

Abstracts

[1] [ORAL COMMUNICATION]

IN VIVO MODELS OF MITOCHONDRIAL DISORDERS

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In vivo models are useful tools to investigate the function of disease genes, help understand the pathogenesis of the corresponding defect, and evaluate the efficacy of treatment. We have been studying three models of mitochondrial disease, namely yeast mutant models for the AAC system, which is ortholog to that of the adenine-nucleotide transporters (ANT) in humans, and mouse and fly mutant models for Surf1, a COX assembly protein. Mutations in ANT1 are responsible for one form of autosomal dominant progressive external ophthalmoplegia associated with the accumulation of multiple mtDNA deletions. Mutations in Surf1 are responsible for the most common form of Leigh syndrome associated with defective COX activity.

We created a yeast recombinant system expressing mutant versions of AAC2, homologous to the ANT1 mutations found in human patients. Three new mutations previously identified in hANT1 were considered. Since the three wild-type amino acid residues are not conserved between yeast and humans, we first replaced specific amino acid residues of Aac2p with the corresponding amino acids of wt Ant1p. The mutant alleles were able to complement the respiratory defect of both AAC-defective strains. However, the introduction of changes equivalent to potentially pathogenic human mutations caused the generation of OXPHOS defective strains. These experiments demonstrate that yeast mutants reflect faithfully the effects of human mutations and can be used to validate their pathogenicity.

To better understand the role of Surf1p and the pathogenesis of LSCOX, we created a constitutive KO mouse model for Surf1. The proportion of Surf1^{-/-} pups was tenfold lower (2.7%) than that expected by mendelian transmission of a recessive trait (25%). This result indicates that the Surf1^{ko} allele had a recessive phenotype, which was lethal in most of the embryos. A total of 34/1236 live pups had the Surf1^{-/-} genotype. In these animals, COX was the only defective enzyme in several tissues, including skeletal muscle, which presented a mitochondrial myopathy with COX-depleted fibers. A significant, consistent reduction in the motor performance of the Surf1^{-/-} mice. However, no evidence for neurodegeneration was detected in Surf1^{-/-} CNS specimens.

We also established transgenic lines for the generation of conditional functional KO via dsRNA interference in *D. melanogaster*. Surf1 dsRNAi constitutive flies die as larvae, which are sluggish and have impaired development. Some larvae can reach the pupal stage but do not progress any further in development. The neurophysiological, cellular and molecular characterization of this line is currently under way.

[2] [POSTER]

EXPRESSION OF THE TRANSCRIPTION FACTOR NF-κB IN DIFFERENT MUSCULAR DYSTROPHIES

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NF-κB is a major transcription factor modulating the cellular immune, inflammatory and proliferative responses. It plays several roles in skeletal muscle but its involvement in neuromuscular disorders is poorly studied. We recently demonstrated the activation of NF-κB pathway in Duchenne muscular dystrophy and inflammatory myopathies. In order to evaluate a possible role of NF-κB in other muscle disorders, we investigated its activation in muscle biopsy specimens from 16 patients with different muscular dystrophies (6 pts with myotonic dystrophy-DM1, 5 pts with FSHD and 5 pts with LGMD2B). NF-κB DNA-binding activity, studied by electrophoretic mobility shift assay, was variably increased in DM1 ($x \pm SD$ of integrated intensity: 49.5 ± 17), LGMD2B (41 ± 6) and FSHD (16.6 ± 12) patients. The latter group, including 3/5 patients with results in the normal range, revealed a significantly lower mean value than DM1 and LGMD2B groups (respectively, $p < 0.0048$ and $p < 0.0034$). Our data suggest that NF-κB pathway may play a pathogenic role in DM1 and LGMD2B, being involved in the response to oxidative stress in the former and in the regulation of inflammation in the latter.

[3] [ORAL COMMUNICATION]

EFFECTS OF STEROID THERAPY ON LUNG VOLUMES IN DUCHENNE MUSCULAR DYSTROPHY

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Ventilatory failure is the major cause of morbidity and mortality among Duchenne Muscular Dystrophy (DMD) patients and is characterized by a progressive decline in Forced Vital Capacity (FVC), consistently with age. To date, steroid therapy has been proven to be effective in improving or stabilizing functional status in DMD for a limited period of time,

but data concerning its efficacy on preventing or delaying pulmonary function deterioration are still lacking. Aim of our study has been to evaluate the effect of steroid treatment on the course of pulmonary volumes in a group of DMD patients.

83 DMD patients were submitted to Pulmonary Function Test (PFT) between 1991 and 2003: 27 patients had received steroid treatment (deflazacort or prednisone) for at least one year (group A) and 56 were in natural history (group B). Respiratory function data were retrospectively evaluated and compared between group A and B; FVC, FVC % predicted, FEV1 and FEV1% predicted were considered. Student's T test was used to compare continuous variables.

81 PFT were available from group A and 255 from group B. Patients of group A between the ages of 11 and 14 yrs showed significantly greater values of FVC% and FEV1% (64.6 ± 21.9 vs 49.1 ± 22 $p < 0.01$; 65.4 ± 26.7 vs 50.9 ± 26.6 $p < 0.05$); older subjects (age: 17-18 yrs) also showed greater FVC and FEV1 absolute values (2.46 ± 1.08 L vs 1.21 ± 0.66 L $p < 0.01$; 2.2 ± 1.07 vs 1.15 ± 0.64 L; $p < 0.001$).

Our data on a large number of DMD patients suggest that an early administration of steroid treatment is effective in delaying the decline of pulmonary volumes; because the onset of ventilatory failure is related to the course of FVC, steroid therapy could be a factor contributing to significant improve morbidity and mortality in DMD boys.

[4] [LECTURE]

MOLECULAR MECHANISMS UNDERLYING CONGENITAL MYASTHENIC SYNDROMES

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Congenital myasthenic syndromes (CMS) stem from genetic defects that affect transmission of information from nerves to muscles at the neuromuscular junction and result in fatiguable muscle weakness. They comprise a disabling – and sometimes potentially lethal – set of disorders with subtly different clinical features. Molecular genetic analysis of the CMS has defined defects in pre-synaptic, synaptic and post-synaptic proteins, with the majority located in postsynaptic acetylcholine receptor (AChR) and its associated protein rapsyn. Functional analysis demonstrates diverse molecular mechanisms through which AChR mutations cause disease. The slow channel syndrome is usually a dominantly inherited disorder in which a single missense amino acid substitution causes prolonged activations of the AChR ion channel and leads to excitotoxic cell damage. The fast channel syndrome shows recessive inheritance and is due to mutations that cause shorter and less frequent ion channel activations. AChR deficiency syndrome shows recessive inheritance with onset in infancy. At patient endplates, the AChR is reduced in number and density, and distributed over an increased area of the postsynaptic membrane. This condition is frequently caused by mutations in the AChR ϵ subunit gene, but in about 25% AChR deficiency

patients, mutations in the AChR genes are not detected. Recently it has been shown that many of these patients have mutations in rapsyn, which plays a key role in clustering AChR at the neuromuscular junction. The mutations are located in various domains within rapsyn and may effect AChR clustering through different molecular mechanisms.

[5] [LECTURE]

COLLAGEN TYPE VI AND RELATED DISORDERS: BETHLEM MYOPATHY AND ULLRICH SCLEROATONIC MUSCULAR DYSTROPHY.

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Collagen type VI is a glycoprotein made up of three genetically distinct α chains, $\alpha 1(VI)$, $\alpha 2(VI)$ and $\alpha 3(VI)$, which assemble in a short central triple helix structure that joins two large globular domains. The heterotrimeric monomers associate in dimers and then in tetramers, intracellularly. Tetramers are secreted out of the cell into the extracellular matrix to assemble in an end-to-end fashion to form microfibrils that are characterized by a band of periodicity of 100 nm. Collagen type VI is ubiquitously distributed in the connective tissues and is particularly abundant around cells, associated with interstitial collagen fibers types I-III, with a possible role as substrate for the attachment of cells and in anchoring collagen fibers, nerves and blood vessels to the surrounding connective tissue.

The three chains are encoded by COL6A1, COL6A2 and COL6A3 genes; the first two are localized on chr. 21q22.3 while the latter is located on chr. 2q37. Between 1996 and 1998 the three genes were found to be associated to Bethlem myopathy, an autosomal dominantly inherited myopathy. The association demonstrates the tissue specific importance of collagen type VI for skeletal muscle. More recently, a second disease, Ullrich scleroatonic congenital muscular dystrophy, has been demonstrated to be associated to COL6A genes by the discovery of recessive mutations in patients and their families.

[6] [POSTER]

ATYPICAL LEIGH SYNDROME ASSOCIATED WITH THE D393N MUTATION IN THE MITOCHONDRIAL ND5 SUBUNIT

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Leigh syndrome (LS) is a neurodegenerative disorder usually starting before one year of age and leading to death within months or years. In most patients, LS is caused by defects of mitochondrial oxidative phosphorylation (OXPHOS), the most common involving pyruvate dehydrogenase complex (PDH), cytochrome c oxidase (Complex IV), and NADH-Ubiquinone oxidoreductase (Complex I). Recently, the 12706T>C and the 13513G>A mutations in the ND5 gene, one of seven subunits of complex I encoded by mitochondrial genome (mtDNA), were identified in 4 LS patients. Here, we report on an additional 4-year-old child harboring the 13513G→A mutation with a LS. He started with ataxia and ptosis at age 12 months. Serum lactate was elevated (4.1 mmol/L, normal values <2). Brain MRI showed hyperlucencies in the hypothalamus, the periaqueductal midbrain, the dorsal mesencephalon, and substantia nigra in T₂-weighted images and in FLAIR, suggesting a diagnosis of LS. Spectrophotometric analyses of OXPHOS complexes showed a moderate reduction of the activity of complex I in muscle (25% of normal values) and in cultured skin fibroblasts (30% of normal). By PCR-RFLP analysis, we quantitated the mutation in tissues from the patient (75, 82, and 70% mutant genomes in skin fibroblasts, skeletal muscle and in peripheral leukocytes) and in blood from his healthy mother (4%) and sister (2%).

Review of the recent literature on LS + complex I deficiency, and the 13513G→A mutation shows that hyperlucencies in the hypothalamus, medial thalamic nuclei, periaqueductal midbrain, and substantia nigra are quite characteristic for this mutation or for LS with complex I deficiency of other origin.

[7] [ORAL COMMUNICATION]

FKRP GENE MUTATION STUDY IN MUSCULAR DYSTROPHY OF UNKNOWN ETIOLOGY

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The fukutin-related protein (FKRP) gene is mutated in Limb girdle muscular dystrophy 2I (LGMD2I). It has been suggested that FKRP may be involved in the glycosylation of alpha-dystroglycan and alpha-dystroglycan deficiency has been reported in LGMD2I.

Two hundred and fourteen patients, who showed muscle histopathology consistent with a muscular dystrophy or myopathy with normal dystrophin, alpha sarcoglycan, calpain and dysferlin, were screened for FKRP gene mutations. We analysed the entire 1.5 kb FKRP coding sequence from patient genomic DNA by single strand conformational polymorphism (SSCP) or denaturing high performance liquid chromatography (DHPLC) of overlapping PCR product in order to identify causative FKRP mutations. Five FKRP missense mutations were identified. Three of the five mutations were novel. Twenty three of the 214 (11%) patients presented a FKRP missense change. In 18 patients both FKRP mutated alleles were identified, and in 5 only one. In 19 patients a C826A missense change was found: 16 patients were homozygous and 3 compound heterozygous for the mutation. In ten of the twenty three FKRP mutation-positive patients, alpha-dystroglycan immunofluorescence was performed and showed a severe reduction of the protein in their muscle biopsies. Clinical presentation in our cohort of patients included proximal muscle weakness, calf hypertrophy, and dilated cardiomyopathy. Disease severity and clinical progression was variable.

In conclusion FKRP gene mutations are responsible of a sizeable portion of LGMDs. In the LGMD2I the C826A missense change appears a common mutation.

[8] [POSTER]

CONGENITAL MUSCULAR DYSTROPHY AND SYRINGOMYELIA: CASUAL ASSOCIATION?

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Congenital muscular dystrophies (CMD) are a heterogeneous group of muscular diseases, with autosomal recessive inheritance, characterized by onset within 6 months of life, marked weakness, generalized hypotonia, joint contractures and, in some cases, CNS (central nervous system) involvement. Syringomyelia (SY) is defined as a tubular cavitation

within the spinal cord. This condition is sporadic, although occasional familial forms have been reported. The disease is rarely symptomatic in children, although symptoms have been observed as early as 2 years of age. In the present study we have evaluated 2 children with merosin positive CMD that have been observed in Neuropediatric Division. In these patients the diagnosis has been made by muscle biopsy showing a typical dystrophic without a muscular reduction of merosin. Cord MRI has revealed for both a thoracic SY without white matter abnormality. Scoliosis was evident in only one patients. The SY has not never considered a diagnostic criteria for CMD. We believe that the association CMD and SY could be not casual and who manifest skeletal abnormalities must be performed in addition to brain a cord MRI.

[9] [POSTER]

THE IMPORTANCE OF EARLY STEROID TREATMENT IN DUCHENNE MUSCULAR DYSTROPHY.

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Steroid administration has been shown to be beneficial in Duchenne Muscular Dystrophy (DMD) by improving muscle strength and functional score. The action of steroids in inhibiting fibrous substitution of the muscles may be more efficacious when the necrosis is maximal. We analysed the effect of an earlier beginning of steroid therapy in DMD.

We screened our DMD database for all patients treated with steroids (prednisone 0.75 mg/Kg/die and deflazacort 0.9 mg/Kg/die) for at least one year. We correlated the age at the beginning of therapy either with a short-term parameter (change in functional score after 12 months) or with a long-term parameter (loss of ambulation). Clinical data included the monitoring of side effects. The Spearman correlation coefficient was used in the statistical analysis. Fifty-six patients treated with steroids were available for the analysis of functional score (FS) after 12 months of therapy. The mean change in the FS was -5.74% (SD ±21.7), with a range of -55% - +50%. Half of the patients showed an improvement. There was a statistically significant correlation between the age of onset of therapy and the improvement in FS after 12 months ($r = 0.52$, $p < 0.0001$). Forty-one treated patients lost ambulation. The age at loss of ambulation correlated slightly with the duration of therapy ($r^2 = 0.15$, $p = 0.01$) whilst a stronger correlation was found with the age at the beginning of therapy ($r^2 = 0.26$, $p = 0.0006$). No dropout for steroid side effects was seen.

Our data on a large cohort of DMD patients suggests that an early beginning of steroid therapy may positively affect the short-term improvement and the loss of ambulation in DMD. Side effects do not appear to be a limiting factor.

[10] [POSTER]

CONSENT FOR SPORT PRACTICE IN SUBJECTS WITH HYPERCKEMIA: A CASE REPORT WITH 3 YEARS FOLLOW-UP

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Sport activity is the best contest for the healthy growth of youngsters and it should be always encouraged: but is difficult give a clear response in subjects with border-line conditions, like hyperckemia at rest.

