroglycan in the Purkinje cell, while no alpha dystroglycan is present in the heterotopic nuclei of the periventricular zone.

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[9] MOLECULAR DIAGNOSIS IN LGMD2A: MUTATION ANALYSIS OR PROTEIN TESTING?

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Limb girdle muscular dystrophy type 2A (LGMD2A) is caused by mutations in the CAPN3 gene encoding for calpain3. The diagnosis of LGMD2A has recently shifted from molecular genetics towards biochemistry. An estimate of sensitivity and specificity of protein analysis is unknown. We correlated protein and molecular data in our large LGMD2A population. By preliminary immunoblot screening of calpain3 of 548 unclassified patients with various phenotypes we

selected 208 cases for mutation analysis: 69 had protein deficiency and 139 had normal expression. We identified 58 LGMD2A patients: 46 had variable degree of protein deficiency and 12 had normal calpain3 amount. The probability of having LGMD2A is very high (84%) when patients show a complete calpain3 deficiency and decreases with protein amount; this new data offers an important tool for genetic counseling when only protein data are available. We detected 37 different mutations, 10 of which are new. Most mutant alleles (87%) were concentrated in 7 exons and 61% correspond to only 8 mutations, indicating the regions where future molecular analysis could be restricted. We report the largest collection of LGMD2A patients so far where both protein and gene mutation were obtained to draw genotype/protein/phenotype correlations and provide insights into critical protein domains.

[10] NOVEL DYSFERLIN GENE MUTATIONS IN A CLINICALLY HETEROGENEOUS GROUP OF ITALIAN PATIENTS.

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The limb-girdle muscular dystrophy type 2B (LGMD-2B) and the distal Miyoshi myopathy (MM) are caused by mutations in the dysferlin gene (DYSF). The type of mutation does not correlate with phenotypic severity and the same mutation has been found to be associated with a wide inter- and intra-familial variation in clinical phenotype. These observations underline the relevance of any description of disease phenotypes caused by dysferlin mutations. We selected 15 unrelated patients with progressive weakness in distal and/or proximal muscles, elevated CK levels, deficiency of dysferlin protein as detected by Immunofluorescence and Western Blot analysis. Nine of them presented clinical features consistent with MM, 5 with LGMD while one patient only displayed hyperCKemia. Direct sequencing of the *DYSF* gene allowed the identification of 18 different mutations: 10 missense and 1 nonsense mutations, 3 microdeletion/insertion and 4 splice site mutations. In particular, 8 mutations (2 in splice sites, 1 microdeletion, 1 nonsense and 4 missense substitutions) had not been previously reported. A variable clinical

phenotype was observed as a consequence of the same mutation (Arg959Trp, Cys158Stop, Val 374Leu), this observation implies that modifying factors are involved in the phenotypic outcome of *DYSF* mutations. In all 15 patients with dysferlin deficiency we were able to identify mutations in the *DYSF* gene, indicating that the detection of dysferlin protein deficiency is a highly specific marker of primary dysferlinopathy.

[11] A MULTICENTER STUDY ON NATURAL HISTORY OF FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

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Facioscapulohumeral Muscular Dystrophy (FSHD), which is usually associated with a 4q35 deletion, represents one of the most frequent hereditary myopathies in Western Countries. The definition of the clinical course of the disease is preliminary to designing a clinical trial. In this perspective, the progressive decline in strength in 96 cases, with the characteristic 4q35 deletion, is under investigation by our multicenter study. The protocol includes Manual Muscle Testing by traditional MRC and four Functional Tests: 1) Gowers' manoeuvre; 2) walking and climbing stairs; 3) raising arms over head; 4) moving a book forwards and backwards; they score 7 (normal) to 0 (loss of function). The general muscular ability was graded in a disability scale, ranging I (mild) to VII (severe weakness). Patients were evaluated at the beginning of the study and subsequently every 12 months. We report data collected after 48 months (22-67), concerning 32 FSHD cases out of 96. They were 18 males and 14 females with a mean age of 48 years (14-76). The clinical course appeared variable. Worsening of strength was evident in 14/32 cases (43%) by at least one of the functional tests. Among them, as expected, F.Test 3) appeared as the most sensitive. Grading in the disability scale changed in 10/32 patients (31%). MRC score, even if very slightly, changed in all. On the whole, our investigation on the clinical course of FSHD indicates that the declining over time in strength is variable, being static in some and very fast in few, however very slow in the majority of them. The type of progression of weakness did not seem correlate to age, gender, age at onset or duration of the disease. However, the patients with more severe 4q35 deletion seem develop a faster decline.

[12] CLINICAL CORRELATES AND SOCIAL CONSEQUENCES OF INTELLECTUAL IMPAIRMENT IN ADULT MYOTONIC DYSTROPHIES

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**Background:** The clinical significance and social consequences of brain involvement (visual-spatial impairment, dysexecutive syndrome and avoidant personality traits) in patients with adult myotonic dystrophies is unclear.

**Aims:** To compare marital status, employment and educational level in myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (PROMM/DM2) and determine whether these social aspects correlate with neuropsychological test scores and neuroimaging.

**Materials and Methods:** 30 patients with DM1 (mean age  $46.9 \pm 15.5$ ; mean disease duration  $19.8 \pm 13.9$ ) and 19 patients with genetically confirmed PROMM/DM2 (mean age  $42.1 \pm 12.1$ ; mean disease duration  $15.9 \pm 11.2$ ) were subjected to a battery of neuropsychological tests, brain MRI and demographic interviews to determine marital status, employment and educational levels.

**Results:** Marital status of DM1 and PROMM/DM2 is comparable to controls. Educational and employment levels are significantly reduced ( $p = 0.005$ ) only in DM1, group with the smallest number of professionals. There is no correlation to neuropsychological tests and neuroimaging.

**Conclusions:** Cognitive impairment effects seem more benign in PROMM/DM2 compared to DM1. These cognitive adverse effects need to be considered in potential therapeutic trials.

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[13] TAUOPATHY IN MYOTONIC DYSTROPHY TYPE 1

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**Background:** Recent data in myotonic dystrophy type 1 (DM1) demonstrate the alteration of tau expression showing that DM1 is a peculiar tauopathy. How this correlates to the degree of cognitive function, neuroimaging and neuromuscular severity is still unclear.

**Aims:** To see whether cerebrospinal fluid total tau expression may be considered a marker for the severity and progression of the neurodegenerative process in DM1.

**Materials and Methods:** We subjected a 28-year-old, graduated woman with a 2-year history of moderately severe DM1 to neuromuscular assessment, neuropsychological tests and to brain MRI. Total tau in cerebrospinal fluid (CSF) was determined by ELISA.

**Results:** There was grade 4 distal and grade 4.5 proximal muscle weakness and mild limb atrophy. Brain MRI and neuropsychological tests were normal. Total tau was 186 pg/ml (normal  $263 \pm 164$ ) and AB42 was 736 pg/ml (normal  $794 \pm 218$ ), both in the normal range, in agreement with normal cognitive assessment in our patient.

**Conclusions:** Our data, although only preliminary and on only one patient, suggest that CSF total tau may correlate with cognitive involvement. Further studies are in progress to confirm or refute this hypothesis.

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[14] TYPE II FIBER ATROPHY TARGETS BIOMOLECULAR DIAGNOSIS IN PROMM/DM2 BUT FISH CONFIRMS FINAL GENETIC ANALYSIS IN MUSCLE BIOPSY

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**Background:** Genetic analysis of myotonic dystrophy type 2 (PROMM/DM2) requires a 3-step protocol (PCR, RP-PCR, SB) and is still not available as a routine diagnostic test. Nuclear clump type II fiber atrophy by immunohistochemistry in muscle biopsy targets subsequent genetic screening for PROMM/DM2, but 30% of patients may be missed by immunohistochemistry alone.

**Aims:** To investigate the specificity and sensitivity of FISH in muscle biopsy as a further diagnostic tool in asymptomatic or mildly symptomatic patients with the clinical diagnosis of PROMM/DM2.

**Materials and Methods:** 18 patients with genetically confirmed PROMM/DM2 were subjected to muscle biopsy of the biceps brachii (mean age  $48 \pm 9.7$  years). Routine histochemistry, immunohistochemistry using antibodies directed against fast and slow myosin chain, quantitative histographic analysis and FISH were performed.

**Results:** Our results show that 12 out of 18 PROMM/DM2 patients display nuclear clump type 2 fiber atrophy. In all patients, including those with normal histograms, FISH demonstrated nuclear foci suggestive of PROMM/DM2.

Conclusions: Immunohistochemistry and FISH combined are mandatory diagnostic procedures to screen patients with the clinical diagnosis of PROMM/DM2, especially if sporadic or asymptomatic, given the complexity of routine genetic testing.

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[15] CARDIAC CONDUCTION ABNORMALITIES IN PROMM/DM2: A 10-YEAR FOLLOW-UP STUDY

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Background: One of the distinctive features between myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (PROMM/DM2) is the less frequent cardiac involvement in PROMM/DM2. However, detailed cardiac follow-up studies in PROMM/DM2 are limited.

Aims: To verify whether patients with PROMM/DM2 develop cardiac conduction abnormalities or cardiomyopathies over time compared to DM1.

Materials and Methods: 70 patients with DM1 (age range 28-72, mean age  $53.5 \pm 11.8$ ; disease duration range 1-46 years; mean disease duration  $16.9 \pm 11.9$ ; mean MRC score  $134.4 \pm 18.3$ ) and 33 patients with genetically confirmed PROMM/DM2 (age range 21-74, mean age  $45.9 \pm 15.8$ ; disease duration range 2-56 years; mean disease duration  $19.1 \pm 13.9$ ; mean MRC score  $135.4 \pm 12.8$ ) were subjected to 12-lead standard EKG, 24-hour EKG Holter monitoring, echocardiograms at initial examination and at mean follow-up of  $10.2 \pm 4.6$  years (follow-up range 2-14).

