5th Aim Congress

ITALIAN ASSOCIATION FOR MYOLOGY

Jolly Hotel delle Terme – Ischia, Naples, Italy,

June 16-18, 2005

Foreward

I am delighted to be asked to write a foreward to the 5th Meeting of Italian Society of Myology, since this is a remarkably comprehensive meeting and contributes significantly to our store of knowledge in Myology with several oral communications, posters and case presentations (Muscle Club) and follows the previous meetings in Camogli, Torino, Padova and Taormina.

During the three days of the Congress lectures will be held on the following topics: Heart in Muscle Disorders and Advances in Therapy of Muscle Disorders.

Moreover I am touched by the fact that there will be before a Meeting Session of the Gaetano Corte Prize winners (last year in Kusadasi the recipients were dr. George Karpati, Dr. Jane Miller and myself) because so many distinguished scientists and doctors and clinical investigators in the field of neuromuscular disorders are here. All doctors and scientists must have an inspiration by Gaetano Conte: it is good to pay tribute to this Neapolitan doctor. In fact all doctors must on occasion believe that they have discovered something new or hope that they bring a significant contribution, to find there that someone else had made the identical discovery many years earlier.

In fact X-linked severe pseudohypertrophic muscular dystrophy has been named by the great French neurologist Duchenne but the contribution of Edward Meryon in pathology has been demonstrated by Alan Emery and probably the first clinical description is the one by Gaetano Conte as prof. Nigro will illustrate in his opening lecture, although I think the disease must have been present several hundred of years before because of the high rate of spontaneous mutation of dystrophin gene. Therefore in beginning research in muscular dystrophy we must acknowledge the contribution all physicians involved in this discovery i.e. Duchenne de Boulogne, Edward Meryon, Gaetano Conte, as well as the molecular biologists that described the gene and its protein product (L.M. Kunkel and E.P. Hoffmann) because muscular dystrophy is world-wide and I believe it will attract medical historians now and in the future.

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Program

Thursday, 16th, 2005

15.00 – 16.00 REGISTRATION

16.00 – 17.00 ITALIAN ASSOCIATION FOR HISTORY OF MYOLOGY
Introduction: C. Angelini – G. Vita
Conte, Duchenne or Meryon Muscular Dystrophy? G. Nigro

17.00 – 19.00 ORAL COMMUNICATIONS
Chairmen: A. Prelle – C.P. Trevisan
De Angelis MV, Di Iorio A, Ferrucci L, Capasso M, Abate G., Uncini A: PREVALENCE OF ASYMPTOMATIC HYPERCKEMIA: AN EPIDEMIOLOGICAL STUDY.
Di Giacopo R, Tartaglione T, Catteruccia M, Chiatamone S, Cianfoni A, SilvestriD'Amico A, Servidei S: PHENOTYPIC VARIABILITY ASSOCIATED WITH THE A3243G "MELAS"MUTATION IN MITOCHONDRIAL DNA: CLINICAL, EURORADIOLOGICAL AND MOLECULAR GENETIC STUDIES.
Doglio L, Orsini N, Scano C, Pedemonte M, Scapolan S, Camoriano R, Minetti C: PROGNOSTIC FUNCTIONAL TEST IN DUCHENNE MUSCULAR DYSTROPHY WALKING PATIENTS.

Ferrari G, Lamantea E, Donati A, Filosto M, Briem E, Carrara F, Parini R, Simonati A, Santer R, Zeviani M: INFANTILE HEPATOCEREBRAL SYNDROMES ASSOCIATED WITH MUTATIONS IN THE MITOCHONDRIAL DNA POLYMERASE-{GAMMA}A.

Previtali S, Menditto I, Grassi S, Rodolico C, Bestini E, Merlini L, Pegoraro E, Palmucci L, Carrera P, Comi G, Quattrini A, Ferrari M, Benedetti S: GENETIC HETEROGENEITY OF LAMIN A/C ASSOCIATED PHENOTYPES.

Ricci E., Frusciante R., Merico B, Silvestri, Di Lella G, Broccolini A, Tartaglione T, Tonali P, Mirabella M.: MR INVESTIGATION IN INFLAMMATORY MYOPATHIES

Rodolico C, Pastura C, Sinicropi S, Ghirlanda P, Toscano A, Messina C, Vita G: AUTOIMMUNE JUVENILE LIMB-GIRDLE MYASTHENIA

Sansone V, Aimè E, Contardi D, Meola G: SLEEP APNEA DETECTION IN MYOTONIC DYSTROPHY USING NASAL PRESSURE HOLTER RECORDING.

20.00 Welcome Cocktails of the Italian Association for Myology (AIM)

Friday, June 17th, 2005

7.00 – 8.00 "NEUROMUSCULAR RUN"

8.30 – 10.30 SESSION I: HEARTH IN MUSCLE DISORDERS

Chairmen: L.I. Comi – E. Bertini

Arbustini E.: GENETIC CARDIOMYOPATHIES

Bellocci F: HEART IN MYOTONIC DYSTROPHY

Boriani G.: HEART IN EMERY-DREIFUSS DYSTROPHY

Politano L.: CARDIOMYOPATHIES IN DYSTROPHYNOPATHIES

10.30 – 11.30 Coffee break and Poster Session 1

11.30 – 13.00 ORAL COMMUNICATIONS

Chairmen: G. Nigro – M. Moggio

Pastorello E, Rigoni MT, Armani M, Cattarin A, Pompato S, Angelici C, Tomelleri G, Tonin P, Mongini T, Siciliano G, Barchetta A,Trevisan CP: FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY AND HEART ARRHYTHMIA: DATA FROM A MULTICENTER STUDY AND REVIEW OF THE LITERATURE

Petretta VR, Palladino A, Sannino P, Passamano L, Nigro Ge, Comi LI, Politano L, Nigro G: LEFT VENTRICULAR DIASTOLIC FUNCTION IN MYOTONIC DYSTROPHY.

Politano L, Messano L, Dello Russo A, Palladino A, Petretta VR, Pace M, Pelargonio G, Sannino P, Nigro Ge, De Martino G, Sanna T, Passamano L, Casella M, Chiodelli R, Mangiola F, Valsecchi S, Parisi Q, Ierardi C, Zecchi P, Comi LI, Della Bella P, Nigro G, Bellocci F: MULTICENTER STUDY TO EVALUATE THE ARRHYTMIC RISK IN MYOTONIC DYSTROPHY TYPE 1: RESULTS OF ONE-YEAR COMMON PROTOCOL

Spinazzola A, Alberio S, Meznaric-Petrusa M, Zidar A, Ferrero I, Calmieri L, Zeviani M: The first recessive mutation of ANT1 is associated with early onset hypertrophic cardiomyopathy and skeletal myopathy.

Lamperti C, Naini A, Lucchini V, Zecca C, Prelle A, Fagiolari G, Bresolin N, Di Mauro S, Moggio M: STATIN RELATED MYOPATHY AND COQ10 DEFICIENCY.