The patient was a white male of 14 years old with a small Ventricular Septal Defect (VSD), that in November 2000 underwent to a preparticipation screening for basket. The first checking of hyperckemia was at 5 years old during a surgery and the same target was found the following years especially after physical effort. The boy referred a training of 6 hours/week from the last 6 years and complained for weakness and muscular pain post exertion. The physical examination showed normal chest, backbone in axis, light winged shoulders and normal muscular mass. The boy underwent further to the screening at the age of 15 and 16 and he gave the same anamnestic informations and the finding of hyperckemia at rest and after training, while the echocardiography didn't show the VSD anymore. Every time the patient was submitted to a maximal stress test with cycloergometer (increase of 25 watt every 2 minutes) and a blood sample for CK, LDH and isoenzymes was taken at 5', 30', 6h, 24h, 48h post stress. Then we evaluate the test he made in three years, and we observed a progressive increase in watt reached at the top (125 watt the 1st year, 175 watt the 2nd, 225 watt the 3th year) and a decrease of HR at rest (74 bpm the 1st year vs 60 bpm the 2nd and the 3th); CK continues to be high even at rest and post stress, while LDH had a values progressively lower. The decrease of LDH rest values (341 vs 228 vs 210 U/l) bound to a decrease of LDH4 and LDH5 and an increase of LDH1 and LDH2 give evidence of adaptation to the work. In this case sport activity doesn't seems to be bad for the subject, that in spite of hyperckemia at rest, have had the right response to training. The consent for sport activity has been given more for psychological benefits bound to it: is however important studying always cardiovascular apparatus because of the risk of latent cardiomyopathy.

screening the entire coding region of *DYSF*, we identified three novel mutations (two missense substitutions and one frame shift microdeletion). The possible existence of a founder effect for the Arg959Trp mutation in the Italian population is discussed.

[14] [POSTER]

A COMPLEX REARRANGEMENT IN THE *DMD* GENE DETERMINES NEW EXON INCLUSION AND BMD PHENOTYPE.

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Duchenne and Becker Muscular Dystrophy (DMD and BMD) are both caused, in the majority of cases, by deletions in the dystrophin gene (*DMD*). A good correlation has been noticed between the severity of the disease and the effect of the deletion on the reading frame: reading frame-disrupting mutations are usually associated with a severe DMD phenotype, whereas in-frame deletions determine a BMD clinical status. However exceptions to the reading frame rule are found in about 8% of patients. Here we describe the unprecedented case of a BMD patient carrying a large out-of-frame intragenic deletion together with an inversion in the *DMD* gene, resulting in the inclusion of a novel exon in the transcript. The patient carried a 48-52 exon deletion, yet skeletal muscle transcript analysis revealed the presence of two unexpected products neither of them including exon 53. The shorter product derived from the juxtaposition of exons 47-54; this in-frame messenger might account for the patient's BMD phenotype. The longer transcript resulted from the inclusion of 73 bp between exons 47 and 54. Sequence analysis revealed that the 73 bp sequence derived from intron 53 but was present in an inverse orientation; its inclusion is predicted to determine protein truncation. These observations are consistent with the presence of a genomic inversion involving exon 53 and flanking regions. The inverted 73 bp sequence displays splicing signals at both ends and thus it is probably recognized as a novel exon when the partially inverted hnRNA is processed. Indeed, the presence of a 5 kb inversion was confirmed at the DNA level;

To our knowledge this is the first reported patient carrying both an intragenic deletion and inversion in the *DMD* locus. This case might provide further insight into both the mechanisms that determine genomic rearrangements in the *DMD* locus and the molecular signals that drive exon inclusion.

[15] [POSTER]

CHOLESTEROL AND ACTIN CAN INFLUENCE CAVEOLAR DYSFERLIN LOCALISATION IN C2C12 MYOTUBES

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Dysferlin is a membrane protein of skeletal muscle whose deficiency causes Miyoshi myopathy (MM), limb girdle muscular dystrophy 2B (LGMD2B) and distal anterior compartment myopathy. The function of dysferlin is not defined but recent studies have reported that dysferlin coimmunoprecipitates with caveolin 3. Caveolin 3 is a principal structural protein of caveolae membrane domains in striated muscle cells and cardiac myocytes. Caveolae are invaginated plasma membrane domains enriched in cholesterol and glycosphingolipids, characterized by triton X-110 insolubility. Mutations of caveolin 3 gene cause different diseases: limb girdle muscular dystrophy 1C, asymptomatic hyperCKemia, rippling muscle disease and a peculiar form of distal myopathy. In limb-girdle muscular dystrophy 1C, where caveolin 3 expression is defective, skeletal muscle shows a severe impairment in caveolae formation and disorganization of T-system, moreover, dysferlin localisation is abnormal. Dysferlin is expressed in C2C12 myotubes and immunoprecipitation technique performed in these cells shows a low affinity link with caveolin 3. We report a study on dysferlin in C2C12 myotubes after induction of caveolae structural alteration by means of cholesterol depletion and actin disorganization. We performed Western blotting analysis of Triton X-110 C2C12 myotubes extracts, and immunohistochemistry in C2C12 treated myotubes in order to clarify whether other molecular components of caveolae are implicated in dysferlin membrane distribution. Western blotting analysis showed dysferlin presence both in Triton-insoluble pellet and in the soluble fraction. These findings show that dysferlin is associated not only with caveolae insoluble fraction but also with other membrane domains. Cholesterol is pivotal for the structural integrity and function of caveolae, the depletion of this lipid produces alterations in caveolae distribution. In the present study we used a cholesterol disrupting agent to explore the role of this lipid in dysferlin localisation in caveolae; the agents chosen were Amphotericin B and Methyl- β -cyclodextrin. Amphotericin B is a polyene antibiotic that binds cholesterol and causes a dramatic redistribution of caveolae. Immunohistochemistry of C2C12 myotubes treated for different time with different drug concentrations showed redistribution of caveolin 3 in endosome compartment without any variation in dysferlin and caveolin 3 triton X-110 solubility, as detected by western blotting analysis. On the contrary, immunohistochemistry of caveolin 3 in C2C12 myotubes treated with Methyl- β -cyclodextrin showed a clustering of caveolae on plasma membranes together with

increase of dysferlin solubility in Triton X-110. Interestingly, cyclodextrin is not only a cholesterol disrupting agent, but it also disorganises cortical actin. To test the possible implication of actin on dysferlin localisation in caveolae we treated C2C12 myotubes with cytochalasin D, which partially disassembles actin filaments. Immunohistochemical analysis of caveolin 3 in cytochalasin D-treated C2C12 myotubes showed a typical clustering of caveolae, and Western blotting analysis of triton X-110 extracts showed a severe increase of dysferlin solubility. To test a possible relation between dysferlin and actin in C2C12 myotubes we performed immunoprecipitation experiments: when myotubes were lysed in 1% triton X-110 anti-dysferlin antibody co-immunoprecipitated actin. Taken together these results suggest a possible interaction between dysferlin and others molecular component of caveolae.

[16] [POSTER]

PATHOGENETIC MECHANISMS OF MYOTONIA IN STEINERT DISEASE: A PHARMACOLOGICAL STUDY

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Several approaches have been in the past used to identify the electrical sarcolemmal alterations responsible for myotonic phenomenon in Myotonic Dystrophy (MyD). Controversial results have been obtained. The first *in vivo* evidence of ionic channels functional alteration in MyD was suggested after the observation that treatment of myotonic muscles with apamin, a peptide from bee venom that specifically blocks Ca²⁺-activated K⁺ channels (SK), reduced "myotonic runs" recorded by needle EMG (Behrens et al., 1994). Very recently, the average rectified value of the compound muscle action potential (ARV), recorded by means of surface EMG, has been used to study, *in vivo*, the sarcolemmal excitability in MyD patients (Chisari et al., 2001). The aim of this study was to evaluate the effect of the local injection of 50 µl of apamin on MyD surface EMG pattern. We tested 8 patients through an experimental protocol consisting of a stimulated contraction, at 15 Hz and lasting 11 secs, of tibialis anterior muscle before and after the administration of 50 µl of apamin. The ARV trend was used to assess the sarcolemmal excitability modifications.

The results obtained showed that the local administration of apamin doesn't significantly modify, during a sustained contraction, the electrical muscle activity in MyD patients.

This work ruled out the hypothesis that SK could play a specific role in the genesis of MyD sarcolemmal excitability alterations and confirms the difficulty to identify a specific mechanism responsible for myotonic phenomenon in these patients.

[17] [ORAL COMMUNICATION] FUNCTIONAL CHARACTERISTICS OF SKELETAL MUSCLE IN α-SARCOGLYCAN-DEFICIENT MOUSE

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α-Sarcoglycan (aSG) is a transmembrane glycoprotein, component of the dystrophin complex, and its absence produces in man a severe form of limb-girdle muscular dystrophy. This study was undertaken in a α-sarcoglycan-deficient (aSG-null) mouse to evaluate whether the absence of the protein from sarcolemma affects the functional properties of slow- (soleus) and fast-twitch (EDL) skeletal muscles. At three months of age, the majority of fibers from soleus and EDL display centrally located nuclei. Muscle mass of both soleus and EDL is significantly higher than controls. However, hypertrophic EDL muscle shows peak tetanic tension (P₀) values lower than control levels and the resultant relative P₀ is significantly reduced. At variance, hypertrophic soleus muscle had values comparable to controls with a resultant specific force not different from control. The initial maximal velocity of rise of tetanic tension is reduced in EDL and unchanged in soleus. The force-frequency relationship is only slightly changed in soleus, whereas it is highly shifted to the left in EDL, an indication of slowing (it should be associated to the increase of CT, however not found), similarly to what was reported in the *mdx* mice. Fatigability is also significantly changes in EDL muscle, whereas no modifications occur in soleus. In fact, EDL presents an initial tension potentiation not seen in control. At variance, SDH analysis shows that the oxidative capacity of EDL and soleus muscles is comparable to control.

Fiber type composition, as determined by immunofluorescence and SDS-PAGE analysis of MHC isoforms expression, demonstrates in 3-month old aSG-null EDL the rise of type 2X-2B at detriment of type 2B fibers, the significant reduction of type 2A fibers and, similarly to control, very rare type 1 fibers. At variance, in soleus muscle the main difference is the significant expression of embryonic MHC, not detected in the EDL, and the rise of type 2X MHC. The expression level of sarcoplasmic reticulum Ca²⁺-pump (SERCA) isoforms was also investigated. While EDL muscle expressed the SERCA1 isoform only both in control and dystrophic muscle, in soleus both isoforms, SERCA1 and SERCA2 were present. The study extended the analysis in single fibers, identified by the expressed MHC isoforms. First, several dystrophic fibers show the contemporary presence of hybrid myosins, a picture noted also in few control fibers. Ca²⁺ sensitivity of myofibrillar proteins, determined by pCa-tension relationships, was apparently not different in EDL and soleus dystrophic fibers in

respect to control. Caffeine sensitivity of sarcoplasmic reticulum Ca^{2+} release caffeine was reduced in EDL (from 3.85 to 2.96) and significantly increased in soleus (from 2.50 to 4.29). Several studies suggest that the dystrophin complex is critical for structural integrity of the myofiber plasma membrane. Muscle physiology studies show that changes in muscle structure and function, downstream of the specific, primary biochemical deficiency, alter muscle contractile properties. In the α SG-null mouse, fast-twitch muscles appear to be significantly more affected than slow-twitch muscles.

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[18] [ORAL COMMUNICATION]
MYOGENIC ATROPHY IN ACUTE QUADRIPLEGIC MYOPATHY (AQM) IS SPECIFICALLY ASSOCIATED WITH TRANSCRIPTIONAL ACTIVATION OF PRO-APOPTOTIC MAPK CASCADE

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AQM is characterized by sub-acute muscle weakness and atrophy in patients with severe systemic illness and exposure to corticosteroids or neuroblocking agents. Myofilaments degradation and selective myosin loss are the main pathologic signs. We have previously shown apoptotic features and proteases activation in atrophic myofibers of these patients (Neurology 55, 854-858, 2000). Nevertheless, the specific chain of molecular events leading to muscle atrophy is not clear yet. We compared gene expression profiling studies, using Affymetrix high-density oligonucleotides arrays, of 5 AQM atrophic muscles, 5 neurogenic atrophies and 8 normal muscles. Confirmatory quantitative RT-PCR and protein analysis of selected transcripts were also performed. We found in AQM muscles a specific activation of MAPK cascade triggering RAS family members transcription followed by MAPKK, MKK and JNKK up-regulation and terminal downstream c-jun and c-fos mediated pro-apoptotic execution. Protein quantitation and phosphorylation states of key components of this pathway were also determined by immunoblotting and immunocytochemistry. This pathogenic cascade is activated by metabolic imbalance and osmolar stress, that are main features in AQM patients, and by oxidative stress, that we have documented by the strong up-regulation of MnSOD, HO-1 and catalase mRNA expression.

In conclusion, we demonstrated that TGF beta-MAPK signaling cascade is specifically activated in myogenic atrophy in AQM and it is regulated at both the transcriptional and protein level through gene expression and protein phosphorylation. This pathway is involved in cell cycle arrest, apoptosis and atrophy. Accordingly, key elements of the cascade are expressed specifically in atrophic fibers with apoptotic features.

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[19] [ORAL COMMUNICATION]
FUNCTIONAL IN VITRO GENE TRANSFER IN ADULT SKELETAL MUSCLE FIBERS

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The postmitotic nature and longevity of myofibers permits the stable expression of transfected genes. The differentiated muscle cells, *in vitro*, are refractory to standard protocols of gene transfer. We describe a new electroporation approach, which allow efficient transfection of adult myofibers *in vitro*. Adult fibers were dissociated from mouse flexor digitorum brevis (FDB) muscles and cultured 24-48 h before gene transfer and confocal microscopy imaging analysis. Muscle fiber transfection was performed by using the 0.5 cm large spatula-like electrodes placed at each side of the cultured adult fibers. Three pulses with a fixed pulse duration of 20 ms and an interval of 200 ms were applied. The ratio of applied voltage to electrode distance was 45 V/cm and 50 µg DNA in 50 µl of 0.9 % NaCl were used. Under the conditions described in methods section, 3-20 % of cultured adult FDB fibers were transfected. The subcellular distribution of transfected gene products was evaluated by immunofluorescent confocal microscope. Snap25GFP showed a typical membrane distribution being mainly localized at sarcolemma membrane. In addition, when mitochondria were stained with TMRM, cytochrome c-GFP co-localized with the TMRM staining. Both snap25- and cytochrome c-products were expressed in the expected position all along the entire. We evaluate whether the correct localization of cytochrome c-GFP to *in vitro*-transfected FDB fibers was associated with a functioning protein, treating transfected fiber with apoptotic stimuli. Treatment of FDB fibers with staurosporine caused the progressive loss of cytochrome c-GFP. We demonstrated that the addition of cyclosporin A, a permeability transition pore inhibitor, completely blocked cytochrome c release. Intracellular concentration of Ca^{2+} started to progressively rise in parallel to cytochrome c release. Colocalization of cytochrome cGFP/cleaved-caspase 3 revealed that activation of caspase3 occurs where cytochrome c was released from mitochondria. Electron microscopy of a 12h staurosporine treated myofiber confirmed that mitochondrial alterations occurred in a limited number of mitochondria that are placed between myofibrils.