Results: None of our patients with PROMM/DM2 developed severe cardiac conduction defects in our time-span compared to DM1 (1<sup>st</sup> degree AV block: n = 28; BBB: n = 22; 1<sup>st</sup> degree AV block and BBB: n = 9; PM implantation: n = 6).

Conclusions: Our data confirm our initial observations that PROMM/DM2 should be considered a relatively benign disorder compared to DM1. This has important prognostic and therapeutic implications.

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[16] PROXIMAL MYOTONIC DYSTROPHY (PDM) MIMICKING PROGRESSIVE MUSCULAR ATROPHY

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Background: Proximal myotonic dystrophy (PDM) although genetically linked to myotonic dystrophy type 2 (PROMM/DM2) locus is characterized by more remarkable proximal muscular atrophy and weakness. Myotonia is clinically undetectable but present at EMG. Misdiagnosis is frequent.

Aims: To describe one patient with PDM mimicking progressive muscular atrophy

Materials and Methods: A 67-year-old man with an initial diagnosis of progressive muscular atrophy had a 17-year history of progressive lower limb proximal weakness. He was subjected to: detailed family history, muscle strength assessment (MRC), EMG, slit-lamp examination, muscle biopsy, DM1 and PROMM/DM2 genetic screening.

Results: Remarkable limb-girdle atrophy and grade 3.5 MRC proximal muscle weakness were present. Clinical myotonia was undetectable. Prominent neurogenic suffering and diffuse spontaneous activity were present on needle EMG. Routine histochemistry revealed aspecific neurogenic suffering; immunohistochemistry using myosin heavy chain (MHC) antibodies demonstrated a selective severe type 2 atrophy. FISH demonstrated nuclear foci. CCTG repeat expansions were found at the PROMM/DM2 locus. EMG myotonia was present in the clinically asymptomatic daughter.

Conclusions: Our data confirm that PDM is a clinical variant of PROMM/DM2. We also recommend that a complete diagnostic work-up is performed in patients with progressive proximal muscle atrophy and weakness including MHC immunohistochemistry and FISH prior to genetic testing.

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[17] SUSTAINED MYOGENESIS IN PERMANENT  
DENERVATED MUSCLES: IMPLICATIONS FOR USING  
SKELETAL MYOBLASTS IN ALLOTOPIC SITES

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Denervated skeletal muscle undergoes a rapid loss of both mass and contractile force. Six-month after irreversible denervation atrophy is complicated by lipodystrophy. However, it has been demonstrated that: 1. permanent denervation is accompanied by a continuous production of myofibers in both rat and human muscles; 2. marcadine injection induces massive necrosis and regeneration of long term denervated myofibers. In this second case, it has been observed that regenerated myofibers, after reaching half-size of normal fibres, undergo denervation atrophy. In spite of that: 1. activated satellite cells, myotubes, and regenerated myofibers are consistently present in atrophic muscles of rats even after a year-long permanent denervation; 2. in human vastus lateralis, from subjects which underwent conus cauda complete injury 1 to 20 years before biopsy, we recently observed that 1% of the myofibers were of recent regeneration (MHCemb+ fibres). These results demonstrate the long-term regenerative capacity of denervated human muscle, thus sustaining use of skeletal myoblasts to heal (or ameliorate the scar of) necrotic core of an infarcted myocardium, a site where reinnervation of the myofibers is hopeless.

[18] CLINICAL OVERLAPPING BETWEEN WOLFRAM  
SYNDROME AND MITOCHONDRIAL  
ENCEPHALOMYOPATHY: A CASE REPORT

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The clinical picture of Wolfram Syndrome is similar to defects that have a mitochondrial involvement and include symptoms such as optic atrophy, diabetes

mellitus, deafness, short stature. We report on a heteroplasmic macrodeletion of the mitochondrial genome in a child with insulin-dependent diabetes mellitus, optic atrophy, neurosensorial hearing loss, ophthalmoparesis, mental retardation, severe epilepsy, cerebellar signs associated to short stature. MRI showed absence of the physiological high signal of the posterior lobe of the pituitary, optic sub-atrophy and hyperintense alteration in brain stem and cerebellum on T2 weighted images. Muscle biopsy showed mild myopathic abnormalities and COX negative/SDH positive fibres. No Ragged-Red fibres were found with modified Gomori Trichrome. Analysis of the respiratory chain, performed on the muscle sample, highlighted a complex III deficiency. Analysis of mitochondrial DNA from muscle showed a heteroplasmic macrodeletion. No mutations in WFS1 gene have been found. We will discuss the peculiarity of our case and the differential diagnosis between Wolfram Syndrome and other mitochondrial disorders.

[19] DOUBLE TROUBLE FOR MUSCLE AND NERVE:  
LAMIN A/C AND PMP22 MUTATIONS IN THE SAME  
FAMILY

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Mutations in the LMNA gene, which encodes the nuclear envelope protein lamin A/C, have been recently shown to be associated with at least six different syndromes. Among them an autosomal dominantly inherited, slowly progressive limb girdle muscular dystrophy with age related atrioventricular cardiac conduction disturbances (LGMD1B) and an autosomal recessive axonal neuropathy (CMT2B1). Missense mutations in the peripheral myelin protein (PMP22) lead to clinical phenotypes ranging from a demyelinating hereditary neuropathy (CMT1A), Dejerine Sottas neuropathy, and an hereditary neuropathy with pressure palsies (HNPP). Here we report an Italian family affected with two autosomal dominantly inherited genetic diseases: LGMD1B and HNPP.

We have clinically evaluated 10 members of the family, among whom 5 were diagnosed as having LGMD1B. Two of these five reported also recurrent painless focal palsies mostly preceded by minor trauma or compression at entrapment sites of peripheral nerves. Pattern of muscle involvement was similar in all and characterized by mild to moderate proximal muscle

weakness without contractures. Heart conduction defect was present in three patients requiring pace-maker implantation. The patients with focal palsies had also pes cavus. Mutations analysis of LMNA revealed a novel missense mutation in exon 9 in lamin gene in the five affected individuals. In the two patients with recurrent palsies a nonsense mutation in the PMP22 gene was also identified. Moreover, PMP22 mutational analysis identified a third mutated individual. Electrophysiological studies in the three PMP22 mutation carriers showed signs of a generalized neuropathy with mixed features of a demyelination and axonopathy. This study provides insights into the relation of nuclear function in muscle and nerve and in the involvement of the nuclear envelope in the development of disease.

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[20] FURTHER EVIDENCE FOR A THIRD GENE CAUSING MYOTONIC DYSTROPHY

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Myotonic dystrophy (DM) is a multisystem disorder and the most common form of muscular dystrophy in adults. One form of the disorder (DM1) is caused by an expanded CTG repeat in the 3-prime untranslated region of the dystrophin myotonia protein kinase gene on 19q13. A subgroup of DM shows a variant phenotype defined as proximal myotonic myopathy (PROMM). Molecular analysis has ruled out the DM1 mutation in these patients. The majority of PROMM families bear a CCTG expansion located in intron 1 of the zinc finger protein 9 gene on 3q21 (DM2). We report a case presenting with a multisystem disease including myotonia, muscle weakness and atrophy, cataracts and azoospermia. The clinical presentation, serological and histopathological changes were similar to those of DM1. Direct gene analysis at DM1 and DM2 loci has not revealed expansions, providing evidence for further genetic heterogeneity in DM. This is the first report of a patient with a DM1-like phenotype in which both DM1 and DM2 are ruled out. The presence of an unstable repeat expansion in a so far unidentified gene, is hypothesized. Such mutation might induce a perturbation of nuclear functions as reported in the others types of DM. This could explain the high clinical and histopathological resemblance between this patient and those with DM1 and, to a lesser extent, DM2.

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[21] A NOVEL HOMOZYGOUS MUTATION IN THE DESMIN GENE CAUSES CARDIAC AND SKELETAL MYOPATHY

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A 41 year old patient with previous pacemaker implantation at age 24th for atrioventricular block, and cardiac transplantation at age 30th for restrictive cardiomyopathy, came to our observation for progressive weakness and cramps at four limbs in the last 3 years. Symptoms were prominent and started distally at the lower limbs. Serum CK was mildly elevated, and needle electromyography showed mild myopathic changes. Skeletal muscle biopsy revealed myopathic features, and bluish accumulations in the sarcoplasm that were immuno-reactive for desmin and other cytoskeletal proteins. Ultrastructural studies revealed electron-dense coarse granular and filamentous aggregates continuous with the Z lines. Molecular genetics excluded mutations in the lamin A/C gene and disclosed an homozygous mutation in the exon 1 (Arg16Cys) of the desmin gene. This is the second report of a homozygous mutation of the desmin gene, and further emphasizes that skeletal muscle myopathy may develop several years after cardiomyopathy.

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[22] HEREDITARY INCLUSION BODY MYOPATHY (HIBM2): THE M712T MUTATION IN AN EGYPTIAN PATIENT

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Hereditary inclusion body myopathy 2 (HIBM2), initially described in Jews of Persian descent, is a recessively inherited distal myopathy, characterized by adult onset and typical sparing of the quadriceps. Different mutations in the UDP-N-acetylglucosamine 2 epimerase/N-acetylmannosamine kinase gene (GNE) were associated with the disease. One homozygous missense mutation in exon 12 (M712T) of GNE is responsible for HIBM2 in Middle Eastern Jews. We identified the M712T mutation in a Muslim Egyptian patient affected with HIBM2. She was the first of four children born to consanguineous parents. The family history was negative for neuromuscular disorder. Neurological examination revealed mild hyposthenia in



the interossei muscles, while a severe weakness was observed in the anterior tibialis and extensor hallucis brevis. All sensory modalities were normal. Reflexes were uniformly decreased. The electrophysiological examination was orienting for a motor axonal neuropathy and a muscle biopsy of the tibialis anterior was carried out. The pathophysiological role of this mutation will be discussed in the light of the clinical, neurophysiological and pathological features.