Vercelli L, Palmucci L, Toscano A, Musumeci O, Rodolico C, Anatrine K, Marena G, Vita G, Mongini T: STATIN-INDUCED MYOPATHY: EVIDENCE OF REDUCED CoQ_{10} LEVELS IN MUSCLE TISSUE OF 32 PATIENTS

13.00 – 14.00 Lunch and AIM Board Meeting

14.30 – 16.30 ORAL COMMUNICATIONS

*Chairmen: V. Nigro – S. Servide*i

Dalla Libera L, Ravara B, Gobbo V, Angelini A, Vescovo V: SKELETAL MUSCLE FIBRES SYNTHESIS IN HEART FAILURE:ROLE OF PGC-1α, CALCINEURIN AND GH.

Ferlini A, Rafani A, Taddei Masieri A, Toscano A, Cudia P, De Grandis D: LINKAGE ANALYSIS IN A FAMILY WITH NON-KINESIGENIC DYSKINESIA REVEALS 2Q33-Q35 MAPPING AND SUGGESTS A MYOFIBRILLOGENESIS REGULATOR 1 GENE INVOLVEMENT.

Galluzzi F, Volpi L, Dolfi A, Segnani C, Falorni M, Pistoleri S, Tornei F, Fontanini G, Siciliano G: EXPRESSION OF RYANODINE RECEPTOR IN PATIENTS WITH CHRONIC FATIGUE SYNDROME AND HIGH EXERCISE LACTATE LEVELS.

Pescatori M, Bernardini C, Bertini E, Minetti C, Bruno C, Mercuri E, Tonali PA, Ricci E: EXPRESSION PROFILING IN EARLY PHASE DMD

Pisani V, Panico MB, Terracciano C, Meola G, Merlini L, Previstali SC, Bonifazi E, Novelli G, Angelici C, Massa R: HISTOPATHOLOGICAL AND MORPHOMETRIC ANALYSIS HELP TO DIFFERENTIATE MYOTONIC DYSTROPHY TYPE 1 AND 2

Traverso M, Ricci E, Donati MA, Assereto S, Stringara S, Broccolini A, Zara F, Bruno C, Minetti C: PREMATURE TRUNCATION OF CAVEOLIN-3 SUGGESTS HAPLOINSUFFICIENCY IN AUTOSOMAL RECESSIVE RIPPLING MUSCLE DISEASE

Vattemi G, Tonin P, Bini L, Lunari C, Rizzato N, Tomeller G: CIRCULATING MUSCLE-SPECIFIC AUTOANTIBODY TO CARBONIC ANHYDRASE III IN A PATIENT WITH MYOFIBRILLAR MYOPATHY

Zecca C, Ciscato P, Fagiolari G, Angeletti B, Gabellini D, Prelle A, Bresolin N, Moggio M, Tupler R: MORPHOLOGICAL FEATURES OF FSHD MOUSE MODEL.

16.30 – 17.30 Coffee break and Poster Session 2

17.30 – 19.00 ORAL COMMUNICATIONS

Chairmen: VR Petretta – C. Minetti

Bertini E, Messina S, Battini R, Berardinelli A, Boffi P, Bruno C, Cini C, Colitto F, D'Amico A, Minetti C, Mirabella M, Mongini T, Moranti L, Orcesi S, Pelliccioni M, Pini A, Swan AV, M. Villanova, Vita G, Main M, Mercuri E: RELIABILITY OF THE HAMMERSMITH FUNCTIONAL MOTOR SCALE IN A MULTICENTRIC STUDY.

Chisari C, Licitra R, Rossi B: THE BLOCK OF CA-DEPENDENT K^+CHANNELS REDUCES MYOTONIA IN STEINERT DISEASE: AN IN VIVO PHARMACOLOGICAL STUDY.

Melone MAB, Petillo O, Calarco A, Torpedine A, Margarucci S, Peluso G: MUSCLE TISSUE ENGINEERING: STRATEGIES FOR REPAIR AND REGENERATION IN HUMAN DEGENERATIVE MUSCLE DISEASES.

Messina S, Aguennouz M, Macellani N, Monici MC, Arcelli D, Seminara P, Volinia S, Squadrito F, Vita G: EFFECTS OF NF-κB BLOCKADE ON MUSCLE GENE EXPRESSION IN MDX MICE.

Piazza S, Alì G, Falorni M, Mancuso M, Pistolesi S, Fontanini G, Siciliano G: MYOPATHY AND MITOCHONDRIAL IMPAIRMENT IN PATIENTS WITH HYPERFERRITINEMIA OF DIFFERENT ORIGIN

Rimessi P, Gualandi F, Spitali P, Calzolai E, Tuffery S, Merlini L, Ferlini A: DYSTROPHIN RE-FRAMING BY ANTISENSE OLIGONUCLEOTIDES: SPECIFIC EXON SKIPPING IN IN VITRO SYSTEMS

19.00 – 20.00 AIM General Assembly

20.30 Social Dinner and Best Poster Award

Saturday, June 18th, 2005

8.15 – 11.15 ROUND TABLE: PERSPECTIVES IN THERAPY

Chairmen: L. Merlini – G. Novelli

Bresolin N., Torrente I.: STAMINAL CELLS FOR MUSCLE DISORDERS: TATE OF THE ART

Bruni S.: PRELIMINARY RESULTS OF ENZYME REPLACEMENT IN TYPE 2 GLYCOGENOSIS

Minetti C.: PROTEASOME INHIBITORS I DYSTROPHIN DEFICIENT MUSCLE

Vita G.: NF-KB INHIBITORS IN MDX MOUSE

Bonaldo P.: CICLOSPORINE IN COLLAGEN VI DEFICIENCY MYOPATHY

Mercuri E.: BUTIRRATE IN SMA

11.15 – 11.30 Coffee break and Poster Session 3

11.30 – 13.30 MUSCLE CLUB: CLINICAL CASES

Chairmen: T. Mongini – L. Politano

13.30 - 14.00 ECM Questionary

14.00 Closing Remarks

ABSTRACTS

DYSTROPHIN GENE AND SHOX GENE MUTATIONS IN A CHILD WITH BECKER MUSCULAR DYSTROPHY AND SHORT STATURE

Aguennouz M, Messina M.F, Rodolico C, Messina S, Valenzise M, Crisafulli G, Vita G

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Growth retardation has been previously described in Duchenne muscular dystrophy (DMD) but its physiopathological mechanism has never been clarified. Short stature has never been reported in children with Becker muscular dystrophy (BMD). We describe a boy, aged 2.7 years, who was referred to our unit of Pediatric Endocrinology due to short stature (-2.8 SDS). His height was lower than target height (-1.9 SD) and bone age was normal. Physical examination showed lumbar lordosis, frontal bossing, mild skeletal disproportions with short legs, shield chest, small hands and hypertrophic calves. Laboratory analyses excluded some of the commonest causes of short stature (celiac disease, thyropathy, inflammatory and chronic diseases), but showed elevated levels of CK (5056 U/L). Neurological examination revealed waddling gait with tip-toe walking and positive Gower's sign. Electromyography was myopathic. Molecular analysis of dystrophin gene detected a deletion of exons 45-52. Diagnosis of BMD was made by dystrophin immunocytochemistry on muscle biopsy. Increased CK level to 4133 U/L and the same dystrophin gene deletion were found in his older brother (6-year-old) who was asymptomatic and had a normal neurological examination. In order to explain the severe height impairment in the younger brother, we searched for mutation in the homeobox gene SHOX (Xp 22.3 locus), causing growth retardation and skeletal abnormalities and associated with Leri-Weill, Langer and Turner syndromes. A novel homozygous mutation, a transition G→A at nucleotide position 972 in exon 3, was found. Such an association of different gene mutations has never been reported. The presence of SHOX mutations as cause of short stature in DMD deserves further investigation in a large cohort of patients.