Thus, present work demonstrates the functional *in vitro* electrotransfer of genes in adult skeletal muscle fibers by a protocol that would be of a great potential for basic research, as well as for gene therapy.

[20] [POSTER]

DETRIMENTAL "IN VIVO" EFFECTS OF OXIDATIVE STRESS IN PATIENTS WITH MYOTONIC DYSTROPHY

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In myotonic dystrophy (MD), despite knowledge on pathogenic CTG repeat expansion mutation in the 3' untranslated region of the myotonin protein kinase gene, the mechanisms by which gene alteration causes involvement of different tissues are still largely obscure. Some evidence has been provided that expanded CTG repeats in myotonin protein kinase increase susceptibility to oxidative stress, and that the effects of this mutation are prevented by thiol antioxidants and by trolox, a vitamin C analog.

In the present study we have analyzed serum gamma-glutamyl transpeptidase (GGT), its fraction bound to low density lipoproteins (LDL-GGT), and advanced oxidation protein products (AOPP) in plasma from 22 patients with myotonic dystrophy and 15 control subjects. The results show significant ($p < 0.01$) increment of GGT, LDL-GGT (27 mU/ml vs. 3 mU/ml), and AOPP (241 $\mu\text{mol/l}$ vs. 127 $\mu\text{mol/l}$) in patients compared to controls, directly related to the severity of the disease. Gamma-glutamyl transpeptidase, an enzyme endowed with a crucial role in GSH catabolism, has been shown to trigger oxidative damage to LDL, thus leading to lipid peroxidation and release of lipid-derived aldehydes. Though the elevated levels of serum GGT in MD are a well known aspect of the disease, this is the first demonstration of increased LDL-GGT in MD. The increased level of AOPP, a selective index of oxidative damage to proteins, demonstrates that oxidative damage occurs "in vivo" despite the natural antioxidant properties of plasma, thus confirming the hypothesis that enhanced oxidative stress can have a causal role in the pathogenesis of MD.

[21] [POSTER]

BEHAVIOURAL PROBLEMS IN DUCHENNE/BECKER MUSCULAR DYSTROPHY PATIENTS

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Duchenne (DMD) and Becker (BMD) muscular dystrophies are allelic diseases characterized by progressive degeneration of muscular tissue without central nervous system (CNS) involvement. The aim of the study is to investigate behavioural adjustment in 30 DMD and BMD patients. 20 patients with DMD and 20 with BMD (all males with mean age 16 years 6 months, range 4 to 18 years) and 20 normally developing chil-

dren (males with mean age 15 years 4 months, range 4 years 18 months) were recruited as control participants. In all patients IQ has been evaluated by WISC. Behavioural symptoms were measured with Child Behaviour Checklist (CBCL). Of the patients with DMD/BMD 22,5% fulfilled the criteria for DSM-IV with separation anxiety disorder being the most common diagnosis. The CBCL total score was in the clinical range for 18% of the patients, 11% of the control children. The externalizing score rates showed a higher score for BMD patients than DMD and control children.

In conclusion patients with BMD have a highest rate of behavioural problems characterized by separation anxiety disorders and this could be the clue that for the patients with less muscular involvement there are more chances of extramuscular involvement.

[22] [POSTER]

LOSS OF CALPAIN-3 CATALYTIC ACTIVITY IN LGMD2A PATIENTS WITH NORMAL PROTEIN EXPRESSION

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The diagnosis of limb girdle muscular dystrophy (LGMD) type 2A, due to mutations in the gene encoding for calpain-3, is currently based on protein analysis, but mutant patients with normal protein expression have been also identified. In this study we investigated 150 LGMD patients with normal calpain-3 protein expression, to identify gene mutations by allele-specific PCR tests, and subsequently analyzed the proteolytic activity of mutant calpain-3. Four mutations were found in 8 patients (5.5%): a frame-shifting deletion (550 A del.) and 3 missense (R490Q, R489Q, R490W). Patients with calpain-3 gene mutations and normal calpain-3 protein expression on western blot are a considerable proportion (20%) of our total LGMD2A population. While in control muscle the calpain-3 Ca^{++} -dependent autocatalytic activity was evident within 5 minutes and was prevented by EDTA, in all mutant patients' samples the protein was not degraded, indicating that the normal proteolytic function had been lost. By this new functional test, we show that conventional protein diagnosis could fail to detect some mutant proteins, and prove the pathogenetic role of R490Q, R489Q, R490W missense mutations. We suggest that these mutations might impair catalytic activity either by affecting interdomain protein interaction, or by reducing autocatalytic activity lowering the Ca^{++} sensitivity. In the future diagnostic procedure of LGMD, the proteolytic activity of calpain-3 could be tested by this biochemical assay to study the function of the protein in those patients with a phenotype compatible with LGMD2A who present a normal calpain-3 protein expression on western blot.

[23] [ORAL COMMUNICATION]
PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA AND
POLG MUTATIONS: CLINICAL AND GENETIC HET-
EROGENEITY

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Autosomal progressive external ophthalmoplegia (PEO) with mitochondrial DNA (mtDNA) multiple deletions has been linked to mutations in three genes involved in mtDNA maintenance or replication: adenine nucleotide translocator 1 (*ANT-1*), *Twinkle* and polymerase gamma (*POLG*). Mutations in *POLG* cause dominant or recessive PEO and are often associated with multisystemic disorders. To further investigate the frequency and genotype/phenotype correlations of mutations in the *POLG* gene, we screened 30 patients with PEO and mtDNA multiple deletions and no mutations in *ANT-1* and *Twinkle* genes. The screening was performed by SSCP analysis and direct DNA sequencing of samples with abnormal SSCP patterns. The mutations were confirmed by RFLP analysis. Mutations on *POLG* were found in 5 patients (16%). The first had PEO and mental retardation and harbored a new heterozygous Gly1176Val mutation. The second patient had PEO and neuropathy, and is a compound heterozygous for Ala889Thr and Arg579Trp mutations. The third patient had PEO and the previously reported Tyr955Cys mutation. Two more patients, one with PEO and exercise intolerance and one with PEO, neuropathy, deafness and hypogonadism, harbored the same new mutation, an heterozygous Pro587Leu change. All the mutations were absent in 120 control alleles.

Our results show the diseases associated with *POLG* mutations are both clinically and genetically heterogeneous. Furthermore, *POLG* mutation account for a significant proportion of patients (16%) with PEO and mtDNA multiple deletions, suggesting that defects in other genes must be involved in the etiology of this syndrome.

[24] [ORAL COMMUNICATION]
NOVEL CAVEOLIN-3 GENE MUTATIONS AND PRO-
TEIN STUDY IN A CLINICALLY HETEROGENEOUS
GROUP OF PATIENTS

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Mutations in the caveolin-3 gene (*CAV3*) cause one mild type of limb girdle muscular dystrophy (LGMD1C) and other clinical phenotypes. The identification of *CAV3* gene mutations in patients presenting with a broad clinical spectrum suggests the role of epigenetic factors in modulating the phe-

notype expression. We screened for immunohistochemical caveolin-3 deficiency 665 muscle biopsies from patients affected with LGMD, proximal myopathy, hyperCKemia, and distal myopathy, in whom normal expression of dystrophin, sarcoglycan, calpain-3, dysferlin, emerin had been previously found. Caveolin-3 immunohistochemistry showed a marked reduction of reaction in 9 cases. Caveolin-3 immunoblot showed a protein amount variable from 0 to 11% of the control. One patient showed classical LGMD phenotype, 3 proximal myopathy, 3 hyperCKemia, and 2 distal myopathy (one mother-daughter couple). Five cases have a positive family history. Direct sequencing of *CAV3* gene allowed the identification of various missense mutations: A46T in exon 2 (4 cases), S61R in exon 2 (1 case), R27Q in exon 1 (1 case), E47D in exon 2 (2 cases). The latter 3 gene mutations are novel. In one case mutation analysis is still in progress. A variable clinical phenotype was observed as the consequence of the same gene mutation (e.g. A46T).

Our study shows that the detection of caveolin-3 protein deficiency is a highly specific marker of primary caveolinopathy; caveolin-3 protein screening is useful in patients with various clinical phenotype; though caveolinopathy is an uncommon muscle disorder, it appears more frequent than previously thought.

[25] [ORAL COMMUNICATION]
BENEFICIAL EFFECTS OF AEROBIC TRAINING ON
EXERCISE-RELATED PEROXIDATION EVENTS IN MI-
TOCHONDRIAL MYOPATHIES

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In mitochondrial diseases deranged oxidative metabolism consequent to aerobic dysfunction is implicated in abnormal production of reactive oxygen species (ROS), this realizing oxidative stress that may lead to cell death with both apoptotic and not apoptotic pathways. Aerobic training has been showed to improve oxidative metabolism in mitochondrial myopathies, probably acting on the balance between wild type and mutated mitochondrial DNA (mtDNA). Aim of this study has been to evaluate, in patients carrying on heteroplasmic large-scale mtDNA rearrangements, the occurrence of in vivo oxidative stress related to exercise and to assess if 11 week supervised aerobic training, other than improve oxidative metabolism, is able to reduce exercise-related ROS production from skeletal muscle. To do that, an indirect marker of oxidative stress, blood lipoperoxide level, measured at rest and during an incremental exercise test, was considered. Mean blood level of lipoperoxides in resting condition was 382 ± 38 AU corresponding to a moderate oxidative stress according to Carratelli et al.. During exercise this value maintained high (379 ± 27 AU at 40% of the maximal predicted normal power

output, 387 ± 27 AU at maximal contraction level), while it was 375 ± 26 AU after a recharge period. After the aerobic training lipoperoxides were decreased by 13.7% at rest ($p < 0.01$), 11.4% at 40% of the power output, 8.6% at maximal contraction level and 8.5% after the recovery period, the absolute values now corresponding to a mild degree of oxidative stress.

These data confirm that aerobic training can be beneficial in patients with mitochondrial dysfunction and indicate that a lower exposure by skeletal muscle to exercise-related peroxidation occurs in such experimental condition.

[26] [ORAL COMMUNICATION]

ULTRASOUND TISSUE CHARACTERISATION PREDICTS MYOCARDIAL STRUCTURAL CHANGES IN CHILDREN AFFECTED BY DUCHENNE MUSCULAR DYSTROPHY

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Duchenne Muscular Dystrophy (DMD) is a progressive genetic disease. Although patients (pts) may be affected by cardiomyopathy, its prevalence in children is still not well known. We studied DMD children (DMDc) by using Ultrasonic Tissue Characterization (UTC) analysis to define the early UTC properties of the myocardium in DMDc and to verify if there are differences among DMDc. At immunocytochemistry pts showed absence of dystrophin in skeletal muscle tissue. All DMDc had normal 2D-echocardiographic parameters. We analyzed with UTC the magnitude of cyclic variation of myocardial integrated backscatter (cvIBS) and the absolute value of integrated backscatter (aIBS) in 8 myocardial regions from the parasternal short-axis views in 20 DMDc aged 7 ± 2 years (range 4-11ys) and in 20 age-matched healthy controls. No significant differences were founded for ejection fraction values between DMDc and healthy controls. DMDc and controls displayed different curves of data distribution for both cvIBS and aIBS ($p < 10^{-6}$ for both). The mean value of cvIBS was 4.4 ± 1.5 dB in DMDc and 8.8 ± 0.8 dB in controls. The mean value of aIBS was 36.4 ± 7.1 dB in DMDc and 26.9 ± 2.0 dB (median 26.7 dB, range 22.2 ± 31.6 dB) in controls

UTC analysis is able to differentiate the myocardium of DMDc from controls. Since the differences among DMDc are relevant, each individual shows a personal UTC profile. This implicates that UTC of the myocardium in each pt is required to assess the progression of the disease. Longitudinal studies in this cohort of pts could assign specific prognostic value to the UTC parameters analysed.

[27] [POSTER]

IMPAIRED HIGH ENERGY PHOSPHATE METABOLISM IN CARDIOMYOCYTES OF DYSTROPHIC MICE

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Changes of high energy phosphate metabolism were found in the heart of humans affected by Duchenne or Becker dystrophies, which are caused by the absence or decrease of dystrophin. It was suggested by Radda (1999) that those changes are in part attributable to cardiac hypertrophy and could account for progression to cardiomyopathy. The present research was performed in mdx mice, a model of Duchenne muscular dystrophy, by ³¹P NMR spectroscopy of beating hearts reper-fused by the Langendorff technique. The PCr/Pi ratio was lower in mdx than in controls (1.11 ± 0.31 ; 2.04 ± 0.16 $p < 0.05$). A decrease was observed in the pH value (mdx 7.03 ± 0.03 ; controls 7.19 ± 0.03 $p < 0.02$). Lower phosphocreatine levels in mdx mice were found also by HPLC methods ($\mu\text{mol} / \text{gr dry wt mdx } 26.3 \pm 4.4$; controls 36.1 ± 6.5 $p < 0.05$). No heart hypertrophy was found in our mdx mice. Degenerative changes, as revealed by optical and electron microscopy (loss of filaments, sparseness of mitochondrial cristae, dilatation of sarcoplasmic reticulum) were present only in rare and very small foci without any apparent relationship with the biochemical changes. The reduction of PCr could be caused by decreased of the level of the creatine kinase. It was reported by Crawford et al (2002) that an aliquot of the enzyme is localized in the sarcolemma of the cardiomyocytes. That aliquot of enzyme may be absent in mdx because of dystrophin lack and associated protein complex delocalisation.

[28] [ORAL COMMUNICATION]

FACIO-SCAPULO-HUMERAL MUSCULAR DYSTROPHY: GENOTYPE-PHENOTYPE CORRELATION IN A POPULATION OF 92 PATIENTS.

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Facio-scapulo-humeral muscular dystrophy is known to be associated to neurosensorial deafness and retinal vasculopathy. The aim of our study is to verify the incidence of extra-muscular involvement, to confirm the existence of a genotype-phenotype correlation and to study the visual central pathway. 92 FSH patients were clinically evaluated by means of two clinical scores. The following exams were performed: Visual Evoked Potentials (VEPs) (48 patients), Retinal Fluorangiography (RF) (35 patients), Oscillatory Potentials (OP) (7 patients), Audiometric Examination (45 patients) and

Brainstem Auditory Evoked Potentials (BAEP) (48 patients). Patients with significant delay of VEP were also studied by automated quantitative MRI. Only one patient (3%) had a RF picture like Coats's syndrome. The VEPs showed in 8 patients (17%) a delay of the P110 wave (mean 123 msec), with normal OP. Neurosensory deafness was present in 11 patients (25%) and BAEPs were abnormal in 27 % of patients. We found a significant statistic correlation between clinical scores and age at onset ($p < 0.005$), disease duration ($p < 0.0001$) and the size of the EcoRI fragment ($p < 0.05$). In our population a fragment of 26 or less kbs is always associated to the expression of the phenotype, while for longer fragments the penetrance decreases. Our results confirm the presence of a genotype-phenotype correlation. We found a lower penetrance for fragments of more than 26 kbs. The quantitative analysis of the white matter in patients with central visual conduction delay is still ongoing.