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[23] PROTEIN KINASE C AND INTERLEUKIN-1BETA IN MYOFIBRILLAR MYOPATHY

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A myofibrillar myopathy is observed in heterogeneous disorders with different clinical presentation such as proximal or distal myopathy or even isolated cardiomyopathy. The morphological hallmark is the myofibrillar disruption with expression of numerous proteins, most consistently of desmin. Familial cases with mutation in the desmin gene and in the alphaB-crystallin gene were reported, nevertheless the underlying pathogenic mechanism is not still unknown. An abnormal expression of cyclin-dependent kinases that regulate cell-cycle progression and of nuclear proteins was reported. We therefore evaluated in eight patients with myofibrillar myopathy, belonging to four families, the expression of some protein kinase C isoforms and of their activator interleukin-1beta, a cytokine that could also mediate myofibrillar proteolysis. The immunohistochemical and immunoblot analysis showed the expression of alpha, eta and zeta isoforms of protein kinase C and of interleukin-1beta in muscle from the patients. Our data suggest that protein kinase C and interleukin-1beta may be involved in the pathogenesis of myofibrillar myopathy.

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[24] BILATERAL PUTAMINAL NECROSIS AND MERRF MUTATION: AN ATYPICAL CASE IN INFANCY

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The myoclonic epilepsy with ragged-red fibers syndrome (MERRF) is one of the major mitochondrial encephalomyopathies. Its main clinical features are myoclonic epilepsy, ataxia, myopathy with Ragged-Red fibers. While there is a strict correlation, reviewing the literature, between the MERRF syndrome and the mt 8344 of the mitochondrial DNA, the opposite is not true. In fact mutations of the MERRF gene are responsible for different other syndromes such as Leigh syndrome, spinocerebellar degeneration, atypical Charcot-Marie-Tooth disease and multiple truncal lipomas syndrome. We describe a child with a MERRF mutation in the mitochondrial DNA and an unusual clinical, neuroradiological and biochemical phenotype, showing early-onset, non progressive cerebellar ataxia and subclinical myoclonias in association with bilateral putaminal necrosis on MRI images and a reduction of the complex V activity. We underline the importance of considering the possibility of the mitochondrial pathology in presence of bilateral symmetrical lesions of the basal ganglia even without any typical clinical picture. Our case confirms the relationship between the alteration in the ATP-ase activity and the basal ganglia involvement.

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[25] GENOTYPE-PHENOTYPE CORRELATION IN NEUROMUSCULAR FORMS OF GLYCOGEN STORAGE DISEASE TYPE IV

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Glycogen storage disease type IV (GSD-IV) is a rare autosomal recessive disorder of the glycogen synthesis due to glycogen branching enzyme (GBE) deficiency, resulting in the accumulation of abnormal glycogen

similar to amylopectin in almost all tissues. The common presentation is characterized by various degrees of liver involvement, which progresses to lethal cirrhosis in most cases. The neuromuscular form of GSD-IV is heterogeneous both in its onset, and in its clinical phenotype, presenting as multiple congenital contractures, severe hypotonia, myopathy, or as APBD. Different mutations in the GBE gene have been identified in patients with different clinical presentation. We studied eight GSD-IV patients with different neuromuscular forms. In all, the promoter and the entire coding regions were sequenced at the RNA and genomic level. Nine novel mutations were identified, including nonsense, missense, deletion, insertion, and splice-junction. This study expands the spectrum of mutations in the GBE gene and confirms the phenotypic and the allelic heterogeneity of GSD-IV. The presence of severe mutations in most of the infantile lethal cases suggests a genotype-phenotype correlation for this disease.

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[26] ASYMPTOMATIC MCARDLE'S DISEASE  
(GLYCOGENOSES TYPE V) IN A CHILD WITH  
ISOLATED HYPERCKEMIA

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McArdle's disease or Glycogenoses type V is a recessive disorder of the glycogenolysis due to myophosphorylase deficiency, characterized by exercise intolerance with premature fatigue, myalgia, and cramps. Although the clinical phenotype is rather uniform, a few clinical variants have been reported, including a fatal-infantile form with weakness, severe respiratory insufficiency and early death, a mild form with excessive tiredness, a late-onset form with fixed weakness in the fifth to sixth decade. In addition, we have previously identified two virtually asymptomatic children with hyperCKemia. Molecular genetic analyses have identified around 30 mutations in the myophosphorylase gene (PYGM) from patients with different clinical presentations.

We report an asymptomatic 9-year-old girl, who presented persistent and markedly elevated serum creatin kinase (CK) levels. She never complained of exercise intolerance or myalgia. General physical and neurologic examinations were normal. Histochemical and biochemical analysis of muscle showed myophosphorylase deficiency. Genetic analysis showed that the patient was a compound heterozygotes for the common mutation encountered in McArdle's disease

(R49X), and for a novel single base-pair deletion. This case suggests that a thorough study of the muscle biopsy is important in patients with isolated and persistent hyperCKemia for correct diagnosis and careful follow-up.

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[27] MODULATION OF MEMBRANE PROTEIN  
EXPRESSION IN MUSCULAR DYSTROPHIES AFTER  
PROTEASOME INHIBITORS TREATMENT

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Previous studies have shown that the proteasomal pathway is involved in the pathogenesis of various muscle diseases. In a heterologous cell system, proteasomal inhibitor MG-132 was shown to successfully block the protein degradation in an autosomal dominant mutant form of CAV-3 seen in limb-girdle muscular dystrophy (LGMD-1C). In collaboration with Dr. M. Lisanti group, at Albert Einstein Institute in New York, we have shown that in vivo administration of the proteasomal inhibitor MG-132 effectively restores the expression levels and the localization pattern of dystrophin and of dystrophin-associated proteins, normally absent or greatly reduced in mdx skeletal muscles. These results were confirmed by the use of two independent methodological approaches, i.e. immunofluorescence microscopy and Western blot analysis. We are now studying the expression of dystrophin associated proteins in primary human muscle cultures from muscle biopsies of patients with Duchenne and Becker muscular dystrophies treated with different inhibitors of cell catabolic process. The current findings directly support the idea that the proteasomal pathway plays a substantial role in protein degradation in dystrophin-deficient muscle. These results may be important in elucidating the molecular mechanisms underlying the pathogenesis of Duchenne muscular dystrophy and may open new perspectives for a possible treatment of this disease.

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[28] VALIDATION OF A GAIT ANALYSIS METHOD TO  
QUANTIFY LOCOMOTION IN MUSCULAR  
DYSTROPHIES

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Our study is aimed at introducing a quantitative method assessing walking disability in myodystrophic patients, based on advanced technologies of gait analysis. The information obtained through this approach, which has already been applied in several pathologies, can be used to monitor disease progression, to plan proper rehabilitative interventions and to quantify the effect of these treatments. The experiment validation will be made on two populations of patients affected by Duchenne and Becker muscular dystrophy. Gait analysis is not at all an invasive method and consists in visualizing patients' movements in a special laboratory by means of a number of TV-cameras. The patients are asked to walk spontaneously on level and to walk on and off for a small foot-block. A system for image processing collects also data from a force plate embedded in the floor. Then it provides biomechanical quantities like joint angles, velocities, joint moments and powers. Proper indexes of performance can be obtained and used to synthetically quantify the functional loss. Preliminary results showed that muscle strength reduction affects walking performance, and specifically changes kinematic and kinetic patterns. A reduction of muscle movement and power can be the result of muscle weakening, and all the observed compensations are aimed at better alignment of the lower limb joint.

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[29] GENETIC TESTING EXPANDS THE MOLECULAR  
HETEROGENEITY OF CARNITINE PALMITOYL-  
TRANSFERASE DEFICIENCY

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Carnitine palmitoyltransferase (CPT) deficiency is the most common inherited disorder of lipid metabolism affecting skeletal muscle. CPT deficiency is caused by mutations in the CPT2 gene located in chromosome 1p13-p11. We performed genetic analysis of CPT2 gene in 13 patients: eleven had the 'classical' muscular form associated with exercise-induced myoglobinuria and

muscle aches provoked by fasting, fever, or high-fat, low carbohydrate isocaloric diet, and one case was affected by the severe, infantile form. Direct sequencing of the entire coding region and intron/exon boundaries of the CPT2 gene allowed the identification of 26 mutations: 24 missense, and 2 frameshift mutations. In particular, 4 mutations had not been previously reported. They include the insertion of an A at nt 2032 within exon 5, and 3 amino acid substitution within the coding region. The Ser113L was present in 14 alleles in heterozygous form, while it was homoplasmic in three patients, thus confirming the pathogenetic relevance of the common mutation. Nonetheless the identification of new mutations in CPT2 expands the molecular heterogeneity of CPT deficiency, ten years after the first characterization of this autosomal recessive metabolic disorder.

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[30] FAMILIAL IDIOPATHIC HYPERCKEMIA

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Persistent hyperCKemia of unknown cause in subjects with normal neurological examination has been defined Idiopathic HyperCKemia (IH). Although the concept of a Familial form of Idiopathic HyperCKemia (FIH) is well known to neurologists only two families with autosomal dominant inheritance have been described. In one family a mutation in the CAV3 gene has been found. Starting from 36 subjects with IH, the extension of CK determination to other family members allowed to identify 11 families for a total of thirty-one subjects (age: 2-72; males 74%). About 25% of subjects complained mild myalgias and/or fatigue. CK were 2-10 times the normal value. A male to male transmission was found in five families with a very high prevalence of males with hyperCKemia (93%). Muscle biopsy of one member from each family was normal by histochemical, immunohistochemical and biochemical investigations, including immunofluorescence for caveolin-3. Morphometric examination was normal in four families, whereas in the remainder it disclosed different changes in fibre size and distribution. One subject developed transient mild proximal weakness during treatment with simvastatin. No muscle weakness developed in the others in a 2-10 year follow-up or in the oldest relatives. Our data suggest that FIH is not rare and autosomal dominant in at least half of cases with higher penetrance in males. FIH seems to be, in the majority of cases, a benign and non-evolutive condition possibly due to mutations of several genes. Non genetic

factors, such as treatment with statins, may cause muscle weakness in some subjects with FIH.