MOTHER AND SON WITH MUSCLE FATIGABILITY AND CARDIOMYOPATHY

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This boy noticed at 18 years easy fatigability, bradycardia and WPW syndrome, and thus he was not enrolled in the army. At age 22 he had very low aerobic resistance and at age 27 an echocardiography showed mild left ventricular dilation, an ophthalmologic exam revealed severe myopia with pigmented retinal dystrophy, an abdominal echography showed hepatomegaly. He was hospitalized at age 28 to investigate his fatigability associated with high CK (1094 U/L, n.v. 0-190). He complained of difficulty in climbing stairs and lifting weights. On physical examination he had waddling gait with hyperlordosis, Gower's sign, abolished foot dorsiflexion with distal leg muscle hypotrophy, mild weakness of proximal, neck flexor and facial muscles. On neuropsychological evaluation he had delayed psychomotor development, IQ (WAIS-R) was 77.

Muscle CT scan revealed moderate proximal and distal atrophy of lower limbs. A mild restrictive ventilatory dysfunction was present on spirometry. Skeletal muscle biopsy showed a vacuolar myopathy with accumulation of PAS positive material.

His mother suffered since age 26 of palpitations and easy fatigability; at age 29 her EKG showed WPW syndrome with paroxysmal atrial flutter, she was cardioverted first electrically, then pharmacologically. At 38 years addominal echography showed hepatosplenomegaly. At age 51 a pacemaker-defibrillator was implanted and one year later she underwent cardiac transplantation. Since then, she reported muscular weakness and myalgia. At age 54, on neurological exam she had weakness and atrophy of proximal limb muscles. Quadriceps femoris muscle biopsy showed mild myopathic changes. A molecular study demonstrated a new mutation in LAMP 2.

INCLUSION BODY MYOSITIS AND RHEUMATOID ARTHRTIS: A CASE REPORT

Azan G, Bono G, Mauro A, Delodovici M.L, Broggini M

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Inclusion body myositis (IBM) is an inflammatory muscle disease characterized by a slowly progressive course.

It has been recognized an association with autoimmune diseases. We describe a patient with rheumatoid arthritis (RA) and IBM. A 37 year-old man was referred because he presented over the past five years a progressive painless, bilateral and asymmetric, upper limb weakness and wasting.

He had seropositive RA since he was 22 years old; he discontinued of his own free will the treatment with prednisone and chloroquine after having taken it for few months. Neurological examination disclosed bilateral upper limb weakness and wasting, asymmetric and predominant on the left side, mainly affecting deltoid, biceps, wrist extensor and flexor muscles; mild and bilateral weakness and wasting of quadriceps were present at lower limbs; deep tendon reflexes were decreased; swallowing was normal. Rheumatoid factor, erythrocyte sedimentation rate, C Reactive Protein were elevated; CK 218 U/L (n.v. <190); thyroid hormones were within normal range. EMG revealed fibrillation at rest

and abnormalities of motor units consistent with myopathic changes; nerve conduction studies were normal. Muscle biopsy showed rimmed vacuoles, endomysial mononuclear cellular infiltrates, Congo red positivity and up-regulation of MHC class I on light microscopy; tubulo-filamentous inclusions both in the nucleus and in the cytoplasm were evident on electron microscopy.

It seems feasible that an immune mechanism may take place in IBM associated with RA; besides, the acknowledgement of the two coexisting diseases may influence the therapy evaluation.

CONGENITAL MUSCULAR DYSTROPHY, MENTAL RETARDATION, UNTRACTABLE EPILEPSY, UNUSUAL GYRATION ABNORMALITIES AND ABNORMAL αDYSTROGLYCAN GLYCOSILATION: CASE REPORT

Berardinelli A (1,2), Cardinali S (2), Colamaria V (3), Lanzi G (2), Morello F (1), Muntoni F (4), Torelli S (4),Veggiotti P (2)

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Congenital Muscular Dystrophies (CMD) are an expanding group of disorders characterised by muscular weakness with or without brain and eye abnormalities. It is well known that some of these disorders are due to O-glycosilation abnormalities. The causative genes of several different CMD have been found in the last years such as different biochemical abnormalities and clinical phenotypes have been described. Anyway there is growing evidence for cases clinically resembling CMD but without any mutation in the known genes. We describe here a 14 years old boy with high CK, severe mental impairment and speech delay, eyes abnormalities, peculiar gyration abnormalities, seizures resistant to therapy with EEG bisynchronism and abnormal dystroglycan glycosilation pattern in muscle. Laminin overlay shows that some ability to bind laminin is retained while agrin overlay shows only a very small amount of residual binding activity. FKRP and POMGtn1 genes are normal.

A MUTANT ACTIN (LYS336GLU) IN A PATIENT WITH NEMALINE MYOPATHY AND HYPERTROPHIC CARDIOMYOPATHY

Bertini E (1), Porfirio B (2), Graziano C (3), Petrini S (1), D'Amico A (1), Tessa A (1), Pacileo G (4), Sewry C (5), Feng J-J (6), Marston S (6)

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Nemaline myopathy (NM) associated to hypertrophic cardiomyopathy (HCM) has been reported only twice, before molecular diagnosis has become available. We report on a sporadic male baby who was referred at age 9 months for delay of motor milestones. He was able to sit independently at the age of 10 months. Now at age 2 years the boy is not able to stand, has moderate reduced facial mimicry, and has frequent episodes of bronchopneumonia. Four serial follow-up cardiac ultrasonographies revealed and confirmed a non progressive HCM with no functional or electrophysiological problems. The parents were healthy and not related, and family history excluded the segregation of a CM. The muscle biopsy showed changes for a NM. Immunofluorescence with rhodamin-phalloidin showed aspects of accumulation of actin filaments in most fibers. Mutations in the in fast alpha-tropomyosin (TPM1) and in the slow alpha-tropomyosin (TPM3) were ruled out. However, sequencing of the ACTA1 gene showed a de novo missense heterozygous mutation A>G in exon 7 changing a Lysine to Glutamate in position 336 (K336E). 2D electrophoresis and immunoblotting for sarcomeric actin showed a leftward-shifted spot of the product of the mutated allele. Quantification of the mutant protein showed that it was only 28% of the myofibrillar actin and 29% of the total actin, indicating that mutant actin was incorporated into thin filaments. This is the first report of a HCM associated with NM and ACTA1 mutation.