[29] [POSTER]
BELOW-THRESHOLD FES IN CP
LONG TERM TRAINING VERSUS ORTHOSIS EFFECT

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Enhancement of muscle function in Cerebral Palsy (CP) can be achieved by Functional Electrical Stimulation (FES). FES can be applied either as a training modality (long-term muscle conditioning) or as a muscle assist (neural orthosis). The objective of this study was to examine usability of EMG signals for monitoring the effect of enhanced muscle function in children with CP by means of below-threshold FES. Special significance was attributed to the co-contraction index (CCI), derived from the EMG of two antagonist muscles, because this parameter has been considered important in motor control. Seven children with CP (study group) and six able-bodied children (control group) took part in the experiments. In addition to conventional physiotherapy, the CP group was trained with FES to the Quads for 12 weeks. At the beginning and the end of this period the children were asked to perform alternate flexion/extension exercises of the knees, with and without FES of the quads. During exercise, the following data were obtained and compared between the groups: velocity, jerk, knee torque and CCI. Comparisons within the CP group and between the groups showed that after training, performance of the CP group significantly improved. Application of FES as an orthosis did not cause, however, significant differences. Hence, it may be concluded that with below-threshold FES supplementing physiotherapy as a training modality is beneficial to CP.

[30] [POSTER]
CONGENITAL MUSCULAR DYSTROPHY PRESENTING
AS INFLAMMATORY MIOPATHY

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Congenital muscular dystrophies are a heterogeneous group of autosomal recessive disorders presenting in infancies with muscle weakness, contractures, and dystrophic changes at skeletal muscle biopsy. Structural brain defects, with or without mental retardation are often present. Recently congenital muscular dystrophy associated with mutations in FKRP gene has been described. This form is characterized by onset in the first weeks of life, inability to walk, cardiomyopathy, muscle hypertrophy, marked elevations of serum CK, normal brain structure and functions. The muscle biopsy showed dystrophic changes, secondary deficiency of laminin alpha 2 and a reduction of alpha dystroglycan molecular weight.

We present a new case of congenital muscular dystrophy characterized by muscle weakness and psychomotor developmental delay with normal brain MRI, and no cardiomyopathy. The main pictures of muscle biopsy were a severe inflammation and a complete absence of alpha dystroglycan at the immunohistochemistry. A described heterozygous C to G transversion in exon 4 at nucleotide 341 in the FKRP gene was found.

[31] [POSTER]
ALPHA DYSTROGLYCAN GLYCOSYLATION DEFECTS
IN A CASE OF CONGENITAL MUSCULAR DYSTROPHY.
HISTOLOGICAL AND IMMUNOCYTOCHEMICAL
STUDIES IN SKELETAL MUSCLE AND IN AUTOPTIC
BRAIN TISSUES

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Congenital muscular dystrophies are autosomal recessive disorders presenting in infancies with muscle weakness, contractures and dystrophic changes at skeletal muscle biopsy. Common structural brain defects as well as lissencephalia, pachygyria, polymicrogyria and heterotopia are often present. Major or minor eyes alterations could be present in WWS, MEB, and Fukuyama Muscular Dystrophy. Defects of alpha dystroglycan glycosylation have been recently described in these syndromes.

We present a floppy baby died at 3 month of age because of respiratory failure. Microcephalia, severe hypotonia, weakness and contractures with arthrogripes were present at birth. No eyes involvement was present. Brain autopsy showed severe

lissencephalia, pachigiria, and no cerebellar malformation. The histological studies showed severe neuronal migration defects with heterotopic nuclei and pseudoependymal cysts.

The muscle biopsy showed dystrophic pattern with severe deficiency of alpha dystroglycan and normal binding for alpha 2 laminin. Alpha dystroglycan glycosylation was also studied in brain.

[32] [ORAL COMMUNICATION]

LMNA MUTATION IN FAMILIAL PARTIAL LIPODYSTROPHY FIBROBLASTS AFFECTS LAMIN A INTERACTION WITH EMERIN, NUCLEAR ORGANIZATION AND RNA POLYMERASE ACTIVITY

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Familial partial lipodystrophy is characterized by abnormal distribution of body fat and insulin resistance. It is an autosomal dominant disease caused by mutations of *LMNA* gene encoding alternatively spliced lamins A and C. Primary fibroblast cultures from a patient carrying an R482L lamin A/C mutation were analyzed by a morphological and biochemical approach. Nuclear abnormalities were observed in cultured cells consisting of altered heterochromatin distribution and nuclear lamin A/C aggregates mostly localized close to the nuclear lamina. Emerin did not co-localize with nuclear lamin A/C aggregates. Interestingly, emerin failed to interact with lamin A in R482L mutated fibroblasts *in vivo*, while the interaction with lamin C was preserved *in vitro*, as determined by co-immunoprecipitation experiments. The presence of lamin A/C nuclear aggregates was restricted to actively transcribing cells. In fibroblasts carrying lamin A/C nuclear aggregates, a reduced incorporation of bromouridine was observed, demonstrating that mutated lamin A/C in FPLD cells affects RNA transcription.

[33] [ORAL COMMUNICATION]
EXPRESSION OF TRANSGLUTAMINASE IN MYOPATHIES

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Transglutaminases (TGases) are enzymes, expressed in different tissues, with a generic role in the stabilization of protein aggregates. TGases may be abnormally activated in autoimmune disorders and neurodegenerative conditions. Overexpression of TGase II (tissue type, tTGase), induced by proinflammatory cytokines, has been found in inclusion body myositis and its role in the formation of β -amyloid aggregates has been postulated.

The aim of our study is to investigate the expression of tTGase in polymyositis (PM), facioscapulohumeral dystrophy (FSHD), Duchenne muscular dystrophy (DMD) and myotonic dystrophy type 1 (DM1) to evidence its possible involvement in the pathogenesis of such diseases. Immunohistochemical and Western blot studies with antibodies against tTGase were performed in muscle specimens from five patients each with PM, FSHD, DMD, DM1 and five normal controls. Northern blot analysis was also done. Immunohistochemical analysis revealed a normal sarcolemmal expression of tTGase in controls, DMD and DM1 muscle fibers. Its overexpression was found in muscle fibers of patients with FSHD and more evidently in PM. These findings were confirmed by Western blot and Northern blot studies.

The marked increase in tTGase expression only found in FSHD and PM suggests its involvement in their pathogenic mechanisms. Such findings may have possible therapeutic implications.

[34] [POSTER]
MUSCLE GLYCOGENOSIS AND MITOCHONDRIAL HEPATOPATHY IN AN INFANT WITH MUTATIONS IN THE MYOPHOSPHORYLASE AND THE DGK GENES

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Here we report a "genetic double trouble" in an infant with myophosphorylase deficiency (McArdle disease) and mitochondrial hepatopathy with liver failure and mitochondrial DNA (mtDNA) depletion. The girl, born to consanguineous Moroccan parents, had severe congenital hypotonia and hepatomegaly. She developed liver failure and died at 5 months of age.

We have studied muscle and liver biopsy specimens histochemically and biochemically, and we have sequenced the

whole coding regions of the deoxyguanosine kinase (*dGK*) and myophosphorylase (*PYGM*) genes. Muscle biopsy showed subsarcolemmal glycogen accumulation and negative histochemical reaction for phosphorylase. Liver biopsy showed micronodular cirrhosis and massive mitochondrial proliferation. Biochemical analysis showed phosphorylase deficiency in muscle and cytochrome *c* oxidase (COX) deficiency in liver. We have identified a novel homozygous missense G-to-A mutation at codon 456 in exon 11 of *PGYM*, as well as a homozygous 4-bp GATT duplication (nucleotides 763-766) in exon 6 of *dGK*, which produces a frame-shift and a premature TGA stop codon at nt 766-768, resulting in a truncated 255-amino acid protein. Both mutations were absent in 100 normal individuals. Our data further expand the genetic heterogeneity in patients with McArdle disease, confirm the strong relationship between MDS, liver involvement and *dGK* mutations, and suggest that genetic “double trouble” should be considered in patients with unusual severe phenotypes.

[35] [ORAL COMMUNICATION]

MITOCHONDRIAL MYOPATHY DUE TO A DEFECT OF MITOCHONDRIAL SULFUR-IRON CLUSTERS TRANSPORT

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Iron-sulfur (FeS) clusters are important cofactors of numerous proteins involved in electron transfer and other metabolic processes. In the respiratory chain of eukaryotic cells, FeS proteins are present in complexes I, II, and III. Thus, defective transport of FeS clusters into mitochondria is expected to impair oxidative metabolism.

We describe a 52-yr-old woman with a clinical presentation resembling facioscapulohumeral muscular dystrophy (FSHD) associated with lactic acidosis. We performed DNA analysis for the diagnosis of FSHD. In a muscle biopsy, we did histochemical and biochemical studies of respiratory chain complexes, direct sequencing of the cytochrome *b* and of the 22 mitochondrial tRNA genes, and Southern blot for multiple deletions.

The patient's DNA showed no rearrangements within the subtelomeric region of 4q, and we found no pathogenic mutations or deletions in her muscle mtDNA. Morphology showed severe myopathy and many fibers were hyporeactive for the succinate dehydrogenase (SDH) stain. Biochemical analysis showed markedly decreased activities of complexes I, II, III, and of aconitase, a Krebs cycle enzyme, all of which contain FeS clusters.

In conclusion our findings are consistent with the hypothesis that the cause of myopathy and impaired muscle oxidative metabolism is due to a defect in FeS cluster proteins, possibly related to defective transport. This same pathogenesis was

proposed in two previous patients with myopathy (Shapira et al, NEJM 1990; Haller et al, JCI 1991).

[36] [POSTER]

NUCLEAR AKAPS ARE MODULATED DURING MUSCLE DIFFERENTIATION: INVOLVEMENT OF THE MAPK/ERK PATHWAY IN THE REGULATION OF AKAP149 EXPRESSION.

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The role of nuclear AKAPs, mAKAP, AKAP149 and AKAP95 in muscle cells, during multinucleate myotube formation, has not been investigated. In an attempt to analyze the fate of nuclear AKAPs during muscle cell differentiation, we evaluated expression and localization of AKAP95, mAKAP and AKAP149 during C2C12 mouse myoblast differentiation. The present study reveals modulation of these AKAPs during myogenic differentiation. AKAP95 is highly expressed in cycling myoblasts, and its expression decreases in differentiating cells. On the other hand, we observe a very low amount of mAKAP and AKAP149 in cycling muscle cells, while expression of mAKAP and AKAP149 is upregulated during C2C12 myoblast differentiation.

Treatment of differentiating myoblasts with PD098059 or U0126, two inhibitors of MAP kinases pathway, enhances AKAP149 expression. In contrast, treatment with bFGF, a growth factor able to induce MAPK/Erk activity, elicits a significant reduction of AKAP149 expression. These data suggest an involvement of MAPK/Erk1/2 pathway in the modulation of AKAP149 expression in myogenic cells.

[37] [ORAL COMMUNICATION]

CLINICAL AND GENETIC ANALYSES OF PATIENTS WITH DYSFERLIN DEFICIENCY

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Dysferlin is a protein of 230 kDa located on the muscle fiber membrane: its deficiency causes distal (Myoshi Myopathy-MM) and proximal (LGMD2B) muscular dystrophies, both recessively inherited. Dysferlin gene (DYSF) maps to chromosome 2p13, spans more than 150 kb and contains 55 exons: the entire coding sequence has been identified as a 6.9 kb mRNA.

We studied 5 families in which the probands, affected by MM (4 cases) or LGMD2B (1 case), showed absence of dysferlin on immunoblot analysis. We collected DNA samples from the 5 probands and 7 family members, 1 displaying calf hypotrophy and very high CK levels, the others asymptomatic except 1 with severe and 2 with mild CK increase. Molecular studies were performed by Multiple Heteroduplex analysis followed by sequencing of fragments showing electrophoretic abnormalities. We found two subjects homozygous for missense mutations: 5626G>A in exon 50 (D1876N) and 2875C>T in exon 27 (R959W). The remaining affected individuals have been shown to carry frame-shift mutations, introducing a premature termination codon: a heterozygous 1639delG (exon 19); a heterozygous 2547_2548insA (exon 25); a homozygous 4872_4876GCCC>CCCCG (exon 44). Among the asymptomatic individuals, we found the condition of compound heterozygote (similar to that of 2 affected sisters) in the case with very high CK, while both individuals with mild increase of CK and 2 relatives with normal CK turned out to be heterozygous.

Muscle MRI was performed in all affected and unaffected individuals showing oedema, fatty replacement, or both only in homozygous or compound heterozygotes.

[38] [ORAL COMMUNICATION]
MUSCLE BIOPSY IN DM2: SPECIFICITY AND SENSITIVITY AS A DIAGNOSTIC TOOL

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Encouraging studies on muscle biopsy have recently demonstrated histopathological differences of myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2/PROMM/PDM). To investigate the specificity and sensitivity of muscle biopsy as a diagnostic tool in asymptomatic or mildly symptomatic patients with the clinical diagnosis of DM2. 21 patients with genetically confirmed DM2 were subjected to muscle biopsy of the biceps brachii (age range 43-72, mean age 57.2 ± 2.1 ; 12 women, 11 men; disease duration range 6-50 years; mean disease duration 16.8 ± 1.7 ; mean MRC score 4.5). Routine histochemistry, immunohistochemistry and immunostaining using antibodies directed against fast myosin chain were performed. Our results show that DM2 patients display a subpopulation of type 2 nuclear clumps and other very small fibers indicating type 2 fiber atrophy. The degree of atrophy increased with disease duration. There was no correlation to MRC score, preferential type 2 fiber atrophy being present even in muscles of normal bulk and strength. The abnormalities observed in patients with genetically confirmed DM2 with muscles of normal bulk and strength strongly supports the initial findings that muscle biopsy in DM2 is a specific and sensitive tool which should be considered as a man-

datory diagnostic procedure to screen patients with the clinical diagnosis of DM2, especially if sporadic or asymptomatic, given the limited availability of the genetic testing on a routine basis in most laboratories as yet.

[39] [ORAL COMMUNICATION]
CONGENITAL FORM OF DISTAL SPINAL MUSCULAR ATROPHY AFFECTING THE LOWER LIMBS: A COMMON CONDITION IN CHILDHOOD

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The most common and severe form of spinal muscular atrophy (SMA I), linked to chromosome 5, has early presentation in the neonatal period. In this form contractures are generally not observed at birth. Arthrogryphosis is however a feature of other neurogenic conditions. A gene for a dominantly inherited distal SMA form, mostly affecting the lower limbs, has been previously localised to chromosome 12q23-q24. The few cases reported were all familial cases with a clear dominant inheritance. We describe clinical and muscle MRI findings in 9 cases (4 familial and 5 sporadic) affected by a form of SMA mainly affecting the lower limbs with presentation at birth. They all had talipes at birth and predominant involvement of the lower limbs. Upper limbs were preserved and trunk and upper limbs appeared to be disproportionately longer compared to lower limbs. Only one of our patients achieved full independent ambulation. The others all use crutches or, in one case, long leg callipers. The course of the disease is only slowly progressive and functional restrictions when present, were mainly due to increased contractures rather than loss of muscle strength. Respiratory and cardiac function are well preserved. EMG was neurogenic in all. Muscle MRI through the thighs revealed diffuse atrophic appearance with relative hypertrophy of the adductor longus. Genetic studies are underway. Our cases suggest that sporadic cases are frequent and that this form should be considered in the differential diagnosis of infants and children with talipes and distal weakness in the lower limbs.