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[31] MUSCLE INFECTION IN CHRONIC HEPATITIS B

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Two patients with chronic HBV infection and mild chronic active hepatitis were referred for persistent hyperCKemia and myalgias in one. Both had normal neurological examination and no familial history of neuromuscular diseases. EMG showed mild spontaneous activity and increased polyphasic potentials in one patient. Muscle biopsy showed a necrotizing myopathy with scarce mononuclear infiltrates and few MHC-I positive fibres in both patients. Histochemical, immunohistochemical and biochemical investigations excluded known causes of myopathy. Electron microscopy, immunohistochemistry and immunoelectron microscopy for HBV antigens, PCR and in situ PCR for HBV DNA showed in both patients but not in controls: 1) immunoreactivity for viral antigens in several structurally intact fibres; 2) positivity of muscle tissue for HBV DNA; 3) fluorescent HBV DNA signals inside several fibres; 4) virus-like particles in the nucleoplasm of a muscle fibre in one patient. HBV is considered strictly hepatotropic with little evidence of replication in other sites. On the other hand, although few cases of myopathies in the course of HBV and other viral illness have been reported, the muscle is considered resistant to viral infection. Our data seem to indicate a direct HBV infection of muscle fibres which may trigger, as in the liver, an immune reaction directed against the infected fibres and eventually induce necrosis. These findings may cast new light on muscle involvement in viral infections and HBV biology.

[32] NULL/MISSENSE CAPN3 COMPOUND  
HETEROZYGOTE WITH A NORMAL AMOUNT OF NON  
FUNCTIONAL CALPAIN-3 IN SKELETAL MUSCLE

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The diagnosis of limb girdle muscular dystrophy 2A (LGMD2A), caused by Calpain-3 (Calp-3) deficiency, is currently based on immunodetection of muscle protein by Western Blot (WB) analysis. One of the pitfalls of WB for Calp-3 detection is that it may result normal also in mutated patients. We report here the case of a young girl (3 years and 6/12), who was referred for asymptomatic hyperckemia at the age of 10 months, whose muscle WB showed a normal amount and pattern of bands for Calp-3. On molecular analysis it was found that she is a compound heterozygote for two mutations of CAPN3 gene (R110X and G222R). R110X is a nonsense mutation on exon 2, G222R is a missense mutation on exon 5, never reported in the Italian population. The latter mutation in homozygosis produced a normal expression of Calp-3 on WB in a Spanish patient. We found at WB that Calp-3 autocatalytic activity was lost despite of a normal amount of protein. At the last neurological assessment the patient has developed some signs of disease (tendency to Gowers manoeuvre). This is the first reported case of compound heterozygosis for R110X and G222R mutations and it further recommends molecular analysis in subjects with normal WB.

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[33] CHRONIC HYPERCAPNIA IN MYOTONIC  
DYSTROPHY: PRELIMINARY RESULTS OF  
PROSPECTIVE STUDY

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Background: Several reports have described the occurrence of ventilatory failure in myotonic dystrophy, especially in patients with severe muscle impairment. Some Authors suggested that it should be suspected in patients with proximal weakness or daytime somnolence/sleepiness.

Methods: 23 patients with the adult form of DM1, who didn't have primary respiratory disease, were ranged in age from 22 to 69 years and were classified with MDRS Scale. Arterial blood gases analysis and

respiratory function tests as forced vital capacity (FVC), maximal inspiratory pressure (MIP) and respiratory muscle strength (RMS), were performed.

Results: Hypercapnia ( $\text{PaCO}_2 > 45 \text{ mm Hg}$ ) was found with the highest value (40%) in class 3 of MDRS Scale, while with the lowest values (6.6%) in classes 1 and 5. In patients with hypercapnia the prevalence of daytime somnolence was 80% but only 26% had sleepiness, without correlation with MDRS Scale.  $\text{FVC\%pred.}$  was related to  $\text{MIP\%pred.}$  so as  $\text{FVC\%pred.}$  to  $\text{RMS\%pred.}$ , but no relationship was found between  $\text{FVC\%pred.}$  and  $\text{PaCO}_2$ . No evidence of familiar occurrence of hypercapnia was detected.

Conclusions: Our data suggest that even if FVC and RMS may be assumed a reliable index of the muscle impairment, they are not related to hypercapnia; the lack of correlation between daytime somnolence/sleepiness and hypercapnia supports the CNS involvement, previously reported.

[34] NEUROPSYCHOLOGICAL AND LINGUISTIC PROFILE OF CHILDREN AFFECTED BY DUCHENNE AND BECKER TYPE MUSCULAR DYSTROPHIES

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It is known that 1/3 of patients affected by Duchenne and Becker muscular dystrophy present varying degrees of cognitive, and consequently linguistic, impairment. Our aim was to assess cognitive, linguistic, visuo-perceptual skills, attention, short-term memory, communication skills (using the parameters of metaphonological processing), reading-writing abilities, and psychological aspects on a population of Italian-speaking dystrophic patients. We performed longitudinally a in-depth neuropsychological evaluation on a sample of 26 children (20 DMD and 6 DMB), aged between 3 and 14 years. We will describe the peculiar and different cognitive and neuropsychological profiles and compare our results with data of the literature. Our aim was to describe longitudinally developmental profile through a follow up study that might allow us to establish the peculiar and different features. The aim, above all in the neuropsychiatric sphere, was to identify heuristic aspects for reflection and relevant to rehabilitation. These could then be used as a basis on which to develop early educational approaches, designed as a means of increasing, finding and for providing alternative support both for the patient and for his/her family.

[35] SPEECH IMPAIRMENT IN DUCHENNE, BECKER MUSCULAR DYSTROPHY AND SPINAL MUSCULAR ATROPHY: ANY RELATIONSHIP WITH RESPIRATORY FUNCTION?

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Vocal fluency impairment has been described in DMD, BMD and SMA patients. We studied so far 10 patients (5 DMD - 2 BMD - 3 SMA), mean age  $9.1 \pm 3.5$  yrs, to find any relationship with speech alterations and respiratory function. All the patients performed spirometry, blood gases analysis, respiratory pattern evaluation, determination of maximal respiratory muscles pressure, both in sitting and supine position. Pattern of breathing, heart rate,  $\text{SaO}_2$  was on-line recorded during spontaneous language, during description of standard figures and during lecture of brief rigmarole. 5 patients (4 DMD and 1 BMD) presented linguistic impairment, including different fluent style of language, according of prosody and irregular parameters fluency. All the patients but 3 presented altered respiratory function (Vital Capacity  $62.5 \pm 27.1\%$  of pred;  $\text{Pimax}$   $34.2 \pm 12.2 \text{ cmH}_2\text{O}$ ). 6 patients developed transient desaturations ( $> 2\%$ ) during talk. Furthermore, brief periods of paradoxical breathing were found in 5 patients. Our preliminary results, suggest that respiratory function may be one of determinant of speech alteration in DMD and BMD patients.

[36] BLOOD GASES ALTERATIONS IN STEINERT DISEASE (SD)

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Chronic hypercapnia was described in Steinert disease, but less is known about the development of chronic hypoxia. We analyzed data of 10 consecutive SD patients (6 F), age  $41 \pm 14.8$  yrs, BMI  $30.9 \pm 6.8 \text{ Kg/m}^2$ , who performed spirometry,  $\text{Pimax}$  and  $\text{Pemax}$ , blood gases analysis and nocturnal oximetry. Vital Capacity ( $62.8 \pm 11.9\%$  of pred) and  $\text{Pimax}$  ( $41.1 \pm 23.7 \text{ cmH}_2\text{O}$ ) were reduced. The mean value of  $\text{PaO}_2$

was  $71.8 \pm 11.4$  mmHg and the mean value of PaCO<sub>2</sub> was  $44.1 \pm 3.7$  mmHg. 5 patients presented PaO<sub>2</sub> < -1.64 SD-score and 6 PaCO<sub>2</sub> > 44 mmHg; alteration in both blood gases was found in 3. 8 patients showed altered nocturnal oximetry (TIB90 > 10%), that is significantly associated with increasing in PaCO<sub>2</sub> ( $x2$  5.48;  $p < 0.05$ ) but not with decreasing in PaO<sub>2</sub>. Patients with nocturnal gas exchange alteration presented higher BMI ( $34 \pm 5$  /  $23.6 \pm 4.5$ ,  $p < 0.05$ ) and higher PaCO<sub>2</sub> ( $45.7 \pm 2.6$  /  $40.3 \pm 3.2$ ). Stepwise forward regression analysis showed a relation between alteration in PaO<sub>2</sub>, sex and Vital Capacity ( $r = 0.85$ ,  $p < 0.01$ ); alteration in PaCO<sub>2</sub> was related to age, Vital Capacity and TIB90 ( $r = 0.86$ ,  $p = 0.03$ ). Our data showed that SD patients are at risk for daytime hypoxia unrelated to alveolar hypoventilation.