RELIABILITY OF THE HAMMERSMITH FUNCTIONAL MOTOR SCALE IN A MULTICENTRIC STUDY.

Bertini E (1), Messina S (2,3), Battini R (4), Berardinelli A (5), Boffi P (6), Bruno C (7), Cini C (4), Colitto F (1), D'Amico A (8), Minetti C (7), Mirabella M (9), Mongini T (6), Morandi L (10), Orcesi S (5), Pelliccioni M (11), Pini A (12), Swan A.V (13), Villanova M (14), Vita G (3), Main M (15), Mercuri E (2)

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The aim of this study was to validate the Hammersmith functional motor scale for children with spinal muscular atrophy in a large cohort of italian non ambulant children with SMA 2 or 3, in a multicentric assessement. Ninety children (M=52; F=38; mean age =6,95; range =2,2 to 12,8 years) had a baseline assessment (T0) and were reassessed either at 3 months (T1) (n=66) or at 6 months (T2) (n=24). Interobserver reliability, tested on 30 children among 3 examiners, was > 95%.Test-retest reliability was high with a mean score variation after 3 months of -0,16 (range: ±2; SD: 0,83) and after 6 months of -0,125 (range: from –5 to +3; SD: 1,59). Of the 66 children examined after 3 months, 4 had adverse effects in between assessments (fractures, prolonged admissions) and were excluded from the analysis. Forty-two (68%) of the remaining 62 reassessed had no variation in scores between T0 and T1 and 13 (21%) were within ±1 point. Only 3 children scored above 1 point while 10 scored lower than 1 point. Nine (37.5%) of the 24 children reassessed after 6 months had no variation in scores between T0 and T2 and another 9 (37.5%) had variations within ±1 point. Only 4 children scored above 1 point while 5 had scores lower than 1 point.

Our study confirms previous observations of the reliability of the scale and helps to establish a baseline of level of functional ability over a 3 and 6 month interval. This information can be valuable in view of therapeutic trials.

BROAD A BAND DISEASE-LIKE STRUCTURAL ALTERATIONS IN A PATIENT WITH IDIOPATHIC HYPERCKEMIA

Boncompagni S (1), Di Muzio A (1,2), Paci M (1,2), Capasso M (1,2), De Angelis MV (1,2), Protasi F, Uncini A (1,2)

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Mrak et al. (1993 and 1996) described two children of about 2-years-old with a particular muscular alteration. The first child had Leber's congenital amaurosis with hypotonia. The second had similar neuromuscular presentation with mild hyperCKemia without ophthalmological abnormality. The ultrastructural analysis of skeletal muscle biopsies revealed in both a specific alteration of the myofibrillar apparatus that they defined as broad A band disease: broadening and smearing of the A band usually found in most of the analysed fiber and extended longitudinally for a few sarcomeres and laterally for 3-4 adjacent myofibrils. A 30-year-old male had idiopathic hyperCKemia (IH) (Reijneveld et al., 2000) with CK values 2-3 times the upper limit of normal, normal neurological examination and EMG. The biceps brachialis muscle biopsy showed mild fiber size variability and normal routine immunohistochemical and biochemical investigations. Electron microscopy (EM) analysis has revealed a specific myofibrillar apparatus alteration in 2/3 of the analysed fibers (8 out of 12), in single or multiple areas within the same fiber. The alteration consist in an apparent loss of the M line with consequent disorganization of the sarcomere A band, loss of transversal striation and Z lines misalignment. It involve a single sarcomere longitudinally but several sarcomeres transversally and does not involve the entire fiber thickness. This abnormality was never found in any other patient we have previously analysed. This case represent an addition indication that altered values of serum creatine kinase may be a sign of possible alteration of muscle fibers of still unknown etiological origin and that the broad A band disease-like structural alterations could be associated to IH.

SLOW-PROGRESSIVE ATROPHY AND SUSTAINED MYOGENESIS MAINTAIN LONG-TERM HUMAN MYOFIBERS EVEN AFTER MUSCLE DEGENERATION DUE TO PERMANENT SPINAL MOTONEURON LESION

Carraro U (1), Rossini K (1), Boncompagni S (2), Protasi F (2), Kern H (3)

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Three years after spinal motoneuron lesion (SML), human vastus lateralis muscles show histological characteristics of long-term permanent denervation (LtPd), i.e., severely atrophic myofibers, with peculiar nuclear groupings, are dispersed among adipocytes and connective tissue (denervated degenerated muscle, DDM). On the other hand, biopsies taken from 9 to 18 months after SML display a pattern of myofibers, which hardly reach the 50% atrophy (Mid-term Permanent denervation, MtPd). Electron microscopy and anti-NCAM immuno-histochemistry confirm permanent denervation of the vast majority of the myofibers in the healthy-looking MtPd muscles. Furthermore, monoclonal antibody to embryonic myosin shows that myogenic events are present from 1- to 37-year post-SML. These results in human muscles suggest that: 1. the stage of mild atrophy lasts up-to 18 months after peripheral denervation and 2. Some myofibers seen in long-term denervated muscles are the result of repeated cycles of myofiber death/regeneration. Altogether, these observations extend the perspectives of denervated human muscles managements by reinnervation and/or functional electrical stimulation.

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SIX NOVEL MUTATIONS IN THE PYGM GENE IN ITALIAN PATIENS WITH GLYCOGEN STORAGE DISEASE TYPE V (GSD-V)

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Deficiency of the muscle isozyme of glycogen phosphorylase is causative of Glycogen Storage disease type V (GSD-V) or McArdle's disease, an autosomal recessive disorder of glycogen metabolism, clinically characterized by exercise intolerance with premature fatigue, myalgia, and cramps. Myoglobinuria occurs in about 50% of patients, and half of these develop renal failure. Although the clinical phenotype is rather uniform, a few clinical variants have been reported, including a mild form with excessive tiredness and poor stamina, a late-onset form with fixed weakness in the fifth to sixth decade, without cramps or myoglobinuria, and a fatal-infantile form characterized by weakness, severe respiratory insufficiency and early death. Various different mutations in the PYGM gene have been identified in GSD-V patients with different phenotype and ethnic background, being the nonsense R49Xin exon 1the most common mutation in the Caucasian population. The aim of this study was the molecular characterization of six unrelated Italian patients with classical clinical features. We identified six novel mutations responsible for the disease, including missense, frameshift and splice-junction mutations, in four patients. The other two cases were compound heterozygous for the common nonsense R49X mutation and for a known single base pair deletion. This study expands the spectrum of mutations in the PYGM gene, and stresses the need of a complete gene analysis for mutation finding.