[40] [POSTER]

RESPIRATORY INVOLVEMENT IN FACIOSCAPULO-HUMERAL MUSCULAR DYSTROPHY (FSHD)

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Respiratory failure has been reported in a few infantile-onset FSHD patients, but the severity of the respiratory involvement and its correlation with the natural history of the disease are unknown. The pathogenic mechanisms of FSHD are still unknown but a deletion in the number of KpnI 3.3 kb repeat units in the telomeric portion of the 4q chromosome has been unequivocally associated with the disease. A simple diagnostic test has been performed that permits to detect, by using the telomeric probe p13E-11, a 4q EcoRI fragment ranging between 11 and 40 kb in up to 95% of sporadic and familial FSHD patients, while longer fragments are observed in normal individuals. We performed pulmonary function tests in 82 FSHD patients (40 males and 42 females) in which the clinical diagnosis had been previously confirmed by genetic analysis. We divided the patients in 2 groups: group A included 37 patients in which the lower limb involvement was absent, mild or moderate; group B 45 patients with severe involvement of the lower limbs (11 were wheelchair bound). The mean value of Forced Vital Capacity (FVC) was $99 \pm 18\%$ in group A and $78 \pm 28\%$ in group B. Maximal expiratory pressure was $79.6 \pm 20.9\%$ in group A and $54.8 \pm 24.6\%$ in group B. Maximal inspiratory pressure was $95.4 \pm 26.5\%$ in group A and $80.9 \pm 31.0\%$ in group B. Mean age and mean 4q35 EcoRI size were similar in the 2 groups (43 $\pm$ 17 years and 22 $\pm$ 4 kb in group A; 46 $\pm$ 14 years and 21 $\pm$ 6 kb in group B).

[41] [Lecture]

CLINICAL PHENOTYPES OF LAMINOPATHIES

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Laminopathies represent a group of human hereditary diseases that arise through defects in genes that encode *nuclear-lamina* and *lamina-associated proteins* (Burke & Collins, Nature Reviews, August 2002).

The first of these disorders to be recognized was X-linked Emery-Dreifuss muscular dystrophy, which is caused by mutation of the *EMD* gene, encoding emerin, a ubiquitous protein of the inner nuclear membrane and a member of the nuclear lamina-associated protein family. Emery-Dreifuss muscular dystrophy (EDMD), is characterised by the triad of: 1) early contractures of the Achilles tendons, elbows and spine; 2) slowly progressive muscle wasting and weakness with a humero-peroneal distribution early in the course of the disease; and 3) a cardiomyopathy that invariably develops by adult-

hood, usually presenting as cardiac conduction defects requiring pacing.

Seven rare human disorders due to lamin A/C mutations have been described so far that include in isolation or combination: muscular dystrophy, cardiomyopathy, peripheral neuropathy, lipodystrophy, insuline resistant diabetes, bone dysplasia, osteolysis, and premature aging.

1. An autosomal form of EDMD, phenotypically similar to the X-linked form, is associated with mutations in LMNA.

2. Limb-girdle muscular dystrophy type 1B (LGMD1B), is an autosomal dominant condition characterised by limb-girdle phenotype with absent or late mild contractures, and heart involvement including atrial paralysis with sudden death and dilated cardiomyopathy

3. Dilated cardiomyopathy with conduction defects (DCM-CD), is a condition with autosomal dominant dilated cardiomyopathy and conduction system defects including sinus bradycardia, atrioventricular conduction block, or atrial arrhythmias.

4. Autosomal recessive Charcot-Marie-Tooth disease type 2 (CMT2B1), is an axonal neuropathy with the age of onset in the second decade, with distal wasting and weakness more prominent in the lower than in the upper limbs, pes cavus, and areflexia. Motor nerve conduction velocity is normal or slightly reduced and sensory nerve action potential is decrease or absent clearly reflecting an axonal degenerative process.

5. Familial partial lipodystrophy of the Dunnigan-type (FPLD), is an autosomal dominant condition that usually becomes evident at puberty and is characterized by selective loss of subcutaneous fat from the limbs and trunk, with accumulation in the face and neck. Affected patients are insulin-resistant and may develop glucose intolerance and diabetes mellitus, hypertriglyceridemia, and low levels of high density lipoprotein cholesterol.

6. Mandibuloacral dysplasia (MAD), is a very rare condition, inherited as an autosomal-recessive trait, characterized by midchildhood onset of mandibular and clavicular dysplasia, acroosteolysis, club-shaped terminal phalanges, stiff joints, alopecia, hand and feet skin atrophy, lipodystrophy, and insulin-resistant diabetes.

7. Hutchinson-Gilford progeria syndrome is a dominant condition characterized by precocious senility, coronary artery disease, absence of subcutaneous fat, congenital contractures, and persistence of fontanelles with pseudohydrocephaly.

More recently Pelger-Huet anomaly has been related to mutations of LBR, encoding the lamin B receptor, a member of the sterol reductase family, evolutionarily conserved and integral to the inner nuclear membrane. PHA is an autosomal dominant disorder characterized by abnormal nuclear shape and chromatin organization in blood granulocytes; it targets heterochromatin and lamins to the nuclear membrane. Affected individuals show hypolobulated neutrophil nuclei with coarse chromatin.

[42] [ORAL COMMUNICATION]
MANAGEMENT OF SCOLIOSIS IN DUCHENNE MUS-
CULAR DYSTROPHY
A 11 YEAR RETROSPECTIVE STUDY

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dic Hospital, Stanmore, London, UK.

Scoliosis is a complication of Duchenne muscular dystrophy (DMD). Spinal surgery is the treatment of choice. In various centres this is offered to all patients at the time of the loss of ambulation. However this approach is controversial. We reviewed our experience of a tailored approach to scoliosis management in DMD. We reviewed case notes of 123 boys with DMD, at least 17 years old and followed at the Hammersmith Hospital Neuromuscular Centre between 1992 and 2002. In our population scoliosis was absent in 12/123 cases (11%), and mild ($< 30^\circ$) in 16 (13%). Other 16 patients (13%) had moderate scoliosis (between 30° and 50°), that was managed conservatively. Surgery was considered in 79 children developed a scoliosis above 50° but only performed in 43/79 (35%) as 16 families (13%) refused it and in other 16 cases could not be performed because the patients had poor cardio-respiratory function. In the remaining 9 patients (7%) surgery was not performed despite curvatures above 50° as the scoliosis had a slow progression and occurred after the age of 14. Our results suggest that a significant number of patients with no or mild scoliosis at 17 years would have undergone unnecessary surgery, had we performed surgery at loss of ambulation. At 17 years however there is no significant difference in survival, respiratory impairment and sitting comfort between patients that had and had not surgery. These results indicate that scoliosis is not an invariable complication in DMD and that, while surgery has been shown to be beneficial in cases with significant scoliosis, its role in milder cases is questionable and needs to be further studied.

[43] [POSTER]
BONE MINERAL DENSITY MEASUREMENT IN CHIL-
DREN WITH DUCHENNE MUSCULAR DYSTROPHY:
EFFECT OF LOW DOSE INTERMITTENT PREDNIS-
OLONE.

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The aim of this study was to assess bone mineral density in boys with Duchenne Dystrophy (DMD) and to establish whether bone density abnormalities, if present, are related to age or level of functional ability. As a proportion of patients with DMD in this study were treated with long-term intermittent prednisolone, we also aimed to establish possible differences between treated and untreated patients. We measured lumbar spine bone mineral density using Dual energy X-ray Absorptiometry (DXA) in a group of 50 boys with Duchenne dystrophy followed at the Hammersmith Hospital of whom 32 had never been treated with prednisolone. The remaining 18 had been treated (prednisolone 0.75 mg/kg for the first 11 days of each month) for 12 months or longer (up to 9 years) and were still on treatment at the time of the study. We used descriptive statistics and statistical comparison of bone mineral density between the 2 groups of patients using independent Mann-Whitney U. Boys with DMD showed reduced bone mineral density when compared with normal age and sex-matched controls, irrespective of steroid treatment and the values of bone mineral density tended to decrease with age. When we compared the scans obtained in the treated and untreated DMD the lumbar spine bone mineral density was significantly higher ($p<0.005$) in the treated than the untreated groups. Our results provide additional evidence that bone mineral density is abnormal in DMD and suggest that the intermittent steroid treatment regime, at variance with the daily or alternate daily regimens, does not further compromise the bone integrity of these children.

[44] [POSTER]

CHARACTERIZATION OF PATTERN OF COGNITIVE IMPAIRMENT IN DM1 PATIENTS.

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A significant involvement of Central Nervous System (CNS) is present in most DM1 patients: a condition of mental retardation is observed in congenital forms while adult patients show a mild cognitive impairment with dysfunction in frontal-executive tasks. Recently neuropathological studies documented an abnormal pattern of tau-protein expression in brains of DM1 patients and transgenic mice. In order to define the pattern of cognitive impairment and evaluate genotype-phenotype correlations, we assessed a detailed neuropsychological study on 67 DM1 patients (9 congenital, 58 classical) including MMSE, memory, linguistic, level, praxis, attentional and frontal-executive tasks. Statistical analysis was performed by ANOVA for multiple tests. A severe, distinct pattern of cognitive impairment was documented both in congenital and adult patients: congenital DM1 show a global cognitive impairment, while adult forms develop a progressive fronto-temporal dysfunction similar to what observed in other tauopathies. No correlation was found between degree of cognitive impairment and n(CTG) in leukocytes or muscle involvement.

Our data suggest that the presence of RNA species carrying very large CUG expansions in embryonic brain tissues would exert a toxic effect on global brain development, while their progressive increase occurring in adults with ageing might specifically affect neurons of frontal and temporal cortex, causing aberrant splicing of tau m-RNA.

[45] [POSTER]

AUTOSOMAL DOMINANT MYOTONIA IN A FAMILY CARRYING A1239H MUTATION OF CACNL1A3 GENE

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Skeletal muscle voltage-gated Ca^{++} channels (L-type) are involved in signal transmission from the outer cell membrane to the transverse tubular system, causing the release of Ca^{++} ions from the sarcoplasmic reticulum and the activation of muscle contraction; like other Ca^{++} -channels, they are composed by distinct tissue-specific subunits: α_{1S} (CACNA1S), which contains the DHP binding site and the voltage sensor element and is coded by CACNL1A3 gene on

chromosome 1q32; α_2 (CACNA2D1), with modulating properties; and three smaller accessory subunits. Mutations of α_{1S} subunit have been described in dominant hypokalemic periodic paralysis (HypoPP-1), malignant hyperthermia susceptibility 5 (MHS-5) and muscular dysgenesis mouse (mdg); more specifically, A1239H point mutations were reported in HypoPP-1 families from different geographic sites. We found a A1239H mutation of CACNA1S in a family featuring myotonic phenomenon and mild hyperCKemia, with normal muscle strength and exercise tolerance. A mild diffuse muscle hypertrophy was observed. Myotonia was cold- and exercise-insensitive, more evident in distal segments. Electrophysiological testing confirmed the presence of myotonic discharges with normal motor unit potentials. The patients never complained of episodic weakness, nor presented serum electrolytes abnormalities. So far, myotonia has never been associated to Ca^{++} -channel mutations: this observation can add important data and new insight to understand the pathophysiology of skeletal muscle channelopathies.

[46] [ORAL COMMUNICATION]

DIAGNOSTIC PROBLEMS IN FACIOSCAPULOHUMERAL DYSTROPHY

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We describe a family in which different clinical phenotypes were present. Patient 1 at the age of 56 years, came to our observation because of diplopia, ptosis and fatigability. After neurophysiological and immunological investigations the diagnosis of myasthenia gravis was performed. Because of persistent pelvic girdle muscle weakness and high CK, a muscle biopsy was performed, that revealed myopathic features. The patient referred that one of her 5 daughters presented severe motor impairment since childhood. Clinical evaluation of this 33 year-old woman (patient2) confirmed marked scapular and pelvic girdle muscle and lower limb muscle weakness. Muscle biopsy detected severe myopathic features; dystrophin and dystrophin-associated proteins, calpain 3 and dysferlin immunohistochemical analysis were normal. Clinical evaluation of 3 additional daughters of patient 1 showed a moderate facial and scapular girdle muscle involvement, confirmed by EMG evaluation, muscle CT and high CK values in 2 of them (patients 3 and 4), and normal phenotype in the other (patient 5), confirmed by normal EMG and CK.

Molecular analysis showed in patients 1, 3, and 4 a 14 Kb fragment at 4q35, which currently indicate a diagnosis of FSHD. This fragment was also present in the asymptomatic patient 5. The same fragment was absent in patient 2, the most severely affected woman, with a limb girdle involvement.

[47] [LECTURE]
GLYCOSYLATION DEFECTS AND CONGENITAL DYSTROPHIES

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Muscular dystrophies are a genetically heterogeneous group of disorders characterised by muscle weakness, muscle wasting and/or pseudohypertrophy and elevated serum creatine kinase levels. Onset and severity of symptoms varies considerably from congenital presentations to adult life. The brain and cardiac muscles are variably affected. Most of the genes responsible for muscular dystrophies identified so far encode for proteins of the extracellular matrix, sarcolemma, sarcomere and nuclear membrane. In the last 2 years however a number of muscular dystrophies have been associated to defects in proteins with putative glycosyltransferase activity. Four of these protein defects give rise to severe, congenital variants of muscular dystrophies (CMD), including Walker Warburg syndrome, Fukuyama CMD and Muscle-Eye-Brain disease, characterized by severe brain involvement. A fourth type, MDC1C, is usually characterized by normal brain function and structure (MDC1C). Milder allelic mutations in the corresponding gene, FKRP, cause a common form of limb girdle muscular dystrophy, LGMD2I. More recently, we identified FKRP mutations in patients with severe brain involvement resembling MEB, providing therefore an unusually wide range of severity among muscular dystrophies.

In all these forms the expression of the highly glycosylated peripheral membrane α -dystroglycan is abnormal, implicating that defective glycosylation of this protein is central to the pathogenesis of muscular degeneration observed in these disorders. As abnormal glycosylation of α -dystroglycan is also found in a number of yet uncharacterized conditions, we believe that the list of glycosyltransferases responsible for muscular dystrophies will considerably increase over the following years.

[48] [ORAL COMMUNICATION]
BIGLYCAN/DECORIN EXPRESSION IN MUSCULAR DYSTROPHIES

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The two small chondroitin/dermatan sulphate proteoglycans, biglycan and decorin, belong to an expanding family of small leucine-rich proteoglycans and were found to be key-active molecules. They are both known to bind different types of collagens. In addition, biglycan binds to dystroglycan, the membrane-bound proteoglycan associated with dystrophin,

while decorin is known to bind other extracellular matrix components. Furthermore, biglycan expression is elevated in muscle from the dystrophic mdx mouse. These findings thus raise the possibility of a role for biglycan and decorin in muscular dystrophy. We investigated the expression of decorin and biglycan both at transcript and protein level in several forms of muscular dystrophies and compared their expression with that in controls of similar age. Both proteoglycans were significantly increased at the mRNA level in sarcoglycanopathies and in dysferlinopathies. They were decreased, although not significantly, in DMD muscles. By immunohistochemistry, an increasing expression of biglycan was detected on the muscle surface with muscle maturation in patients and controls. Decorin similarly increased with age but was more localized in the perimysial and endomysial connective tissue. There was an apparent reduced expression of biglycan around muscle fibers and an increase in the perimysium, in sarcoglycan- and dysferlin-deficient patients. Decorin and biglycan appeared reduced at both sites in DMD patient muscle. The multiple roles of these two proteoglycans are reflected in their variable expression in muscular dystrophies. The significant increase of biglycan and decorin in some dystrophic forms indicates a role of these proteoglycans in the sarcolemma and extracellular matrix organization.