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[37] INVOLVEMENT OF CYTOSKELETAL AND EXTRACELLULAR MATRIX STRUCTURAL COMPONENTS IN LGMD2I MUSCLE: REPORT OF ONE CASE

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Fukutin-related protein (FKRP) is a putative glycosyl transferase localized in the Golgi apparatus. Recessive mutations in FKRP gene cause Congenital Muscular Dystrophy type 1C (MDC1C) and a milder allelic variant Limb Girdle Muscular Dystrophy type 2I (LGMD2I).  $\alpha$ -dystroglycan glyco-epitope abnormalities have been reported in MDC1C and LGMD2I muscle biopsies suggesting that FKRP is involved in  $\alpha$ -dystroglycan glycosylation pathway. To gain insight in the pathogenesis underlying FKRP related pathologies, we report an electron microscopy and immunohistochemical study of a muscle biopsy from a patient affected by LGMD2I carrying heterozygous mutations in FKRP gene. A severe deficiency of  $\alpha$ -dystroglycan glycosylated protein and a moderate laminin  $\alpha 2$  deficiency was detected in most muscle fibers. Electron microscope examination of non-necrotic fibers revealed striking alterations of basal lamina consisting of frequent detachments from the plasmalemma, duplications and discontinuities. The plasmalemma beneath areas of basal lamina alterations appeared discontinuous in some cases. Basal lamina disruption was associated with alterations of membrane associated cytoskeleton: the characteristic cortical cytoskeletal structures linking the contractile apparatus to the extracellular matrix through the membrane, the

dense plaques, were absent. We investigated the expression of some dense plaque components as integrin  $\alpha 7B$ , talin and vinculin, by immunofluorescence. An irregular distribution of all these proteins along the plasmamembrane was observed. Our findings, consisting of alterations of basal lamina and loss of dense plaques in definite sites in muscle fibers suggest that FKRP mutations may alter the stability and organization of extracellular matrix-cytoskeleton binding in LGMD2I muscle.

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[38] REDUCTION OF LYSOSOMAL MEMBRANE PROTEINS 1 AND 2 AND ACTIVATION OF UBIQUITIN-PROTEASOME PATHWAY IN 19P13.3-LINKED LATE ONSET AUTOSOMAL DOMINANT DISTAL MYOPATHY

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We describe a second large Italian kindred with autosomal dominant vacuolar myopathy characterized by variable severity (asymptomatic to severe), onset in adult life, weakness of distal limb muscles and no cardiac involvement. At least 19 individuals, over 4 generations are affected. We clinically examined 9 members and performed haplotype analysis in 17 members from two generations. In the examined patients, CK was slightly increased. EMG revealed repetitive high frequency discharges and myopathic potentials in proximal and distal limb muscles. A muscle biopsy, performed in 6 patients, revealed myopathic features of variable severity with presence of vacuoles in several muscle fibers. Vacuoles were filled with basophilic granular material partially positive for acid phosphatase. Electron microscopy showed the vacuoles to be autophagic. Immunohistochemistry revealed almost complete absence of lysosome-associated membrane proteins 1 and 2 (LAMP-1, LAMP-2) and increase in ubiquitin-proteasomal components. Linkage analysis excluded loci associated with similar pathological conditions and localized the defect to the 19p13.3 locus. Histopathological and immunochemical features of the vacuoles suggest abnormalities of protein degradation with dysregulation of the lysosomal compartment and activation of the ubiquitin-proteasomal pathway. We speculate that the primary defect may be an abnormality in the lysosomal degradation pathway or in lysosome-related components.

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[39] MUSCLE MORPHOMETRY OF HUMAN LONG-TERM LOWER-MOTONEURON DENERVATED MUSCLE IN SCI

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While early denervation has been widely studied both in animal models and humans, long-term effects of denervation have attracted much less attention since the general opinion is that all myofibers disappear within twelve months of denervation. In Spinal Cord Injury (SCI), muscle atrophy is especially severe when injury permanently involves lower motoneurons. Through a small skin biopsy, needle muscle biopsies were taken in SCI patients from the right and left vastus lateralis muscle. Though after irreversible denervation, atrophy is complicated by fibrosis and fat substitution, we recognized atrophic myofibers even after 200-month post-SCI. Myofiber, collagen and fat contents were evaluated by morphometric analysis. In spite of severe progressive atrophy, substantial fat substitution appears very late after SCI injury in humans (at around 50-month after injury) and then surprisingly decreases, while fibrosis continue to increase. Since biopsy cryosection area strictly depends on myofiber content, which could or couldn't be functional, we performed SDS PAGE analyses on serial sections. MHC content, over total proteins, is a good molecular marker to follow the atrophic process in long-term permanent denervated human muscles. Myosin heavy chains are also present 450-month after SCI.

[40] ROLE OF THE OXIDATIVE STRESS IN MYOTONIC DYSTROPHY

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Steinert's myotonic dystrophy (MD) is an autosomal dominant disease and the most frequent muscular dystrophy in adulthood, characterized by a multisystem involvement. The pathogenetic mechanism responsible for the multisystem involvement in MD is still unclear. An increased susceptibility to the oxidative stress together with increased levels of circulating free radicals in MD, suggests a role of the oxidative stress in this disease. We investigated 39 patients affected by MD to evaluate serum gamma-glutamyl transpeptidase (GGT), GGT fraction bound to low density lipoproteins

(LDL-GGT) and advanced oxidation protein products (AOPP) levels, as indicators of oxidative stress. Total GGT ( $127 \pm 18.98$  mU/ml vs.  $11 \pm 3.05$  mU/ml;  $p=0.0005$ ) and absolute LDL-GGT ( $19.08 \pm 6.27$  mU/ml vs.  $1.86 \pm 0.83$  mU/ml;  $p=0.0021$ ) values were increased in MD, with higher levels of GGT in the older patients ( $p=0.0117$ ). Plasma AOPP levels were significantly higher in patients than in controls ( $52.08 \pm 5.86$   $\mu$ mol/l vs.  $29.9 \pm 4.67$   $\mu$ mol/l;  $p=0.021$ ), with higher values in those patients with extra-muscular signs of the disease; AOPP showed a significant correlation with serum GGT levels ( $r=0.5831$ ;  $p=0.0022$ ), but not with age. The relationship between GGT and AOPP indicates a possible role of the former in generation of oxidative stress in MD, while the association of AOPP levels with extra-muscular signs of the disease suggests that oxidative stress is important in the systemic manifestations of the disease.

[41] LOWER LIMB INVOLVEMENT IN FSHD: A MR STUDY

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Introduction: FSHD is an autosomal dominant disease in which lower limb involvement always follows that of facial and shoulder muscles and represents an unequivocal index of disease progression and severity. Methods: We studied 50 patients, aged 21 to 71 years, with a genetically confirmed diagnosis of FSHD. Muscle examination was performed on a 1.5-Tesla MR scanner, with T1-W SE images (TR/TE=500/35 msec) and T2-W STIR images (T1=1 50 msec). Axial slices were obtained from psoas to distal foot muscles.

Results: MR study showed pelvic/lower limb muscles involvement in 45 patients, including 4 cases with normal clinical evaluation. The overall severity of muscle involvement was in keeping with the level of functional severity, although MR sensitivity was higher than clinical examination in detecting the extent and severity of single muscle involvement. The most frequently involved muscles were: semimembranosus, biceps femoris (long head), gluteus medius, semitendinosus, and soleus, while quadratus femori, obturator internus, biceps femoris (short head) and iliacus were only rarely involved. A correlation was found between age and severity of muscle involvement ( $r=0.58$ ;  $p=0.006$ ). Focal areas of hyperintensity on T2-W STIR images indicated the presence of "oedema" in muscles that showed minor features on T1-W sequences.

Conclusions: Muscle MR examination is a powerful tool in the evaluation and follow-up of FSHD patients.

[42] NOVEL GNE MUTATIONS IN ITALIAN FAMILIES  
WITH AUTOSOMAL RECESSIVE HEREDITARY  
INCLUSION-BODY MYOPATHY

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The most common form of autosomal recessive (AR) hereditary inclusion-body myopathy (HIBM), originally described in Persian-Jewish families, is characterized by onset in early adult life with weakness and atrophy of distal lower limb muscles, which progress proximally and relatively spare the quadriceps. AR HIBM is associated with mutations in the UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene (GNE) on chromosome 9p12-13. In the present study we have identified seven novel GNE mutations in patients from five unrelated Italian families with clinical and pathologic features indicative of AR HIBM. Four were missense mutations (N519S, P27S, A600T and G206S), two consisted in a single-base deletion and one in an intronic single-base insertion. These latter findings further extend the type of GNE abnormalities associated with HIBM. Furthermore, in one patient we also identified the R246Q missense mutation that corresponds to the one previously reported in a family from Bahamas. Interestingly, in two of our families distinct mutations affected nucleotide 667 in exon 3 (667delG and 667 G>A). The possibility of specific portions of the gene being more prone to mutations remains to be elucidated.

[43] A NOVEL MUTATION IN MTDNA ASSOCIATED  
WITH MELAS

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The syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is clinically and genetically heterogeneous. Investigating a group of MELAS patients negative for the common 3243A>G mutation in the mtDNA, we identified a 22-year-old girl who was hospitalized because of recurrent headache, visual disturbances, and clonic movements in

upper limbs. Past medical history was significant for clumsiness and delayed development in early childhood and headache when she was in primary school. At age 16, the patient had had a transient ischemic attack with residual left hemiparesis that had resolved in three days. During hospitalization, EEG showed epileptic activity on the left occipital lobe. Brain MRI showed hyperintense signal in T2-weighted images in the left occipital cortex. Serum lactate levels were elevated at rest (2X normal). In a muscle biopsy sampled specimen, histochemistry showed near absent cytochrome c oxidase (COX) stain whereas biochemistry evidenced an isolated COX deficiency (residual activity was 18% normal). Direct sequence of the whole mtDNA identified a new mutation which satisfied consensus criteria for pathogenic variants. Our findings add to the growing array of mtDNA variants associated with human pathologies.

[44] EXPERIENCE WITH "DA VINCI" SURGICAL  
SYSTEM FOR THYMECTOMY IN PATIENTS WITH  
MYASTHENIA GRAVIS

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Background: thymectomy is a widely accepted surgical therapy for treatment of patients with myasthenia gravis. Several surgical approaches are used, but there is no agreement on which technique is the best. Minimally invasive technique, such as "Da Vinci" robotic surgical system, have recently been introduced, but large scale studies are still lacking.