THE BLOCK OF CA-DEPENDENT K+CHANNELS REDUCES MYOTONIA IN STEINERT DISEASE: AN IN VIVO PHARMACOLOGICAL STUDY

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Introduction

Many studies have been carried out in order to clarify the mechanism underlying the altered electrical properties of sarcolemma in Myotonic Dystrophy type1 (MyD) but univocal results have not been reported. The first in vivo evidence of ionic channels abnormality in MyD was suggested

after the observation that the local treatment of muscle with apamin, a toxin that specifically blocks a class of Ca-activated K+-channels (SK), reduced the "myotonic runs" recorded by needle EMG (Beherens et al., Muscle & Nerve 1994). This evidence suggested a role of SK in sarcolemma "instability" responsible for myotonia in this disease. Recently we showed a characteristic surface EMG pattern, strictly leaked to myotonia, in MyD patients (Chisari et al., Clin. Neurophysiol. 2002). In this study we evaluated the effect of the local administration of apamin on sarcolemma excitability alteration recorded through surface EMG.

Patients and Methods We applied a stimulation protocol to 8 MyD patients and recorded an amplitude parameter (ARV) before and after the local injection of 50 µl of 10 µM apamin. This was administered by intramuscular injection, using an insulin needle, under the detection electrode. The stimulation protocol was applied 10 min before and 5,10,15 and 20 min after the toxin injection. To verify the reliability of our approach, in two patients we recorded the needle EMG "myotonic runs" before and after the local injection of apamin. Results According to Beherens et al., we observed a clear reduction of myotonic discharge recorded by means of needle EMG. On the other hand, in 2 out of 8 patients tested trough the surface EMG we observed a complete but transient normalization of the basal ARV. The other 6 patients did not show significant modification of EMG pattern. Conclusion This work confirmed the role of SK in sarcolemma "instability" represented by the needle EMG "myotonic runs" and did not rule out the hypothesis that SK could play a specific role in the genesis of MyD sarcolemma excitability alterations responsible for the phenotypic expression of myotonia. Of course further studies are necessary to validate this hypothesis but we consider this approach a good starting-point to study in vivo muscle functional alteration in Myotonic Dystrophy type1.

SKELETAL MUSCLE FIBRES SYNTHESIS IN HEART FAILURE: ROLE OF PGC-1a, CALCINEURIN AND GH

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Background. Patients with congestive heart failure (CHF) have decreased exercise capacity because of muscle fatigability. Symptoms are due to a specific myopathy with increased expression of fast type II fibres, fast MHCs and muscle atrophy. PGC-1a, a potent transcriptional co-activator for nuclear receptors, induces mitochondrial myogenesis and the preferential synthesis of slow fibres. IGF1-Calcineurin stimulation can lead to increased expression of PGC-1a. Methods. We investigated the levels of PGC-1a during progression and regression of skeletal myopathy in the soleus muscle of rats with right heart failure secondary to monocrotaline-induced pulmonary hypertension. We used GH to stimulate the IGF1-calcineurin- PGC-1a axis. Results. The slow MHC1 decreased from 90.6 ± 0.5 to 71.7 ± 2.2 in the CHF rats ($p<.00001$) and increased to 82.1 ±1 .8 after GH ($p<0.00002$). Western blot analysis showed that PGC-1a is significantly decreased in CHF, while it came back to control values after GH. Cytochrome c was decreased in CHF and returned to control values with GH. Troponin I was expressed solely as slow isoform in the control soleus, while the fast isoform appeared in CHF. Its expression returned to control values after GH.

Conclusions. We conclude that PGC-1□ plays an important role in regulating slow fibres expression. PGC1-1a is in turn regulated by the IGF1-calcineurin axis. GH by increasing the circulating levels of IGF1, enhanced the expression of slow MHC1, TnI and the synthesis of mitochondria.

PREVALENCE OF ASYMPTOMATIC HYPERCKEMIA: AN EPIDEMIOLOGICAL STUDY

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Introduction: Asymptomatic hyperCKemia is an alarming sign for affected subjects, a puzzling diagnosis for physician and an increasing cause of referral to Neuromuscular Centres. Despite the fact that persistently increased serum CK levels are commonly encountered in healthy individuals, prevalence data does not exist. The aims of this study were to determine: 1) the prevalence of asymptomatic hyperckemia; 2) the influence of age and gender on the CK levels. Methods: We analysed the data of 1271 Italian subjects (Inchianti study). The presence of causes of increased CK level (drugs, cancer, alcoholism, physical exercise, thyroid disease, cardiac disease, trauma) was determined by a questionnaire. A neurological examination was performed by a physician at the inclusion. Systematic differences between group were evaluated by the mean of Pearson $\chi 2$ test. Multiple Logistic Regression model was used to evaluate the independent association with the hyperCKemia status. Results: The prevalence of hyper CKemia in this population is 9.2% (n = 117). Among recognized cases, 4.48% had at least one cause for hyperCKemia (n=57); yet 4.72% had an asymptomatic hyperCKemia (n=60). The levels of CK were highest among the youngest age groups ($p=0.006$). No influence of gender on the CK level was found. Conclusions: The prevalence of asymptomatic hyperCKemia in the Italian population is 4,72%. We have found a significant variation by age group with highest rates among the young.

THE INLUENCE OF SENSITIZATION ON M. PHRENICUS PHENOTYPE FROM GUINEA PIG

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The m. phrenicus of mammalia belongs to mixed type of muscles with insignificant prevalence of fast muscle fibers (MF). The presence of oxidative, glycolytic and mixed MF in m. phrenicus by determination of succinate dehydrogenase activity was demonstrated. In three weeks after double ovalbumine sensitization the increasing of fast MF amount by using the monoclonal antibodies to the different myosin's was shown. The determination of succinate dehydrogenase activity have demonstrated, that in those conditions not only the enzyme activity, but also amount of steady against exhaustion MF were significantly decreased. These data allow to understand the possible reasons of external breath dysfunctions at the patients with bronchial asthma.

PHENOTYPIC VARIABILITY ASSOCIATED WITH THE A3243G "MELAS" MUTATION IN MITOCHONDRIAL DNA: CLINICAL, NEURORADIOLOGICAL AND MOLECULAR GENETIC STUDIES

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We study 18 patients with the A3243G mutation: 6 from 5 families had the full MELAS syndrome (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes), while the others manifested Progressive External Ophthalmoplegia, familial hypertrophic cardiomyopathy, or encephalopathy with a phenotype generally homogeneous in the family. Age of onset was between 6 and 50 years. Age of first stroke ranged from 7 to 34 years. The percentage of the A3243G mutation was variable and did not correlated with phenotype. Acute neurological deficits consisted in one or more of the follows: hemianopsia, aphasia and or apraxia, cortical deafness, psychosis, hemiparesis. Strokes always followed headache or seizures. By serial MRIs, infarcts occurred at different times and regions moving from the temporal cortex toward parietal and occipital cortex and spread slowly over a few weeks up to six months after the initial symptoms. There was a relative sparing of deep white matter. DWI imagines and the early elevation of ADC map demonstrated vasogenic edema in the acute/subacute lesions. Finally, proton magnetic resonance spectroscopy showed abnormal elevation of lactate and reduction or inversion of NAA/cholin ratio in the affected area suggesting a severe neuronal damage due to mitochondrial dysfunction. Conventional MRI revealed also cerebral and or cerebellar atrophy. Thus, two mechanisms seem involved in the pathophysiology of MELAS: abrupt loss of function associated due to cell injury followed by partial recovery and a slowly progressive degenerative process. Steroids in the acute phases and chronic supplementation therapy with high doses of coenzyme Q10 stabilized the disease in most patients.