[49] [ORAL COMMUNICATION]
TUBULAR AGGREGATES AND CRYSTALLINE INCLUSIONS IN SKELETAL MUSCLE: REVIEW OF 12 CASES

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Tubular aggregates are sarcoplasmic reticulum proliferations apparently arising from dilated terminal cisternae in muscle cells; their occurrence is considered an aspecific and casual finding in muscle biopsies from patients with a variety of conditions, including muscle channelopathies and secondary myopathies. Moreover, they were the major pathologic abnormality in cases of congenital myasthenia, limb girdle weakness, familial and sporadic muscle pain, and in ornithine aminotransferase (OAT) deficiency presenting with retinal degeneration. Their functional role is poorly understood, and a correlation to intracellular Ca^{++} flux regulation has been hypothesized but not proved. Interestingly, in an animal model (MRL+/+ mouse) castration of male mutants completely abolished the occurrence of tubular aggregates, suggesting a hormonal influence. On the contrary, intracellular crystalline inclusions in muscle fibers have been only rarely described in human biopsies and their pathogenesis is unknown. We reviewed 12 cases featuring either tubular aggregates or crystalline inclusions out of about 2000 skeletal muscle biopsies, and compared their clinical and pathologic characteristics. All patients were males and presented with myalgias and/or cramps and a variable degree of weakness; CK levels were always significantly incremented. Clinical work up disclosed a disorder

der of androgen hormones in two of them; in one case a neoplastic disease was found. One patient complained transient episodes of weakness. Interestingly, a patient had a history of chronic licorice ingestion, without evident serum ion concentration abnormalities. Intracellular crystalline inclusions were observed in a patient complaining fatigue and muscle pain, with a familial history of asymptomatic hyperCKemia.

In our case serie, tubular aggregates and crystals inclusions in muscle biopsy were a very rare finding, strictly correlated to male sex, muscle pain and hyperCKemia; the relationship with male hormones and electrolite balance needs further investigation.

[50] [POSTER]

ELECTROPHYSIOLOGIC STUDY IN TEN PATIENTS WITH MEROSIN-POSITIVE CMD

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The congenital muscular dystrophies (CMDs) form a heterogeneous group of autosomal-recessive disorders presenting in early life with muscular weakness, hypotonia and joint contractures. Current classification of CMD identifies merosin-deficient (merosin-negative) CMD as a severe form (with nonmuscular involvement including the central and peripheral nervous system and the heart) and merosin-positive CMD as a mild form (typically non progressive or slowly progressive and usually resulting in only minor disabilities). We performed neurophysiologic studies in ten MP-CMD children to establish the presence or absence of peripheral nervous system involvement. From our hospital records of patients with congenital muscular dystrophy over the last 11 years, ten children (six males and four females), aged 9 months to 14 years, were found to be positive for merosin on immunostaining. Nerve conduction study was performed on the peroneal and median nerve (motor nerve conduction velocity) and then for either on the sural nerve (sensory nerve conduction velocity). All the patients had normal sensory and motor nerves conduction velocity and amplitude. Merosin, or laminin M chain, is a predominantly extracellular protein that forms an essential link between the extracellular matrix and the dystrophin-associated glycoprotein component of the muscle cytoskeleton; her absence explains, in accordance with several autors, the find of a progressive slowing in nerve conduction in the patients with merosin-negative CMD, suggesting in this subjects a myelination arrest. In conclusion our study, performed in ten children, demonstrates the absence of axonal or demyelinating neuropathy in patients with merosin-positive CMD.

Reference: H.J. Gilhuis, H.J. ten Donkelaar, R.B. Tanke et al. Nonmuscular involvement in merosin-negative congenital muscular dystrophy. *Pediatr Neurol* 2002 Jan; 26(1): 30-6.

[51] [POSTER]

ASSESSMENT OF BODY COMPOSITION IN DUCHENNE MUSCULAR DYSTROPHY: A SIMPLE PROTOCOL WITH MR.

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One of the problems facing clinicians in management of subjects with Duchenne Muscular Dystrophy (DMD) is to evaluate the body composition and to quantify accurately the decrease in lean mass and increase in adipose tissue. Common anthropometric measures are not suitable, as they evaluate only the amount of subcutaneous fat. We suggest here a simple quantitative MR protocol with very short acquisition time and good reliability in volume construction for the evaluation of body composition in patients affected by DMD. Nine boys with DMD, ranging in age from 6 to 12 years, were selected to undergo MR examination. The MR data were compared with anthropometric evaluation and functional ability. Quantitative data we obtained confirm that mean fat mass in the DMD group is higher than in reference healthy controls, what can be interpreted as being due to intramuscular fat deposition and to an increase in subcutaneous fat increase. The results are reliable and there is a close correlation between MR grade and patient's age and clinical functional grade. We propose this MR protocol as being a valid, accurate and suitable method in quantifying body composition in DMD and, because of its very short time of acquisition, it is particularly suitable for the pediatric population. We recommend this protocol for pilot studies in assessing progression of neuromuscular diseases, possible treatment efficacy and for nutritional evaluation of those patients who may require a particular diet based on their specific body composition.

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[52] [POSTER]

VISCERAL INVOLVEMENT IN X-LINKED MYOTUBULAR MYOPATHY LONG-TERM FOLLOW-UP: A CASE REPORT

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We report a 2.5-year-old male born at 41 weeks of gestational age. At birth he presented severe hypotonia and required intensive clinical intervention with ventilatory support;

he required gavage feed for 1 month. Severe motor retardation than developed with slow improvement. On examination at the age of 5 months he had hypotonia, generalized muscle weakness with subgravity power in neck and trunk, some degree of antigravity power in the limbs, and failure to thrive. Limbs contractures, limitation of ocular movements, ptosis or diaphragmatic breathing were absent. A congenital myopathy was suspected but parents refused muscle biopsy. Prader Willy disease, SMA and myotonic dystrophy were excluded on clinical ground and appropriate laboratory examinations. CK was normal; transaminase and ammoniemia were elevated and aminoacids showed a profile compatible with liver dysfunction. At the age of 8 months he was admitted at the Paediatric Intensive Care Unit because of severe jaundice and anemia, hepatosplenomegaly, drowsiness and pancreatic failure. Many metabolic disorders and diseases characterized by liver and pancreatic involvement were excluded. Muscle biopsy was finally performed and was consistent with myotubular myopathy (MM) confirmed by molecular genetic analysis (de novo mutation at R421Q). The patient was assisted with mechanical ventilation, gastrostomy was performed and appropriate supportive medical therapy was performed. Hepatic and pancreatic functions gradually improved.

We consider visceral involvement in this case due to the underlying congenital MM. X-linked recessive myotubular myopathy (MM) is characterized by severe neonatal hypotonia, generalized muscle weakness and severe respiratory involvement, caused by mutations in the MTM1 gene on Xq28. MTM1 encodes the protein myotubularin, which is ubiquitously expressed. Death usually occurs in infancy or early childhood from respiratory failure. Non neurological manifestations are reported in long-term survivors, including visceral involvement (biochemical liver dysfunction, liver hemorrhage). This case suggests that long term-survivors affected by MM should be monitored for medical complications in organ systems.

[53] [POSTER]

THE STRUCTURAL CHANGES OF ACUTE PHYSICAL EXERCISE IN RAT TISSUES

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It is well accepted that acute, excessive exercise can cause skeletal muscle damage, including apoptosis or necrosis of the myofibers. One of the proposed mechanisms responsible for the tissue damage after a physical exercise is oxidative stress, which is widely known to be an activator of apoptotic process. The most common index of oxidative stress is an increase in oxidative damage biomarkers, such a lipid peroxidation or decrease in levels of antioxidants and antioxidant enzymes. The relationship between the generation of oxygen free radicals

and tissue damage after physical exercise still remains to be clarified. The aim of our work was to study the acute exercise effects on skeletal muscles and on other organs (e.g. kidney, liver) and the role of exercise-induced oxidative stress in these organs. The responses to oxidative stress induced by acute exercise (treadmill running to exhaustion) were investigated in the EDL muscles and kidney of rats 6 and 96 hr after exercise. Apoptosis was detected on the paraffin-embedded sections of EDL and kidney using TUNEL method. In tissue's homogenates oxidative damage biomarkers (lipid peroxidation) and endogenous antioxidant (glutathione) were measured. Moreover the serum level of myoglobin was estimated. Using TUNEL method we showed the presence of only single apoptotic myonuclei in EDL muscle samples in both time-course samples and the presence of apoptosis in collecting ducts of kidney's medulla, in particular 6 hr after exercise. On the basis of our biochemical observation we can say that the level of lipid peroxidation marker MDA + 4HDA in kidney was lower after 6h after exercise in comparison to the control group. After 96 hr the level of MDA + 4HDA was augmented in comparison to the value from 6h and was almost the same when compared to the control group. Level of reduced glutathione, the second biochemical parameter measured in kidney, was also lower at 6 hr post exercise in comparison to the control. After 96 hr its level has risen to the value comparable with the control. Myoglobin levels were also measured in blood: neither at 6 hr post exercise nor at 96 hr their levels changed in comparison to control values.

Our results show that the responses of the skeletal muscle and kidney to oxidative stress induced by acute exercise are very different and they do not confirm the hypothesis that oxygen free-radicals evoke tissue damage in this extreme conditions.

[54] [POSTER]

THE DISTRIBUTION OF KIDNEY TUBULAR DAMAGE AFTER PHYSICAL EXERCISE

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Physical exercise disturbs organism homeostasis and leads to the changes in the hemodynamic and metabolic processes in the kidneys. Physical exercise is connected with a reduction of effective renal plasma flow. The result is a decreased glomerular filtration and altered tubular functions. The lysosomal enzymes are sensitive markers of changes of kidney. The origin of these enzymes is mainly of proximal tubules. The experiment was carried out on male, 3 month-old rats subjected to the treadmill running to exhaustion. The EDL muscle and kidney were removed after 6 and 96 hr after exercise. Tissue samples were analysed in electron microscopy and the lysosomal enzymes activity was determined in homogenates. The ultra-

structure of skeletal muscles do not reveal the presence of peculiar features of post-exercise damage. However, in the EM observation of the kidney we demonstrate the presence of significant number of apoptotic tubular cells. It was shown the increase of N-acetyl- β -D-glucosaminidase activity in kidneys after 6 hour 4,3-fold and after 96 hour 3,7 –fold related to control group. The activity of β -glucuronidase was increased 3-time in all examined group. The activity of arylsulfatases was slightly decreased 6 hr and about 20% decreased 96 hr after exercise.

In conclusion, both ultrastructural and biochemical changes in tubular cells confirmed the exercise-induced kidney damage after an acute exercise. Change of activity of lysosomal enzymes testifies the high permeability of lysosomal membrane caused by exercise.

[55] [POSTER]

LAMININ α 2 – NEGATIVE CONGENITAL MUSCULAR DYSTROPHY (MDC1A) PRESENTING WITH A MILD PHENOTYPE

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MDC1A is due to mutations in the laminin α 2 gene. Both severe and milder phenotypes are reported. Clinical severity correlates with the amount of the laminin α 2 protein in skeletal muscle. Laminin α 2 negative CMD are due to loss of function mutations and are characterized by neonatal onset of muscle weakness, marked delay in motor milestone with the inability to reach independent ambulation and abnormal white matter signal at brain MRI.

Partial laminin α 2 deficient CMDs mostly due to laminin α 2 missense mutations, are clinically more heterogeneous and a LGMD-like phenotype has been described. Here we present a female patient diagnosed with laminin α 2 negative CMD (MDC1A) who is still ambulant at 14 years of age. The patient presented at birth with severe hypotonia and an abnormal white matter signal on MRI. She reached independent ambulation by the age of two and currently, at 14 years of age, she is still able to walk unsupported and to raise from the floor with Gower's manouvre. Laminin α 2 protein analysis showed a complete absence of the protein in her muscle biopsy by both immunofluorescence and western blotting using antibodies directed against the carboxyl and amino terminus domains of the protein. Laminin α 2 mutation analysis revealed a homozygous out of frame deletion mutation in exon 56. To our knowledge this is the first patient reported with a loss of function mutation in laminin α 2 gene with mild phenotype. Our data strongly suggest that modulating factors can ameliorate clinical phenotype in MDC1A.

[56] [POSTER]

RECURRENCE OF KEARNS-SAYRE SYNDROME IN A FAMILY DUE TO MOTHER-TO-OFFSPRING TRANSMISSION OF A SINGLE MTDNA DELETED SPECIES.

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Mutations in mtDNA include either large-scale rearrangements, that are usually sporadic, or point mutations, that are usually transmitted through the maternal lineage. These two concepts, however, have recently been challenged by two observations. The first was the identification of at least one family with a mother-to-offspring transmission of a single mtDNA deletion; the second concerned a family in which a deleterious point mutation in a mtDNA gene was present in a patient's mtDNA that had clearly been inherited by the proband's father. We present here a second case of maternal transmission of a single heteroplasmic deleted mtDNA species in two subjects, both affected by Kearns-Sayre syndrome (KSS). A single, 8.5 kb deletion was detected in muscle DNA from both probands. Two elder daughters of the KSS mother were normal and no deletion was found in their blood DNA. The rearrangement was present also in blood DNA of both subjects. Further molecular investigation showed that mutant mtDNA in muscle consisted of deleted species only. However, both deleted and duplicated species were present in blood mtDNA from the mother and, in much lesser amount, from the affected son as well. Whether the mother-to-offspring transmission of deleted mtDNA occurred directly in our family, or through the generation of partially duplicated species, remains to be elucidated. In any case, our results conclusively demonstrate that the risk of recurrence for mtDNA deletions, although very low, is not 0%. This notion has important implications in the clinical management and genetic counseling of KSS.

[57] [POSTER]

NEW TECHNIQUES IN MECHANOMYOGRAPHY (MMG): A HIGH DYNAMIC SENSOR-ACTUATORS SYSTEM TO RECORD TWITCH RESPONSES AND MUSCLE SOUND

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Surface myomechanical responses, as elicited by electrical stimulation or voluntary contraction, arise from those active fibers in the muscle which undergo dimensional changes. As these responses potentially contain clinical information, the recorded signal was termed mechanomyogram (MMG). State of the art for MMG recording are microphones, contact sen-

sors and accelerometers. We developed a new high dynamic active system consisting of a scanner galvanometer equipped with lever and indenter that allows to record surface myomechanical responses ranging from muscle sounds during voluntary contraction to single twitch responses during FES test patterns. The device also can be used to measure surface muscle stiffness as the contact force of the indenter is programmable. The galvanometer was mounted on an isometric ankle dynamometer to sense calf muscle responses. The mean twitch contraction time from the surface responses (60 ± 11 ms) was shorter than from the contraction responses (115 ± 7 ms), indicating more fast contracting fibers under the indented area. In a second protocol voluntary target contractions were produced and the surface responses were simultaneously recorded on an accelerometer. After double differentiation of the galvanometer signal, both acceleration MMG's showed a high coincidence in time and frequency domain. A specific application of this galvanometer-dynamometer test system is the assessment of regeneration processes in paraplegics with long term denervated muscles.