Methods: from April 2002 to October 2003, 24 patients with myasthenia gravis underwent thoracoscopic thymectomy with "Da Vinci" surgical system. A left side approach with 3 accesses.

Results: mean operative time was 129 minutes. No intra-operative complications were reported, post-operative complications occurred in two patients. Mean hospital stay was 2,5 days. Histological analysis of specimens revealed 16 hyperplasia, 4 normal thymus, 2 atrophy and 2 thymoma; in 11 (45,8%) patients were found ectopic thymic tissue.

At follow-up evaluation (mean 13 months, range 3-21) three patients (12,5%) were in complete remission and 16 (66,7%) showed significant clinical improvement, for a global benefit rate of 79,2%. All patients judged excellent cosmetic results and nobody needed analgesics at three months from operation.

Conclusions: "Da Vinci" robotic surgical system seems to be a well tolerated, safe and efficient technique. 3-D view and high dexterity of surgical instruments make robotic thymectomy safer than VATS and transcervical



thymectomy and equally radical. A longer follow-up is necessary to verify long term results.

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[45] GM2 GANGLIOSIDOSIS MANIFESTING AS FAMILIAL MOTOR NEURON DISEASE

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GM2 gangliosidosis (Sandhoff disease) is a lysosomal disorder due to beta-hexosaminidase subunit (HEXB) deficiency. Its most frequent clinical manifestation is indistinguishable from Tay Sachs' disease. Juvenile forms of spinocerebellar ataxia and, rarely, motor neuron diseases (MND) have been described. We report a HEXB deficiency manifesting as an autosomal recessive MND. A 62 year-old sister and a 49 year-old brother came to our observation because of progressive limb muscles wasting, weakness, dysarthria and dysphagia. Their parents both originated from a village of South Italy. Other two out of six germans were similarly affected, with onset of symptoms between ages 30 and 40. Both patients showed wasting and weakness of proximal muscles, predominantly at lower limbs, and absent R.O.T: the sister had lost deambulation. The brother, still able to walk, showed as striking feature marked, diffuse fasciculations. EMG and VCS showed acute and chronic motor neuron damage and a sensitive axonal neuropathy, confirmed by muscle and nerve biopsies. Brain MRI revealed cerebellar atrophy. MEP, EEG , fundoscopy were normal. Biochemistry documented absence of HEXB form activity in patients' leukocytes. Genetic studies are in progress. In conclusion, we must consider HEXB deficiency in the differential diagnosis of juvenile MND for prognostic reasons: in fact animal models' studies indicate substrate deprivation as a potential treatment for HEXB deficiency.

[46] VACUOLAR MYOPATHY DURING CHRONIC HEPATITIS C AND INTERFERON- $\alpha$ 2A THERAPY

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Several cases of Rhabdomyolysis and myopathy during the course of hepatitis-C (HC) have been described in the recent years. The muscular involvement in HC has been related to the interferon (IFN) therapy, but some authors reported cases of myopathy in non-treated patients hypothesizing a direct pathogenic role of HC virus. Herein, we report on a 46 years old man, with chronic HC, who began to complain muscle weakness and iperckemia in the last two years. The patient underwent one year later IFN  $\alpha$ 2A and ribavirine treatment. Neurological examination revealed muscle weakness and hypotrophies in both shoulder and pelvic girdles. Creatine-kinase was mildly increased (570 U/L); EMG revealed a myopathic pattern in the four limbs muscles. A muscle biopsy showed an increased variation of fiber size; several fibers contained vacuoles, in both peripheral and central position. Biochemical analysis as well as immunohistochemistry were normal. In the six months following IFN discontinuation, the clinical feature of the patient did not significantly improve. A second muscle biopsy confirmed the variability of fiber size and the presence of vacuoles. Conclusion: We report on vacuolar myopathy during chronic HC treated with IFN; the exclusion of any other known causes of vacuolar myopathy as well as the lack of improvement of both clinical and histological features, following IFN discontinuation suggest a possible correlation between muscular and hepatic disorders.

[47] A NEW FAST AND RELIABLE SDS PAGE  
PROTOCOL TO QUANTIFY TOTAL PROTEIN AND  
MYOSIN HEAVY CHAIN IN CRYO-SECTIONS OF  
NEEDLE BIOPSY FROM LONG-TERM PERMANENT  
DENERVATED HUMAN MUSCLE BEFORE AND AFTER  
FES

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We describe a new SDS PAGE method, which allows to quantify total protein and Myosin Heavy Chain Content in cryo-sections of needle muscle biopsies. Severe atrophy of skeletal muscles occurs during long-term permanent denervation (LT-PD). We studied 14 human muscle biopsies taken from patients suffering lower motoneuron denervation from 2 to 30 years. Patients were treated with Functional Electrical Stimulation (FES) for different periods of time (0.5 to 6 years). Electrical stimulation of denervated muscles increase size of the myofibres and maintains sarcomeres, by preventing/reversing secondary degeneration of the long-term denervated tissue. Here we show results of the quantitation of myosin content in long-term permanent denervated muscles, which strongly support the use of FES to meliorate muscle trophism in long-term lower-motoneuron denervated human muscles.

[48] EXTENT OF EARLY DAMAGE AFTER FREE-  
RUNNING IN DYSTROPHIC MICE

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In order to evaluate the effects of exercise in normal or dystrophic muscles of rodents, three-month old control (c57 Balb/6J) and alpha-sarcoglycan-null (alpha-SGko) mice were housed in cages provided of a rotanting wheel and allowed to spontaneously run the full night. Activity of mice was quantified as covered distance. At 1 day post-running mice were sacrificed and muscles from both legs removed. Muscle cryosections were stained with Hematoxylin and Eosin and anti-embryonic MHC antibodies to valuate the percent of swollen fibers. alpha-SGko mice normally run a distance of 68% in respect to the control 6J. Before running, fast and

slow muscles of alpha-SGko mice presented the majority of fibers with central nuclei, together with rare swollen, dying fibers, foci of macrofage infiltration and areas with fibers expressing the embryonic MHC, evidence of muscle regeneration. The day after running muscles of control mice showed modest edema and some infiltration of phagocytes. Fibers positive to embryonic MHC were as rare as in pre-running controls. In the alpha-SGko running mice, the day after run, both fast and slow muscles showed diffuse infiltration and swollen fibers. Results of morphometric analysis demonstrate that whereas in control 6J mice all fibers are within normal range, alpha-SGko mice have twice the amount of swollen myofibers.

[49] RAPIDLY REVERSIBLE MYOPATHY RELATED TO  
FINASTERIDE THERAPY

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A seventy-three year-old man, admitted to dermatological ward for evaluation of erythematous-papular-desquamative skin lesions, was referred to neurologist because of three months lasting progressive muscle weakness and atrophy, with 10 kg body weight loss. Physical examination revealed severe weakness (MRC 3/5) in proximal districts of limbs confirmed by clear EMG myogenic pattern and mild active denervation in proximal muscles. Leukopenia, increase of serum inflammatory markers and myoglobin, autoantibody titers and muscle enzyme values within normal range were observed. Skin biopsy was consistent with discoid lupus erythematosus, whereas muscle biopsy showed atrophy of type II fibers, mild type grouping and no inflammatory changes. The patient had been treated with finasteride 5 mg/die for five years because of benign prostatic hyperplasia and never had used corticosteroids. Owing to similarity of clinical findings with a previously described case, the drug was withdrawn (Haan, 1997). One month later, recovery of muscle strength was remarkable and body weight had increased by 3 kg. Antiandrogen finasteride, a 5-alpha-reductase inhibitor, is employed in benign prostatic hyperplasia and alopecia. Sexual dysfunctions are most frequent side effects, whereas abdominal-pelvic pain, headache and asthenia are rarely reported. The current case confirms rapidly reversible myopathy as a possible adverse effect of finasteride treatment, attributable to its structural affinity to corticosteroids.

[50] PILOT TRIAL OF SALBUTAMOL IN CONGENITAL MYOPATHIES

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Several studies have documented positive effects of beta-adrenergic agonists on human skeletal muscle with regard to muscle strength and lean body mass. The aim of this pilot study was to evaluate the effect of Salbutamol in a group of children with Central Core Disease (CCD) and Multi-minicore disease (MmD). Thirteen patients, 8 with CCD (mean age 17.5 years) and 5 with MmD (mean age 13.6 years) were started on oral Salbutamol 2mg qid. Measures of efficacy were the change from baseline at 3 and 6 months in muscle strength, assessed by MRC score, myometry and functional measures and forced vital capacity. Statistical analysis was performed using repeated measures ANOVA (significance level <0.05). Two patients stopped the medication after a month because they did not notice any improvement (1 with CCD and 1 with MmD) and another one after 4 months because of increased tremors and palpitations. The remaining ten (6 with CCD and 4 with MmD) completed the course of Salbutamol without any significant adverse effects. There was a significant increase in myometry, MRC score and forced vital capacity between baseline and the six-month assessments. For both myometry and MRC score the difference was already significant at 3 months and this was associated with a significant increase in functional abilities assessed with a structured functional scale. Our results suggest that Salbutamol was overall well tolerated and might be beneficial in CCD and MmD patients. Larger prospective randomised, double-blind, placebo controlled trials with Salbutamol will be needed to confirm these preliminary findings.