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PROGNOSTIC FUNCTIONAL TEST IN DUCHENNE MUSCULAR DYSTROPHY WALKING PATIENTS

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Functional evaluation of global locomotor movement and prognosis of locomotor autonomy in muscular dystrophies is limited to a systematic description based on a scale of items and final score to define walking ability (i.e. Vignos Functional Scale). The biomechanical expression of muscular stenic defect consists of progressive difficulty in rising the body's barycentre against the force of gravity. For this reason, the higher is the required barycentre elevation, the more evident is the loss of performance. Preservation of walking capability as longer as possible is an important objective and needs a careful planning for possible interventions. Aim of our study was the assessment of walking disability in Duchenne Muscular Dystrophy (DMD), obtained by a motion analysis system compared with clinical/functional assessment yielding to a functional classification.

We studied 26 DMD walking patients(age 4-11 yrs). Gait analysis was performed by Vicon 512 and Kistler platform. The following functional tests were considered: capability of rising from lying on the floor without assistance, possibility of walking fast, capability of standing up progressively from a lower chair (45-30-20-10 cm of seat high). A relevant quantify of kinematic and kinetic variables was obtained: pelvic orientation in space, as a mean to reveal a dynamic compensation along progression of disease, and flexion-extension angles at hip, knee ankle joint, as a mean to put in evidence the anti-gravity effort in presence of progressive muscle weakness. At the present time, we were able to correlate pathognomonic graphs of kinematics variables with each Vignos class examined. Further kinetics analysis are in progress.

IDENTIFICATION OF TWO NOVEL MUTATIONS IN POMGNT1 GENE IN ITALIAN PATIENTS WITH MUSCLE-EYE-BRAIN DISEASE (MEB)

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MEB, an autosomal recessive disorder characterized by congenital muscular distrophy, brain malformation and ocular abnormalities, is caused by loss of function in the POMGnT1 gene encoding Protein O-Mannosyl b-1,2-N-acetyl-glucosaminyltransferase 1, responsible for a correct glycosilation of the a-dystroglycan. To date, around 25 POMGnT1 mutations, located throughout the gene, have been reported with a world-wide distribution, and only one Italian patient has been fully genetically characterized. Here, we report the identification of two novel disease-causing mutations in two Italian MEB patients. Patient 1, a 6 year-old girl, presents diffuse hypotonia, retinal degeneration, buphtalm, hyperckemia (1500 U/L), and mild mental retardation. Brain MRI reveals fronto-temporal "cobblestone complex", white matter changes, a flat pons and cerebellar cortical cysts. Patient 2, a 7-year-old boy, presents diffuse hypotonia with pyramidal signs, buphtalm, cataract and congenital glaucoma, hyperckemia (1000 U/L), and severe mental retardation. Brain MRI reveals frontal polymicrogyria, white matter changes, a flat pons and cerebellar cortical cysts. Both muscle biopsies showed a dystrophic pattern with absence of a-dystroglycan. The entire coding region and exon/intron flanking sequences of the POMGnT1 gene were analysed in both patients. Patient 1 carries a mutation at the splice acceptor site at exon 16 and a novel nonsense mutation in exon 10. Patient 2 carries a previously reported mutation in exon 17, and a novel missense mutation in exon 7. Our findings enlarge the number of POMGnT1 mutation in MEB disease, confirming the presence of "private mutations" also in Italian patients.

LINKAGE ANALYSIS IN A FAMILY WITH NON-KINESIGENIC DYSKINESIA REVEALS 2Q33-Q35 MAPPING AND SUGGESTS A MYOFIBRILLOGENESIS REGULATOR 1 GENE INVOLVEMENT

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Paroxysmal dystonic choreoathetosis (PDC, MIM 11880) represents an episodic movement disorder characterised by attacks of dystonia, chorea, and athetosis. These attacks involve the extremities, trunk, and face and may cause dysarthria or dysphagia. The PDC attacks are triggered by several stimuli including alcohol ore caffeine and to lesser extent fatigue, hunger and emotional stress. The disease is inherited in an autosomal dominant fashion and linkage analysis has mapped the locus in 2q33-q35. Recently, heterozygous mutations in the myofibrillogenesis regulator 1 gene (MR-1) have been identified in these patients. The MR-1 gene shows three major isoforms: MR-1L, MR-1M and MR-1S, possessing tissue specificity. All the mutations identified so far localise within exon 1. We describe a family with PDC recurrence (father and two sons affected). We performed linkage analysis by using several VNTRs flanking the MR-1 locus. We demonstrated that the three affected members share the 2q33 haplotype, indicating a linkage with the MR-1 locus. We are currently searching for the presence of MR-1 mutations by RNA analysis on the muscle biopsy of the father. Transcription studies will confirm the causative effect of MR-1 mutations in this family.

INFANTILE HEPATOCEREBRAL SYNDROMES ASSOCIATED WITH MUTATIONS IN THE MITOCHONDRIAL DNA POLYMERASE-{GAMMA}A

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POLG1 is a major disease gene in mitochondrial disorders. Mutations in this gene can be associated with multiple deletions, depletion, or point mutations of mtDNA. In turn, these different molecular phenotypes dictate an extremely heterogeneous spectrum of clinical outcomes, ranging from adult-onset progressive ophthalmoplegia to juvenile ataxic syndromes with epilepsy, to rapidly fatal hepatocerebral presentations, including Alpers' syndrome. Recently, we studied nine infant patients with a combination of progressive neurological and hepatic failure. Eight children, including two sibling pairs and four singletons, were affected by Alpers' hepatopathic poliodystrophy. The latter is a rare progressive

infantile disorder characterized by a combination of liver failure and spongiotic degeneration of the brain grey structures, leading to early onset encephalopathy dominated by severe refractory seizures. A ninth baby patient suffered of a severe floppy-infant syndrome associated with liver failure. Analysis of POLG1, the gene encoding the catalytic subunit of mitochondrial DNA polymerase, revealed that all the patients carried different allelic mutations in this gene.

NEW DGK GENE MUTATIONS IN THE HEPATOCEREBRAL FORM OF MITOCHONDRIAL DNA DEPLETION SYNDROME

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Objectives: To document novel homozygous mutations in the gene for deoxyguanosine kinase (dGK) in two children with mitochondrial DNA depletion. Methods: Clinical features included liver failure, hypotonia, cerebral atrophy, and nystagmus in one patient, and liver cirrhosis, optic dysplasia, nystagmus, and microcephaly in the other. We sequenced the whole coding region of the dGK gene. Results: We identified two novel homozygous mutations, G352A and C269T, which lead to truncated proteins. Conclusions: These data confirm that dGK mutations typically affect liver and brain.