[58] [POSTER]

INHIBITION OF APOPTOSIS OF SKELETAL MUSCLE FIBERS IN HEART FAILURE AS A THERAPEUTICAL TOOL TO ANTAGONIZE MUSCLE ATROPHY

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Heart failure is a syndrome leading to a skeletal muscle myopathy with atrophy and shift toward fast contracting fibres type. It has been shown that the major cause of atrophy is muscle waste due to skeletal muscle myonuclei apoptosis. Apoptosis is triggered by circulating cytokines and their second messengers, in particular sphingolipids. Several attempts to block apoptosis have been tried: TNF α has been blocked with Embrel, Infliximab and thalidomide without success and this experimental avenue has been diverted. There are recent observations showing that apoptosis and atrophy can be prevented by blocking the Angiotensin II receptors. Another possible approach is to block the apoptotic cascade involving sphingomyelinase and caspases. This can be done in vivo with natural occurring substances such as carnitine that have been shown able to block both sphingomyelinase and mitochondrial caspases, or in vitro with other substances like N-oleoylethanolamine. Aim of our study is to shed some light on the pathophysiology of the heart failure myopathy, but at the same time to see whether apoptosis can be stopped with subsequent prevention of the myopathy (atrophy and Myosin Heavy Chains shift) and improvement of exercise capacity and symptoms. We decided to test this hypothesis both in vivo, in a well known and accepted animal model of heart failure (the

monocrotaline treated rat, that is a model of right ventricle failure secondary to pulmonary hypertension) and in vitro (on cultured myotubes where apoptosis can be induced either with staurosporine or doxorubicine). In case it was possible to block the apoptotic cascade and prevent muscle waste and atrophy, these research could be extended to heart failure in humans and to other myopathies, even genetic, in which it has been shown that apoptosis plays a determinant role in producing myocyte loss such as Duchenne Dystrophy.

[59] [ORAL COMMUNICATION]

CLINICAL STUDY AND IMMUNOPATHOLOGICAL PROFILE OF FOCAL MYOSITIS

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Focal myositis (FM) is a rare condition of skeletal muscle that presents as an inflammatory pseudotumour. No immunopathological study has been so far published. MHC class I and II molecules are expressed in muscle fibers in polymyositis (PM) and dermatomyositis (DM), but no difference in MHC I or MHC II staining patterns between PM and DM has been found. The study of various metalloproteinases (MMPs) in inflammatory myopathies has provided equivocal data. The aim of our study is to investigate MMP-2, MMP-7, MMP-9 in relation to the expression of MHC I and II in FM.

5 patients (3 F, 2 M; 18-71 yrs) were studied. Muscles involved were: gastrocnemius (two cases), quadriceps (one case), thigh muscles (two cases). EMG, muscle MRI study and biopsy were performed. An immunohistochemical study with antibodies against CD4, CD8, B cells subset, macrophages, C5b9, MHC class I and II, MMP2, MMP-7, MMP-9 was done.

Expression of MHC I antigen was found in muscle fibers independently of inflammatory infiltrates. No immunostaining for MHC II and MMP-7 was observed. MMP-2 was slightly expressed in few muscle fibers. A strongly cytoplasmic and sarcolemmal expression of MMP-9 was found.

Our results indicate that MHC class I molecules only are involved in the pathogenesis of FM. Absent staining for MMP-7 differentiate FM from PM.

[60] [ORAL COMMUNICATION]
 SIZE AND ASSORTMENT OF KPN1 REPEAT ARRAYS IN
 SUBTELOMERIC REGIONS OF HOMOLOGOUS 4Q35
 AND 11Q26 LOCI IN NORMAL SUBJECTS AND FSHD
 PATIENTS

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy caused by the deletion of a variable number of KpnI repeats on chromosome 4q35: similar contractions at the homologous 11qter locus are never associated with the disease. The identification of a BlnI restriction site within the 11qter repeat units that is absent in the homologous 4q repeat removed the interference of small 11qter fragments and led to a marked improvement of FSHD molecular diagnosis, but could not avoid the interference of 2 other factors: 1) interchromosomal exchanges between 4q and 11q KpnI arrays; 2) overlap of 4q35 EcoRI fragments between patients and normal individuals.

We analysed by PFGE the polymorphic 4q and 11q alleles in 110 normal individuals and 70 FSHD patients and families to assess: a) the size distribution of 4q alleles as compared with 11q alleles; b) the frequency of interchromosomal exchanges between 4qter and 11qter alleles c) the structural organization of the hybrid alleles.

Only 67% of healthy subjects and 75% of FSHD probands showed a canonical pattern of alleles, while the remaining subjects displayed different kinds of chromosomal rearrangements. In subjects carrying a canonical pattern of alleles the size of 4q alleles ranged between 30 and 200 kb while the size of 11q alleles ranged between 11 and 250 kb in size (p=0.002). 11q alleles shorter than 30 kb were detected in 12 individuals (1 of 17 kb, the others ranging 22-29 kb).

We did not find differences in the structural organization of the 4q-11q hybrid alleles between healthy and FSHD subjects.

[61] [ORAL COMMUNICATION]
 REGENERATIVE MYOGENESIS IN FES-INDUCED
 FUNCTIONAL RECOVERY OF HUMAN LONG-TERM
 PERMANENT DENERVATED MUSCLE

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Following denervation, skeletal muscle undergoes rapid loss in both mass and contractile force. Myofibers undergo atrophy, with an accompanying series of changes in structure, biochemistry and physiology. Several-months denervation finally results

in loss of myofibers, and their substitution by adipocytes and collagen sheets. Morphologic and molecular features of the long-term denervated muscle suggest that the original fibers are lost and those seen are the results of repeated cycles of cell death and regeneration. Markers of myogenic events in adult muscles are activated satellite cells and the presence of embryonic myosin and myogenic transcription factors. Functional electrical stimulation of permanent denervated muscle recovers from severe atrophy the mean size of the myofibers, and prevents apoptosis/necrosis and secondary degeneration.

We here describe histological, immunohistochemical and molecular methods, which identify markers of damage, regeneration, and repair on a muscle sample of just a few milligrams. A suitable source of material is a small biopsy, whose cryostat sections allow fiber typing and histopathology by immunochemical analyses. A few additional serial cryostat sections are used for molecular analyses. By electron microscopy we identify abnormalities of sarcomeric organization, nuclear distribution, mitochondrial content, and activated satellite cells. By light morphometry percent fat and/or interstitial tissue area, and fiber size distribution are determined. Myosin ATPases and immunohistochemistry are used to quantify fiber types, including early or late regenerated myofibers. Myosin Heavy Chain (MHC) patterns are indicators of regenerative events and of muscle adaptation to changing demands. Total protein content, myosin: total protein and myosin: actin ratios are determined by SDS-PAGE to reliably complement or substitute morphometry in quantifying muscle trophism in skeletal muscle biopsies.

Minimum diameter of the myofibers is 54.0+/-7.2 µm in normal muscle, 26.0+/-11 in upper-motoneuron denervated muscle and 21.7+/- 14.7 in lower motoneuron denervated muscle, while 48.53+/-25.60 in lower motoneuron denervated muscle after 2 years of FES. Frequency distributions of myofibers according to their minimum diameter in semi-thin sections show that about 30% are atrophic (minimum diameter smaller than 40 µm), while in not-FES denervated muscle about 90% are atrophic). A large proportion (60%) of myofibers are eutrophic i.e. with a minimum diameter larger than 40µm (11% in not-FES denervated muscle) or hypertrophic (11%), i.e. minimum diameter more than 80 µm. Similar results are present in spastic muscle after FES. Two years of FES regimen rise muscle trophism to almost normal values.

The re-established muscle trophism is confirmed by electrophoretic analyses showing that myosin:actin ratio is in between those of normal and severely atrophic myofibers of long-term denervated rat muscles. Hence results of molecular analyses are consistent with morphometry results, and have the advantage of being performed in a much shorter time and with substantial less man-power.

Analyses of biopsies from FES patients show regenerating myofibers. In denervated muscle 2.8±1.27 of myofibers are stained by anti-embryonic myosin antibody, 0.6±0.28 in spastic muscle after FES and 1.03±1.03 in completely denervated muscle after FES. Both small and large regenerated myofibers are present. When positive MHC-emb are more than 1% we can suppose that possible causes are the increased physical

activity during the two weeks, which precede biopsy. Indeed, a few myofibers have central nuclei, a feature suggesting they are no more than 11-day old.

Taken together the results of morphometry and molecular analyses solidly demonstrate that two-year-long FES substantially reverses the severe atrophy of many years of lower motoneuron denervation of human muscles, and that generative/regenerative myogenic events significantly contribute to the functional recovery of long-term paralyzed human muscles.

[62] [ORAL COMMUNICATION]

COLLAGEN TYPE VI DEFICIENCY DISRUPTS BASAL LAMINA-EXTRACELLULAR MATRIX BINDING IN ULLRICH CONGENITAL MUSCULAR DYSTROPHY AND COL6A1 NULL MUTANT SKELETAL MUSCLE

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Collagen type VI is a major extracellular matrix (ECM) protein expressed in several tissues, including skeletal muscle and skin. It consists of three genetically distinct α -chains ($\alpha 1$, $\alpha 2$, $\alpha 3$) secreted in the ECM where they form an extended microfilamentous network. In skeletal muscle, collagen type VI has been found abundantly at the basal lamina of muscle fibers, suggesting a role in anchoring basal lamina to the adjacent ECM. Recessive mutations in *COL6A2* and *COL6A3* genes, encoding $\alpha 2$ and $\alpha 3$ chain of collagen type VI, respectively, have been demonstrated to cause Ullrich congenital muscular dystrophy (UCMD) with both severe and mild phenotypes. UCMD is characterized by muscle weakness at birth, proximal joint contractures with distal laxity, and dystrophic changes in muscle biopsy. *Col6a1* null mutant mice develop muscle weakness and myopathy suggesting a key role of collagen VI in maintaining muscle integrity. To gain insight into the function of collagen type VI in muscle, we examined, by electron microscopy, the skeletal muscle from 4 patients affected by UCMD with partial deficiency of collagen type VI, and from *Col6a1* null mutant mice. We detected an empty space between the basal lamina and the ECM and no banded collagen fibrils were detected tightly associated to the *lamina reticulata*. Striking evidence of altered binding with the underlying connective tissue was detected in contracted fibers that showed absence of ECM components at level of sarcolemmal invaginations. Electron microscope examination of replicas obtained from cultured skin fibroblasts of the same patients revealed altered collagen type VI network. The secreted monomers formed microfilaments with a low ability to develop an interconnected network. These results suggest that

collagen VI deficiency induces disruption of ECM integrity in UCMD and *Col6a1* knockout mice muscle.

[63] [ORAL COMMUNICATION]

ECTO-ATPDASE ACTIVITY OF α -SARCOGLYCAN. POSSIBLE ROLE IN THE PATHOGENESIS OF SARCOGLYCANOPATHIES

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Extracellular nucleotides are important signaling molecules modulating various physiological responses. Nucleotides, liberated in the extracellular fluids as a result of cell lysis, exocytosis or efflux from transport proteins, exert their action through the binding to specific cell surface receptors: P1 and P2, activated by nucleosides and nucleotides, respectively. A central role in modulating nucleotides signaling is played by a family of ecto-enzymes, ectonucleotidases (NTPDases), that sequentially degrade ATP to eventually form adenosine. Thus, activity of ectonucleotidases both terminate the signaling, generate new signaling molecules and mediate the salvage of purines. Striking past and recent evidences suggest the occurrence of extracellular ATP signaling in skeletal muscle, even though its physiology remains elusive.

We previously demonstrated that α -sarcoglycan is an ATP binding protein that attributes to the purified dystrophin complex the ability to hydrolyze ATP (Betto et al., J Biol Chem 19, 7907-12, 1999). As the putative ATP binding site is located on the extracellular domain, our data suggested that α -sarcoglycan could be an ecto-nucleotidase. Genetic defects responsible for the primary or secondary deficit of α -sarcoglycan generate severe muscular dystrophies, suggesting that α -sarcoglycan is recipient of an essential function for muscle physiology and survival. Therefore, we decided to further investigate the putative ATP hydrolyzing activity of α -sarcoglycan. During differentiation of C2C12 cells, the expression level of α -sarcoglycan rises in parallel to the level of total NTPDase activity of cells. Importantly, about 20% of the activity is inhibited by an antibody specific for the extracellular portion of α -sarcoglycan. This result demonstrates both that α -sarcoglycan contribute to the ATP-hydrolyzing activity of C2C12 myotubes and also that these cells express other ecto-enzymes. To better characterize the activity of α -sarcoglycan, we then transfected the protein in HEK 293 cells, normally low in NTPDase activity. HEK 293 cells transfected with α -sarcoglycan demonstrate a large rise of activity, which requires as substrate ATP or ADP (at a ratio 3:1) while is not effective with other triphospho-nucleosides. Moreover, at variance with other NTPDases, α -sarcoglycan activity is stimulated by the contemporary presence of both Ca^{2+} and Mg^{2+} . Our results conclusively demonstrate that α -sarcoglycan is an enzyme that hydrolyze extracellular ATP, thus partici-

pating to the signaling of extracellular nucleotides. These finding offers new clues on the possible effects of the lack of α -sarcoglycan activity in the pathogenesis of muscular dystrophies.

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[64] [POSTER]

REGULATION OF MUSCLE GROWTH BY DIFFERENT
SIGNALING PATHWAYS. ROLE OF IGF 1 AND AT-
ROGIN1

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Increasing evidence indicates that in eukaryotes cell growth (mass accumulation) is finely regulated in response to environmental and developmental conditions and can be deranged in diseases such as diabetes and cancer. The rate of muscle mass accumulation is controlled not simply by the availability of nutrients but by signaling pathways that coordinate nutritional, hormonal, and mitogenic signals. Increasing evidence suggest that IGF1, through Akt/PKB pathway, is involved in mass accumulation. On the other side two muscle-specific ubiquitin ligases (E3s), called Atrogin 1/MAFbx and MuRF 1, have recently emerged as crucial factors in the regulation of mass reduction. Both the E3s are upregulated in different catabolic conditions like diabetes, cachexia, denervation, fasting etc. We studied which signal transduction regulates Atrogin 1 and MuRF 1 expression by using an in vitro and in vivo approach. Different adeno- and retro-viral constructs for IGF1 pathway have been used. The results show that IGF 1 controls Atrogin 1 by suppressing its expression. Moreover IGF 1 suppressed protein breakdown induced by dexamethasone and T3 treatments and lowered the proteolysis below the basal level. These results indicate that muscle mass is maintained by the inhibition of ubiquitin/proteasome system performed by IGF 1 and open new perspectives in the design of new drugs for the intervention in muscle disease.