[51] NEUROLEPTIC MALIGNANT SYNDROME AFTER RISPERIDONE ADMINISTRATION. MUSCLE BIOPSY FINDINGS

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Morphological observations of muscle biopsy in the neuroleptic malignant syndrome are very rare. We report on a 14-year-old female patient who presented,

after abuse of unknown psychotropic drugs, acute delirium with auditory hallucinations and insomnia. Five days later a physician prescribed Risperidone 1,5 mg/daily and 2 days after therapy the patient started to present oculo-facial dyskinesia and respiratory failure. The patient lost her ability to breathe on the air a few days later and needed mechanical ventilation and later tracheotomy. Brain and spinal MRI as well as EEG were normal. Laboratory findings showed increased CK and myoglobinuria. The patient regained spontaneous respiration after 2 months. The muscle biopsy was performed after 27 days from ventilatory failure, and showed marked diffuse endomysial edema, and increased diameter variability. We found no signs of necrosis and regeneration. The calcium ATPase staining showed that exclusively and many type I fibers showed wide central areas that were devoid of activity. Staining for oxidative mitochondrial enzymes showed central core-like areas. The ultrastructural examination showed that the central areas, which were negative for calcium ATPase staining, corresponded to loss of thick filaments. To the best of our knowledge morphological abnormalities observed in this case have never been reported in the neuroleptic malignant syndrome and show some similarities with the acute quadriplegic myopathy.

[52] OCULAR AND DISTAL MUSCULAR INVOLVEMENT ASSOCIATED WITH RIMMED VACUOLES

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Oculopharyngeal muscular dystrophy and oculopharyngodistal myopathy share ptosis, ophthalmoplegia, dysphagia, skeletal muscle involvement, and the pathological finding of rimmed vacuoles. We evaluated a 28 years old woman who had eyelids drooping and difficulty in walking and running since childhood. CK, lactic and pyruvic acid, thyroid hormones, ACh receptor antibodies were normal. On physical examination marked bilateral ptosis with gaze impairment was evident; dysphagia and dysarthria have not appeared in the course of the disorder; muscle weakness and atrophy were present in the legs; extensor muscles of the hands were also affected; inability to walk on the heels and toes was manifest. EMG showed a mixed myopathic and neurogenic pattern; motor and sensory nerve conduction velocities were normal as well as repetitive stimulation. On muscle biopsy ragged red fibres and respiratory chain defects were absent. There was no evidence of inflammatory cells and amyloid deposition. The prominent feature in many fibres was

the presence of rimmed vacuoles. The genetic analysis did not display a pathological expansion of PABP2 (Poly A Binding Protein 2) gene. On electron microscopy the rimmed vacuoles paralleled multilaminated membranous structures, granular and fibrillar material; no intranuclear tubulo-filamentous inclusions were found out. This sporadic case genetically uncharacterized, although sharing some clinical and pathological features with oculopharyngeal muscular dystrophy and oculopharyngodistal myopathy, leaves the relation phenotype-genotype unsolved.

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[53] INFLAMMATORY GENES EXPRESSION IN FOCAL MYOSITIS AND POLYMYOSITIS USING HUMAN MICROARRAYS

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**Objective:** The purpose was to identify genes that are differentially expressed in skeletal muscle of patients with focal myositis (FM) and polymyositis (PM), searching for a molecular hallmark responsible of the restricted inflammatory phenomenon in FM.

**Methods:** Microarray experiments were performed using amplified RNA isolated in muscle specimens from five patients each with FM and PM, and five normal controls. A GeneChip microarrays panel of 160 human inflammatory genes was used.

**Results:** 58 genes showed significantly increased expression in both FM and PM. They included genes involved in immune response, T-cell cytotoxicity, extracellular matrix breakdown, propagation of the inflammatory process. We found higher activation of metalloproteinases (MMP) 9,12,13 in PM versus FM, and interferon gamma (IFN-gamma) in FM versus PM. Adhesion molecule ICAM-1 resulted abundantly expressed only in PM. Mn super-oxide-dismutase, inducible nitric oxid synthase and interleukin-1-beta (IL-1 beta) were overexpressed only in FM.

**Discussion:** Microarray analysis is an effective tool for identifying genes differently involved in the inflammatory/immune response of skeletal muscle during FM and PM. Exaggerated expression of MMP-9, 12,13, and ICAM-1, facilitating lymphocyte adhesion and enhancing T-cell mediated cytotoxicity by degrading extracellular matrix proteins, could contribute to extend inflammatory process in PM. Conversely, selective upregulation of some genes seems to characterise FM. These findings may have practical implications in considering therapeutic different approaches in inflammatory myopathies.

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[54] CAVEOLIN-3 SEQUENCE VARIATIONS IN A PATIENT WITH PERSISTENT HYPERCKEMIA

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Caveolin-3 (CAV3) is the muscle specific product of the caveolin gene family and constitutes an integral membrane component of caveolae. Mutations within CAV3 coding sequence underlie five distinct muscle disorders: the limb-girdle muscular dystrophy -1C, Rippling Muscle Disease, Distal Myopathy, Hypertrophic Cardiomyopathy and sporadic and familial forms of hyperCKemia (HCK). We performed CAV3 molecular analysis in a patient presenting with persistent HCK (783-1084 U/L) at repeated sampling. The patient was free of symptoms of muscular impairment (apart from occasional myalgia) and neurological examination failed to demonstrate significant reduction of muscular strength. Immunocytochemical and molecular analysis allowed to exclude the occurrence of a dystrophinopathy. We performed sequence analysis of CAV3 coding region, intronic boundaries and 3'UTR region alternatively spliced in transcript variants of the gene. We identified the presence of two synonymous nucleotide variations (C to T at nucleotides 27 and 99 of exon 1), occurring in heterozygosity. Both nucleotide changes occur adjacent to a GAGG motif, resembling a purine-rich exonic splicing regulatory sequence. We are currently testing their possible pathogenic significance by RNA analysis on patient skeletal muscle biopsy.

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[55] MOLECULAR CHARACTERIZATION OF ATP2A1 GENE IN A PATIENT WITH BRODY MYOPATHY

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Brody disease is a rare disorder of fast-twitch skeletal muscle function, characterised by exercise-induced impairment of skeletal muscle relaxation, stiffness and cramps. The disease is generally autosomal recessive (AR). Mutations in the ATP2A1 gene, encoding for the fast-twitch skeletal sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase have been discovered in the majority of AR Brody patients. We have molecularly characterised a sporadic case of clinically diagnosed Brody disease. The

patient's parents originate from a small Italian village, supporting the possibility of an AR inheritance model. Ca<sup>2+</sup>-ATPase activity was found to be significantly reduced in patient's muscle biopsy (50% of control values). We have sequenced both the entire coding region of the ATP2A gene (23 exons) and the intronic boundaries. No exonic mutations (apart from two known polymorphisms in exons 7 and 23) or variations in canonical splice sites were identified. Two previously unreported intronic variations were detected, both located in the proximity of acceptor splice sites (in intron 19 and intron 22) and both representing frequent allelic variants in our population. We are proceeding in RNA analyses in muscle tissue in order to explore the possibility that deep intronic mutations could be responsible for the Brody myopathy in this patient.

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[56] ROLE OF SPLICING IN MODULATING THE PHENOTYPE OF DYSTROPHINOPATHIES

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Pre-mRNA splicing represents an important process able to increase the capacity to encode multiple protein products by alternative splicing events. Splicing mutations are responsible for the 15% of human genetic diseases and are often regulated in a tissue/cell-specific manner. A relevant category of sequences required for both constitutive and alternative splicing are splicing enhancers known to participate to the exon definition process. We have identified and functionally characterised dystrophin splicing abnormalities which significantly contribute to the phenotypic spectrum of dystrophinopathy (either DMD/BMD and X-linked dilated cardiomyopathy or XLDC). A deletion mutation occurring within intron 11 creates a novel exon which is inserted in the dystrophin mRNA and produces an out of frame transcript predominantly in the heart. In patients with isolated exon 5 deletion a complex splicing event of exon skipping and scrambling correlates with the phenotypic differences between DMD and BMD. We identified four putative splicing enhancers, and two of these, located within intron 11, are able to modulate the dystrophin RNA configuration and consequently the clinical picture. Our results suggest that splicing plays a relevant role in determining the phenotypic spectrum of dystrophinopathies, reinforcing the importance of studying muscle tissue for the appropriate definition of both clinical diagnosis and genetic prognosis.

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[57] EPISODIC MYOGLOBINURIA IN TWO SIBLINGS WITH REDUCED CPT ACTIVITY AND CYTOCHROME C OXIDASE NEGATIVE FIBRES

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A 48-year-old male was admitted to an intensive care unit for acute respiratory insufficiency following fever and muscle pain. Myoglobinuria was referred and serum creatine kinase was over 100000 U/l. Muscle biopsy performed two weeks after the acute onset was sent to us with a clinical diagnosis of polymyositis. Histological examination disclosed slight fibre size variability, occasional internal nuclei and two fibres negative for cytochrome c oxidase (COX) staining. Immunohistochemical markers for inflammation were negative. Carnitine palmitoyltransferase (CPT) was reduced to 30% residual activity. The patient came to us after two months. Family history revealed that he was born from first cousins, last of five children, one of whom with similar symptoms. The patient had been complaining of episodic weakness and myalgia after strenuous walks since the age of 9; later he presented several episodes of myoglobinuria and respiratory insufficiency diagnosed as acute alcohol intoxication. At the moment of our observation neurological examination was normal. Also his 59-year-old sister referred episodes of myoglobinuria and had moderate proximal weakness. Muscle biopsy demonstrated reduction of CPT activity (40%), and mitochondrial alterations with one ragged red fibre and two COX negative fibres. Genetic analysis of both cases is under way. The interest of the family is the coexistence of two metabolic muscle disorders, probably both contributing to episodic myoglobinuria.