EXPRESSION OF RYANODINE RECEPTOR IN PATIENTS WITH CHRONIC FATIGUE SYNDROME AND HIGH EXERCISE LACTATE LEVELS

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Chronic fatigue syndrome (CFS) is characterised by at least 6 months of disabling fatigue accompanied by symptoms, such as headache, low-grade fever, sore throat, arthralgias, muscle pain, memory and sleep disorders. Different mechanisms have been postulated to explain its pathogenesis. We selected 10 subjects with abnormal ischemic lactate test and aspecific muscle biopsies by a questionnaire profile (SF-36, Fatigue Severity Scale, CFS Impairment Index, and a Pain Scale), for the assessment of lipoperoxides and lactate during exercise. Since some studies showed a possible role of oxidative stress on sarcoplasmic reticulum ryanodine channels (Ryr1), we also searched for immunoenzymatic alterations of this Ca2+ channels, in comparison with the G protein Rho-A. At a myometer submaximal fatigue test patients showed a significant ($P<0.05$) reduction of the basal strength (-16.4%) compared to controls (-6.1%). At exercise aerobic test there were reduction of maximal output, anticipated lactate anaerobic threshold with significantly increased exercise peak lactate ($P<0.05$): 402.7 ± 108.2% of the resting value, compared to 293.5% ± 55.1 in controls. Lipoperoxide mean level in resting condition indicated mild oxidative stress. Skeletal muscle Ryr1 mean grey immunostaining intensity was significantly higher than in control samples ($P<0.0005$). These data suggest that oxidative stress and impairment of oxidative metabolism could play a role in the pathogenesis of pain and exercise intolerance in CSF patients, and that fatigue could result from a dysfunction in Ca2+ transport through the sarcoplasmic reticulum membranes.

INFLUENCE OF RAT HINDLIMB SUSPENSION ON SARCOLEMMAL DYSTROPHIN AND PERMEABILITY OF SARCOLEMMA TO MACROMOLECULES

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Dystrophin is located at the muscle sarcolemma in a membrane-spanning protein complex that connects the cytoskeleton to the basal lamina. Dystrophin complex represents both a membrane structure with mechanical functions and a scaffold to locate sarcolemma signaling proteins. Mutations in many components of the dystrophin protein complex cause different forms of muscular dystrophy, indicating the importance of this complex in normal muscle function. Two experiments performed on Wistar rats were purposed:

– to study influence of unloading on the number of sarcolemmal dystrophin disruptions in skeletal muscle fibers

– to compare damage induced by downhill running in normal and unloaded muscle

– to study the number of dystrophin disruptions during recovery from hindlimb suspension

– to compare sensitivity of different parts of dystrophin molecule (the central rod domain and the COOH-terminal domain) to damage

– to estimate the number of muscle fibers containing Evans blue dye and serum creatine kinase levels

– to investigate whether the increased level of intracellular calcium plays role in dystrophin destruction during hindlimb suspension.

Muscle unloading was modulated by hindlimb suspension for 14 days. As a standard damaging action we used a

downhill running on a motor-driven treadmill at a constant speed of 31 m min-1 with 15 deg decline. Duration of running was 10 min (2 bouts of 5 min separated by 2 min rest interval). Duration of recovery from hindlimb suspension was 3 and 7 days. Animals were injected with Evans blue dye and blood samples were taken in order to estimate the permeability of sarcolemma to macromolecules. In order to decrease the accumulation of calcium in sarcoplasma during hindlimb suspension in the second experiment animals were injected with calcium-binding agent EGTA. Cryosections of m. soleus were stained with monoclonal antibodies against dystrophin. It was shown that:

– hindlimb suspension leads to destruction of dystrophin, which became more during recovery

– different parts of dystrophin molecule have the same sensitivity to the damage induced by downhill running in normal conditions and the different sensitivity to the damage induced by unloading, downhill running after hindlimb suspension and reloading

– after hindlimb suspension the damage induced by downhill running is the same with the damage induced by reloading

– calcium-binding agent EGTA decreases destruction of dystrophin during hindlimb suspension.

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IDENTIFICATION OF DELETIONS AND DUPLICATIONS OF THE DMD GENE IN AFFECTED MALES AND CARRIER FEMALES BY THE MULTIPLE LIGATION PROBE AMPLIFICATION (MLPA)

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Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) are caused in the majority of cases by deletions of the DMD gene, easily detectable in affected males by multiplex PCR. However, different approaches must be used for the identification of female carriers, in which deletions are not detectable by PCR, due to the presence of a normal X chromosome. In this study we used the Multiple Ligation Probe Amplification (MLPA) tool for the identification of female carriers of DMD deletions or duplications in 12 families with a single affected male, ten of which previously diagnosed as carrier of a DMD rearrangement, and the remaining two with unknown disease-causing mutation. In all the investigated affected males MLPA analysis confirmed the presence of a DMD rearrangement, and in six of them allowed the refinement of the breakpoints. In 12 female relatives of the affected patients MLPA analysis showed a DMD deletion or duplication, confirming their carrier status. Two of these were the mother and the sister of a patient whose disease-causing mutation was not known. MLPA analysis proved to be an useful tool for the analysis of both affected males and female carriers of DMD rearrangements also in cases in which the disease-causing mutation in the affected male is not known, providing useful information for the genetic counselling of the family.

α-DYSTROGLYCAN DOES NOT PLAY A MAJOR PATHOGENIC ROLE IN AUTOSOMAL RECESSIVE HEREDITARY INCLUSION-BODY MYOPATHY

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Autosomal recessive (AR) hereditary inclusion-body myopathy (HIBM; MIM# 600737), originally described in Persian-Jewish families, is a neuromuscular disorder characterized by onset in the early adult life with weakness and atrophy of distal lower limb muscles and relative sparing of the quadriceps. HIBM is associated with mutations in the UDP-N-acetylglucosamine 2-epimerase/N-acetylmannos-amine kinase gene (GNE; MIM# 603824) on chromosome 9p12-13. UDP-N-acetylglucosamine 2-epimerase/N-acetyl-mannosamine kinase is a bifunctional enzyme with epimerase and kinase domains, that is expressed in different tissues and has a critical role in the biosynthesis of N-acetylneuraminic acid, the precursor of sialic acid. Sialic acid is normally present on the distal ends of N- and O-glycans and is involved in many biological functions including cellular adhesion, formation or masking of recognition determinants, stabilization of glycoproteins structure and signal transduction. In this study we searched for the presence of any significant abnormality of α-dystroglycan (α-DG), a highly glycosylated component of the dystrophin-glycoprotein complex, in 5 HIBM patients which were previously clinically and genetically characterized. Immunocytochemistry and immunoblot analysis on total protein extracts from muscle biopsies showed that α-DG was normally expressed and displayed its typical molecular mass. Immunoblot analysis on the wheat germ lectin-enriched glycoprotein fraction of muscles and primary myotubes showed a reduced amount of α-DG in 4 out of 5 HIBM patients, compared to normal and other diseased muscles. However, such altered lectin-binding

behaviour, possibly reflecting a partial hyposialylation of α-DG, did not affect the laminin binding properties of α-DG. Therefore, the subtle changes within the α-DG glycosylation pattern, detected in HIBM muscles, likely do not play a key pathogenic role in this disorder.