[65] [POSTER]

FROM CLINICAL TO BIOMOLECULAR FINDINGS IN 17
KINDREDS WITH DM2

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Myotonic dystrophy type 2 (DM2/PROMM/PDM) is still often misdiagnosed and unrecognized. Genetic testing is not available yet on a routine basis in most laboratories. The diag-

nosis of DM2 is still in fact one of exclusion. To describe clinical, laboratory and genetic findings in 46 patients from 15 unrelated kindreds with genetically confirmed DM2 and provide a useful clinical diagnostic approach to an adult patient with a myotonic syndrome. 94 patients with a myotonic syndrome having normal CTG repeat expansions (age range 48 $\pm$ 7, age range 24-70, 52 women, 42 men) were subjected to: (i) a detailed clinical protocol including manual and quantitative muscle strength assessment (QMA); quantification of myotonia using subjective, functional, and measures of relaxation time using QMA protocol and by EMG; (ii) laboratory investigation to determine multisystem involvement including ocular, cardiac, cognitive, behavioral and endocrine involvement; (iii) genetic screening using PCR, rapid PCR assays and Souther Blot on leukocyte DNA; (iv) biceps muscle biopsy.

Of these 46 patients included in the protocol study, the clinical diagnosis of DM2 was confirmed genetically in 13 of 17 kindreds (73%). Of these one kindred shares linkage to chromosome 7q for the C1CN1 channel gene. The remainder 3 kindreds are subject of ongoing genetic and laboratory screening.

Our clinical and laboratory approach to the myotonic syndromes demonstrates that a strict diagnostic clinical and laboratory protocol is mandatory for the genetician to confirm the diagnosis of DM2 especially for sporadic cases and non-informative families.

[66] [POSTER]

HOMOPLASMIC T3394C MTDNA MUTATION AND GENETICALLY DOCUMENTED CPT DEFICIENCY IN A PATIENT WITH RAGGED RED FIBERS AT MUSCLE BIOPSY AND MYOGLOBINURIA

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We describe a 45 y. o. male patient who came to our observation after an episode of acute renal failure due to myoglobi-nuria, accompanied by intense myalgias and generalized weakness. Serum CK levels were over 30,000 U/L at onset of symptoms (normal values < 195 U/L) and still around 8,000 U/L one week later, after dialysis treatment. He completely recovered, with serum enzyme normalization, in a few weeks. In the past, the patient had presented similar episodes of myalgias and exercise intolerance with dark urine. Neurological examination was normal and family history negative.

Left biceps muscle biopsy was normal except for the presence of some both COX-positive and COX- negative ragged red fibers. The clinical picture was highly suggestive for a Carnitine Palmitoyl-Transferase (CPT) deficiency, but a respiratory chain disorder, particularly cytochrome *b* gene mutations (Andreu et al, 1999), was also suggested by the morphological pattern. Biochemical CPT assay showed a marked

CPT deficiency (isotope 64.5 pmol/min/mg, nv 452 +/- 160; forward 147 pmol/min/mg, nv 367 +/- 111) and genetic screening (1p32 gene) revealed that the patient is heterozygous for the common S113L substitution. Search for the other mutation is underway. Complete mtDNA sequence did not reveal any cytochrome *b* gene mutations, but disclosed a homoplasmic T3394C point mutation, normally related to the Leber's hereditary optic neuropathy (LHON) phenotype, but also described in association with the A3243G mutation in patients with diabetes mellitus (Ohkubo et al, 2001).

Two further patients with identical clinical and morphological features are presently being studied.

[67] [ORAL COMMUNICATION]

MUTATIONAL SCREENING OF THE ENTIRE MTDNA IN 66 UNRELATED PATIENTS WITH MITOCHONDRIAL DISEASE

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Mutations in mtDNA account for approximately 40% of adult-onset and 11% of paediatric mitochondrial encephalomyopathies. We analyzed the sequence of the entire muscle mtDNA in a cohort of 66 mitochondrial patients. Inclusion criteria were (1) absence of genetic diagnosis, and (2) presence of biochemical OXPHOS defects, or (3) typical morphological abnormalities in muscle, or (4) both. Approximately 41% of the patients were adults, while the remaining were paediatric cases. A total of 43 muscle biopsies were analyzed biochemically. Ten samples had isolated complex I (CI) deficiency; five had isolated complex IV (CIV) deficiency, four had a combined deficiency of CI and CIV, two had isolated complex III (CIII) deficiency. Twenty samples had multiple respiratory defects, while the remaining samples were normal. Most of the adult cases had muscle morphological abnormalities indicating a mitochondrial myopathy. A total of five pathogenic heteroplasmic mutations were found. Four mutations have already been described: two mutations in ND3 and ND5 genes were associated with Leigh syndrome with CI deficiency. An A3291G change in tRNA-Leu(UUR) was found in a MELAS patient, while a 4272Cins mutation in tRNA-Ser(UCN) was present in a proband with a complex phenotype including hearing loss, cognitive deterioration, and a monomelic second motor neuron disease affecting the left upper limb. A fifth mutation, in tRNA-Asn, was novel and associated with severe, adult-onset, isolated mitochondrial myopathy in a sporadic patient. Several new homoplasmic changes were also detected, whose pathogenic significance remains uncertain. The absence of clearly pathogenic mtDNA mutations in 61/66 samples strongly indicates the nuclear origin of the primary genetic defect in most of our patients.

[68] [POSTER]

ULTRASONIC TISSUE CHARACTERIZATION AND DOPPLER TISSUE IMAGING IN THE ANALYSIS OF LEFT VENTRICULAR FUNCTION IN GENETICALLY CONFIRMED FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant inherited disorder with an incidence of 3 to 5 cases per 110000. Lack of evidence exists on the presence of cardiac involvement in FSHD. Twelve patients with genetically confirmed FSHD (7 males, aged 49.2±17.5 years) and 12 matched healthy subjects underwent assessment of left ventricular (LV) structure and function through the combined use of conventional echocardiography, ultrasonic integrated backscatter signal (IBS) and tissue Doppler imaging (TDI). The cyclic variation of the IBS (CV-IBS) of both septum and posterior wall were analyzed. Myocardial velocities from the apical view were sampled at septum and lateral wall levels by pulsed-wave TDI. On conventional echocardiography, LV function was comparable between the two groups. CV-IBS at the septum (4.9±1.6 dB) and the posterior wall (7.3±1.9) were lower in the FSHD compared to controls (septum: 9.2±1.2 dB; lateral wall 11.4±2.3 dB, p<0.001). On regards to TDI measurements, no significant differences were seen in the diastolic myocardial velocities between the two groups, whereas FSHD exhibited a lower peak of the systolic wave of both septum (7.5±1.2 cm/sec) and lateral wall (8±1.8 cm/sec), as compared to controls (septum: 8.9±0.7 cm/sec; lateral wall: 11.6±1.1 cm/sec, p<0.001). No correlation was found between CV-IBS or TDI parameters and KpnI-BlnI 4q fragment size. This investigation provides the first secure evidence of cardiac involvement in FSHD. Ultrasonic tissue characterization with the intrinsic contractility study and the evaluation of the regional function should, therefore, represent a new integrated diagnostic modality for the evaluation of left ventricular function.

[69] [POSTER]

LAMP-2 DEFICIENCY: TWO NEW ITALIAN CASES

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Primary LAMP-2 deficiency is an X-linked glycogen storage disease characterised by the clinical triad of cardiomy-

opathy, vacuolar myopathy and mental retardation, known previously as Danon's disease. LAMP-2 is a lysosomal membrane structural protein and mutations in the lamp-2 gene have been reported in 11 patients, only one of them being an Italian case. Here, we describe two new Italian cases of LAMP-2 deficiency. A 23 year-old man had received a diagnosis of Wolf-Parkinson-White syndrome at the age of 21 and one year later developed an hypertrophic cardiomyopathy. He was admitted to our department because of muscle fatigability and persistent increased serum CK (783 UI/L). Neurological examination was normal. EMG revealed increased percentage of polyphasic motor unit potentials. The second case, a 17 year-old boy, had had a floppy infant syndrome at birth and a slight delay in the development milestones. At the age of 4 a brain MRI showed a partial corpus callosum agenesis. Since childhood he complained of proximal muscle weakness and exercise intolerance. Clinical examination showed waddling gait, mild proximal weakness, brisk reflexes and bilateral Babinski sign. Mild mental retardation was also evident. Increased serum CK (500 UI/L) was found. EMG showed a myopathic pattern with presence of myotonic discharges. ECG and echocardiography revealed an hypertrophic cardiomyopathy. Muscle biopsy revealed in both patients a vacuolar myopathy with increased glycogen content. Biochemical analysis showed normal acid maltase activity. LAMP-2 immunoreactivity was absent but vacuoles strongly stained for limp-1. C5b-9 complement fraction immunodelineated vacuoles.

[70] [ORAL COMMUNICATION]

FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY: A MULTICENTER STUDY ON OCCURRENCE OF AUDITORY ALTERATIONS

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Little information is reported on the involvement of extra-muscular tissues in Faciocapulo humeral Muscular Dystrophy (FSHD), a degenerative myopathy usually associated with a 4q35 deletion. In classical FSHD patients, the few investigations aimed to detect the possible impairment of hearing functions carried out controversial results. The clinical features of 96 cases with the characteristic 4q35 deletion are under evaluation by our multicenter study: currently, it is mainly focused on occurrence and definition of their possible auditory alterations. Until now, we had considered 49 out of our 96 FSHD cases: they were 23 males and 26 females with a mean age of 48 years (range: 14-76). These patients had clinical hearing examination by an otologist, who also investigated possible previous exposure to noise trauma, ototoxic sub-

stances and ear infections. Auditory function was evaluated by an electronic audiometer: audiograms of both ears were obtained by measuring hearing ability for 125 to 8000 Hz. Far-field Brainstem Auditory Evoked Responses (BAER) were also evaluated in 16 of them. In the absence of auditory symptoms, in some of our patients the audiometric study showed a mild to moderate hearing impairment. This sensorineural alteration was detected in 16 of them (33%) and concerned mainly the high-frequency tones, 4000-8000 Hz. They were 9 males and 7 females and the majority of them (13/16) were aged over 50 years (50 to 76). Comparison with standardized tables and with the frequency of hearing pathology in controls of the same age, indicates that the auditory alterations found in our FSHD patients were similar to those found in normal population. BAER detected mild alterations of cochlear type in three of the 16 cases examined. On the whole, our investigation appears to point out that hearing impairment in classic FSHD is not more common than in the normal population.

[71] [ORAL COMMUNICATION]

TUMOR NECROSIS FACTOR (TNF)- α IN MITOCHONDRIAL MYOPATHIES

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TNF- α , a pleiotropic cytokine, is considered to play an important role in the pathogenesis of inflammatory and autoimmune diseases. Mitochondrial dysfunction leads to hydrogen peroxide production and, subsequently, to oxidative stress which is a potent inducer of TNF- α expression. We investigated in muscle biopsies from patients with mitochondrial myopathies the immunohistochemical expression of TNF- α and of TNFR I and TNFR II (TNF- α receptors). Ragged red fibers, fibers with subsarcolemmal mitochondrial accumulation and COX negative fibers without evident structural abnormalities were positive for TNF- α . TNF- α immunoreactivity corresponded to the distribution of mitochondrial proliferation observed by SDH stain and by confocal microscopy co-localized with COX subunit IV immunoreactivity. TNF- α positive fibers were also immunoreactive for TNFR I and TNFR II. We previously demonstrated that TNF- α immunoreactive muscle fibers were also reactive for the antioxidant agents MnSOD and GSH. Taken together these data suggest that in muscle fibers of patients with mitochondrial myopathies (1) TNF- α expression might be induced by the oxidative stress, (2) TNF- α could modulate the antioxidant activity and (3) TNF- α action might be mediated by its receptors TNFR I and II on mitochondrial membrane.

[72] [SCARLATO LECTURE]
NEW ANTIBODIES IN MYASTHENIA GRAVIS

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Myasthenia gravis (MG) is caused in the majority of cases by circulating autoantibodies to the nicotinic muscle acetylcholine receptor (AChR), the main neurotransmitter receptor at the neuromuscular junction. In MG, anti-AChR antibodies are generally measured by immunoprecipitation of ¹²⁵I- α -bungarotoxin-labelled AChRs. α -bungarotoxin is a neurotoxin from the venom of *Bungarus multicinctus*, the banded krait, that binds very specifically and irreversibly to the AChR. MG is being increasingly recognized in the elderly. Several studies have now documented a higher incidence of AChR positive MG in both men and women over age 60 years than among younger individuals (Vincent et al JNNP in press). Since MG can be misdiagnosed as stroke or motor neuron disease in the elderly, it is likely that there is considerable under-diagnosis of MG in this age group.

Maternal antibodies to the fetal isoform of the AChR are found in rare cases of arthrogryposis multiplex congenital. The cross the placenta and cause paralysis in the developing fetus leading to joint contractures and other deformities.

Antibodies to AChRs are present in about 85% of patients with generalized disease, but only in about 50% of patients with purely ocular muscle weakness. Recently, antibodies to the muscle specific receptor tyrosine kinase, MuSK, have been identified in 40-70% of SNMG patients; those with MuSK antibodies often present with predominant ocular and bulbar weakness, or neck extensor weakness, and be more difficult to treat effectively with conventional immunosuppression (Scuderi et al Lab Invest 2002; Vincent et al Lancet Reviews Neurology 2002; Sanders et al, Neurology in press).

SNMG patients without MuSK antibodies also have an immune mediated disease. Their plasma or sera inhibits the function of AChR expressed on TE671 cells, or on the subline that expresses adult AChRs. The effect appears to be indirect, perhaps acting on another muscle surface receptor that activates a second messenger system leading to reduced AChR function. This plasma factor is not an IgG and co-purifies with IgM (Plested et al Neurology 2002).

AChR antibodies are present in only 50% of ocular MG patients, but MuSK antibodies and the non-IgG factor are not present in those tested so far. Therefore, there may be another, ocular muscle-specific target, in this form of MG.

[73] [ORAL COMMUNICATION]
EXPRESSION OF PLECTIN IN MUSCLE FIBERS WITH
CYTOARCHITECTURAL ABNORMALITIES

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Plectin is a protein belonging to the cytoskeletal anchoring system, concentrated at sites of mechanical stress in different cell types. In normal skeletal muscle, plectin is located at level of Z-discs, sarcolemma, post-synaptic membrane, and intermyofibrillar network. We investigated plectin immunocytochemistry in lobulated fibers, fibers with tubular aggregates, target fibers, central core disease and centronuclear myopathy. 30-40% of lobulated fibers had patchy increase of plectin immunoreactivity at sarcolemmal level with focal subsarcolemmal increases. Tubular aggregates revealed a low binding for plectin. 10% of central cores exhibited faint focal increase of plectin immunoreactivity. Target formations had a normal plectin pattern. In centronuclear myopathy, plectin immunoreactivity was increased around the centrally located nuclei in 8-12% of the fibers, at the sarcolemma of 51% of type 2 fibers, and at the membrane of small vacuoles located peripherally around the central nuclei. We postulate that plectin may play a role in the subsarcolemmal aggregation of mitochondria in the lobulated fibers, and in the central position of nuclei as well as in shape formation, positioning and moving of the vacuoles in centronuclear myopathy.