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[58] MUSCLE COMPLICATIONS OF TREATMENT WITH STATINS

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Statins are highly effective drugs to reduce serum cholesterol and low-density lipoprotein cholesterol levels. Clinical trials have shown that they also reduce the risk for coronary heart disease events, coronary procedures and stroke. About 1-5% patients treated with these drugs develop myopathy, in some cases with severe myoglobinuria, acute renal failure and even death. We collected 20 patients who came to our Centre during the last two years because of clinical or

laboratory signs or symptoms developed during treatment with statins. Most of them had symptoms as muscle pain, cramps, weakness, and/or high serum creatine kinase (CK) levels (from 46 to 2000U/L). In no case discontinuation of the therapy effected normalization of CK or clinical signs. In 12 patients muscle biopsy was performed. One patient showed inflammatory findings, another had normal muscle biopsy; in all the other cases we found non specific alterations such as small fibers, variation in fibre size, internal nuclei, moth-eaten fibres, occasional ragged red fibres and increased lipids. In conclusion, statins seem to lead to permanent muscle damage in patients with no symptoms or signs of neuromuscular disorders before therapy. The pathogenetic basis of muscle abnormalities has to be investigated.

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[59] EFFECT OF TREATMENT WITH PDTC AND IRFI 042 ON STRENGTH AND FATIGUE IN MDX MICE

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Previous studies provided evidences that generation of reactive oxygen species and activation of transcription factor NF-kB may play important roles in the pathogenesis of Duchenne muscular dystrophy. We tested whether IRFI 042, a vitamin E-like antioxidant, and PDTC, a NF-kB inhibitor, could have an effect on muscle weakness in mdx mice. We treated 48 5/6-week old mdx and wild type mice with intraperitoneal injections of PDTC (50 mg/kg), IRFI 042 (20 mg/kg), or vehicle, three times a week for five weeks. Data regarding weight, survival and forelimb strength and fatigue were collected. Motor performance measurements were carried out using a grip meter attached to a force transducer which measures peak force generated. Mdx mice treated with IRFI 042 or PDTC showed at the end of treatment a significantly higher forelimb strength than vehicle controls (IRFI 042: +53.6%,  $p < 0.001$ ; PDTC: +53.1%,  $p < 0.05$ ) as well as higher strength normalised to weight (IRFI 042: +57.8%,  $p < 0.001$ ; PDTC: +54%,  $p < 0.05$ ). Furthermore PDTC-treated mdx mice had significantly less fatigue than vehicle animals (-120%,  $p < 0.004$ ). Our results suggest that PDTC and IRFI 042 might have a beneficial effect on weakness and fatigue in mdx mice. Further studies are needed to investigate the morphological and biochemical substrates of such encouraging preliminary results.

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[60] EXPRESSION AND ACTIVATION OF TRANSCRIPTION FACTOR NF-KB IN DMD: RELATION TO AGE

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**Objective:** The aim of the present study was to investigate expression and activation of NF-kB in relation to age in Duchenne muscular dystrophy (DMD) patients.

**Background:** Our previous studies demonstrated an important activation of nuclear factor-kappaB (NF-kB) in muscle specimens from DMD patients. In particular, NF-kB immunolocalization was found in all regenerating fibers and in 20-40% of necrotic fibers.

**Methods:** Muscle samples from 10 DMD patients (age range: 2-9 years) were studied by immunocytochemistry for the activated form of NF-kB. NF-kB DNA binding activity was investigated by electrophoretic mobility shift assay (EMSA).

**Results:** There was a significant decrease in number of regenerating and necrotic fibres with increasing age. All regenerating fibers and some necrotic fibers were NF-kB positive. EMSA analysis evidenced activation of NF-kB pathway in all patients but at variable degree which inversely correlated with age of patients ( $r: -0.89$ ;  $p < 0.005$ ).

**Discussion:** The present study confirms that NF-KB pathway may play a role in DMD, modulating the immune response and regulating myogenesis and muscle repair. Further studies are needed to investigate the biochemical correlates of NF-kB activation.

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[61] ACUTE QUADRIPLAGIC MYOPATHY (AQM): CLINICAL AND MOLECULAR FINDINGS

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We reviewed a cohort of 15 patients with AQM. All patients had muscle weakness and atrophy and a history of sepsis, ICU treatment and corticosteroids and/or NMBAs administration. CK were low-normal and electromyography showed myopathic features. Morphology revealed a variable degree of myofiber atrophy with thick filaments loss. Biochemistry revealed a marked increase of acid maltase activity which correlated with severity of clinical involvement. We have recently proved by gene expression profiling studies a specific induction of TGF beta/MAPK pathway in AQM (Ann Neurol 55:195-206, 2004). This pathway is involved in cell cycle arrest, apoptosis and atrophy. Accordingly, we now demonstrated in this

large series of patients that atrophic myofibers showed co-expression of TGF beta receptors, p38 MAPK, c-jun, and c-myc, including phosphorylated active forms, and these same fibers showed apoptotic features as shown by TUNEL and activated-caspase 3 immunoreactivity. Moreover we found a strict correlation between TGF beta/MAPK induction and degree of atrophy. TGF beta/MAPK is activated by metabolic imbalance and osmolar stress, that are main features of our patients, and by oxidative stress, that we have documented by the strong up-regulation of MnSOD, HO-1 and catalase mRNA expression. Our data suggest a pathogenic model for AQM where a combination of cellular stress, inactivity of muscle, and drug stimulation all converge on the TGF beta-MAPK leading to acute stimulation of apoptosis and myofilament loss.

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[62] UNUSUAL CLINICAL AND GENETIC FEATURES IN A PATIENT WITH MUSCLE PHOSPHOFRUCTOKINASE DEFICIENCY

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Muscle phosphofructokinase deficiency is a metabolic myopathy characterized by early muscle fatigue, proximal weakness, compensated hemolytic anemia and hyperuricemia. We report on a 45 year-old man who, since he was 25, complained of diffuse myalgias, vomiting and myoglobinuria after physical exercise. Often hospitalized because of high serum CK levels (160.000 IU/l). He also had a mild cardiopathy with hypertrophic ventriculi. Neurological examination was unremarkable. Family history was negative for neuromuscular disorders but his 15 year-old son had slightly elevated serum CK (600 IU/l). Laboratory tests revealed mild reticulocytosis 2% (v.n.0-1.5 %), increased serum bilirubin 1.8 mg/dl, (n.v. 0.4-1) and hyperuricemia 8.5 mg/dl (n.v. 5-7). Ischemic forearm test showed a normal lactate rise. Muscle biopsy failed to show glycogen storage and biochemical studies revealed a 4% PFK residual activity. Direct sequencing of PFK entire coding region evidenced two mutations in heterozygosity, one point mutation A to C determining an aminoacid change D591A, located in a quite conserved protein region, the second one (reported in an Italian patient) was a IVS6-2A/C. His three brothers and one son were heterozygous for the point mutation D591A whereas the son with hyperCKemia harbored only the IVS6-2A/C in heterozygosity. Absence of chronic muscle weakness and muscle glycogen storage as well as normal lactate rise are quite unusual features

of a long-standing PFK deficiency, but biochemical deficiency was confirmed by presence of two mutations in heterozygosity.

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[63] INCLUSION BODY MYOSITIS AND HCV INFECTION

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The association of chronic HCV infection and immunomediated disorders is firmly established; several reports describe co-occurrence of myositis and HCV infection, whereas cases of Inclusion Body Myositis (IBM) are very rare. A seventy-one-year-old man got HCV infection (HCV-RT-PCR: 2a/c genotype, 7.2x10<sup>5</sup> viral load) and presented with mild chronic hepatitis at the age of fifty-six; he has never been treated by interferon. He was referred to neurological evaluation because of one-year-history of dysphagia, muscle weakness and CK increase. Physical examination showed weakness of neck, distal upper limbs (MRC 3 on forearm) and proximal-distal lower limbs (MRC 4) muscles. Electrodiagnostic examination revealed a myogenic-neurogenic pattern and mild axonal impairment of peroneal nerves. Rheumatoid Factor, anti-peroxidase, anti-microsome, anti-tyreoglobulin, ANA, ENA, anti-mitochondria, LK-Ab, C3, C4 were in normal range and cryoglobulins were negative. Vastus lateralis muscle biopsy showed endomysial inflammation, rimmed vacuoles and eosinophilic inclusions consistent with IBM. Immunohistochemistry for HCV non-structural protein NS3 showed reactivity of some infiltrating cells; no stain was observed within muscle fibres. In HCV-associated myositis, including the previously reported five cases of HCV-associated IBM, published data are not univocal regarding immunocytochemical and PCR detection of HCV within skeletal muscle. A direct role of HCV is thus not demonstrated, but its systemic modulatory immune hies.effects might contribute to pathogenesis of different inflammatory myopathies.

[64] AUTONOMIC NERVOUS SYSTEM IMPAIRMENT IN  
MITOCHONDRIAL DISEASES

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Autonomic dysfunction including gastrointestinal dysmotility, cardiac arrhythmias, altered sweating and postural hypotension has been rarely reported in mitochondrial cytopathies (MC). The aim of our study was to evaluate autonomic control on cardiovascular system in a group of patients with different mitochondrial disorders. 21 subjects, aged 15-75 years, free from diabetes, severe cardiac impairment or diseases compromising autonomic function were investigated as well as 20 sex and age-matched controls. A battery of cardiovascular reflex tests including beat-to-beat variability during quiet and deep breathing, heart

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rate (HR) responses to Valsalva manoeuvre and standing, and blood pressure responses to cold stimulus, mental arithmetic and sustained handgrip were carried out. Power spectral analysis (PSA) of HR variability was performed. Plasma catecholamines levels were detected while supine and after 10 minutes head-up tilt. Four patients (19%) showed normal autonomic function, seven (33%) evidenced borderline autonomic impairment and ten (48%) showed definite autonomic damage. Results showed parasympathetic dysfunction in 2 patients, sympathetic dysfunction in 7 patients and a mixed impairment in 8 patients. PSA of HR variability mean values showed a lower lying high frequency (HF) band in MC ( $p < 0.02$ ). Adrenaline plasma rise from supine to standing position was reduced ( $p < 0.03$ ). These data suggest that an impaired autonomic control of cardiovascular system is not rare in MC patients mainly affecting the sympathetic nervous system.

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