DOWN SYNDROME AND GUILLAIN BARRÈ: A CASE-REPORT

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It has been reported an higher incidence of certain autoimmune disorders in Down syndrome (thyroiditis, insulin-dependent diabetes mellitus, celiac disease), but only 1 case of Down syndrome associated to a chronic idiopatic demyelinating polyneuropathy (CIDP) and none with Guillain Barrè. It has, in fact, been hypothesized the existence of a possible "protective factor" in Down Syndrome against autoimmune demyelinating diseases. We here report the case of a 15 years old girl affected by Down Syndrome that came to our attention for a progressive decrease of muscle strength, manifested after a gastroenteric infection. The weakness was pronounced especially proximally at the lower limbs, leading to the inability to raise from the floor autonomously or to walk up the steps.

The peak of symptoms manifested in 15 days, the clinical picture stabilized soon after and was still unchanged when we saw her 2 months after the beginning of the symptomatology.

The EMG was characterized by the presence of increased F-wave, CSF showed albumino-cytological dissociation and spine MRI showed abnormal enhancement of the nerve roots in the region of the cauda equine. She was treated with IVIg (400 mg/Kg/die) for 5 days without beneficial effects and currently she is being treated by Deflazacort (0.9 mg/Kg/die) and carefully monitored.

IDENTIFICATION OF A NOVEL CALPAIN-3 SPLICING MUTATION OCCURRING IN HETEROZYGOSITY IN A SPORADIC LGMD2A PATIENT

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Calpain 3 (CAPN3) is a muscle specific Ca++-activated protease which mutations underlie the most common type of autosomal recessive limb girdle muscular dystrophy (LGMD2A). Despite of a recessive model of inheritance, in an high proportion of LGMD2A cases (ranging from 22% to 43%) only a single CAPN3 mutation is detectable raising the possibility of double heterozygosity. We performed CAPN3 molecular study in a sporadic LGMD2A patient with a total deficit of CAPN3 at the immunoblot analysis on muscle biopsy. RNA analysis showed the presence of an aberrant CAPN3 transcript carrying a 75 nucleotides intronic insertion between exons 19 and 20. Genomic analysis demonstrated the presence of a T>A mutation at the canonical intron 19 donor splice site. The identified splicing mutation is present in heterozygosity and coexists with a normal CAPN3 allele as demonstrated by the sequencing of the full coding region. The identified splicing mutation induces the recognition of both novel donor and acceptor sites within intron 19 and we are currently performing in vitro splicing assays in order to investigate this peculiar splicing behavior. We are currently investigating a possible involvement of FKRP and Titin genes which mutations are known to cause a secondary CAPN3 reduction.

MOLECULAR DIAGNOSIS OF COMMON NEUROMUSCULAR DISORDERS BY REAL-TIME PCR

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We currently utilise Real-Time PCR for the identification of female carries of deletions/duplications in the dystrophin gene, for the molecular detection of SMN1 gene deletions and PMP22 gene deletions/duplications. All Real-Time PCR assays are based on the relative quantification of a target sequence versus a reference sequence and utilise the DDCt comparative method. For the identification of female carriers of dystrophin rearrangements, we have available Real-Time PCR assays for exons 5,7,8,16,43,44,48,50,51, corresponding to dystrophin mutational hot spots. We have analysed a total of 18 females at risk for being carriers of a previously identified dystrophin mutation and in 8 cases we confirmed the carrier status. Duplication or deletion of the genomic region 17p11.2-12 cause CMT1A or HNPP, respectively. We performed the molecular diagnosis of these diseases by using Real Time PCR systems in 5 CMT1A and 2 HNPP clinically diagnosed patients. Among these 2 CMT1A patients resulted with 3 copy of the PMP22 gene, therefore confirming the clinical diagnosis. SMN1 gene copy number was analysed by Real Time PCR in 15 subjects at risk to be carrier of deletion mutation and in 2 SMA patients. This analysis allowed us to identify 6 carriers and to confirm the homozygous deletion of SMN1 gene in the affected patients. A prenatal diagnosis of SMA was also performed.

USE OF LIPOIC ACID AGAINST PEROXIDATIVE DAMAGE IN MITOCHONDRIAL MYOPATHIES

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Mitochondrial disorders are degenerative diseases characterized by decreased mitochondrial capability to supply cellular energy requirements. We performed an open pilot study in which we analyzed the effect of lipoic acid in 9 patients with mitochondrial myopathy with external ophtalmoplegia. Lipoic acid is an important antioxidant which reduces the accumulation of toxic metabolites. A previous work by Barbiroli et al. in a case of CPEO showed improvement of muscle performance and rate of energy metabolism by magnetic resonance spectroscopy. The patients were evaluated before and after 3 and 6 months of oral treatment with 600 mg lipoic acid daily. The following parameters were considered: muscle strength (MRC scale), degree of ptosis and dysphagia, timed functional tests (10 meters walking, rising from the floor, climbing 3 steps); laboratory investigations (serum creatine kinase and lactic acid). Before and during the study all the patients had been regularly assuming carnitine. During the study most patients reported subjective improvement of general conditions and muscle performance; 6 patients showed improvement in functional tests. In 3 patients serum lactic acid was reduced and the only 2 cases with hyperCKemia normalized. No patients reported worsening of symptoms. No relevant side effects were observed, nobody dropped out. In one case diabetes improved. Our results indicate that treatment with lipoate may have a role in mitochondrial myopathies to prevent or limit peroxidative damage.

PHASE 1 MULTIPLE-DOSE SAFETY AND PK STUDY OF PTC124 FOR NONSENSE MUTATION SUPPRESSION THERAPY OF DUCHENNE MUSCULAR DYSTROPHY

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OBJECTIVES: To assess the safety, PK, palatability, and nonspecific stop codon

readthrough effects of multiple sequential doses of orally administered PTC124

BACKGROUND: PTC124 is a novel, orally bioavailable, nonantibiotic, small molecule that promotes ribosomal readthrough of mRNA containing a nonsense mutation (premature stop codon) and has the potential to restore dystrophin function in patients with nonsense-mutation-mediated Duchenne muscular dystrophy (DMD). Daily dosing with PTC124 has been shown to induce full-length dystrophin protein production and a decrease in muscle injury in mdx mice harboring a nonsense mutation in the dystrophin

gene. Toxicology studies demonstrated that the drug was well tolerated in rats and dogs at doses ¡Ý1500 mg/kg, showed no evidence of genotoxicity, did not induce QT-interval prolongation, and did not undergo extensive metabolism. In a Phase 1 single-dose study in healthy young adult volunteers, PTC124 safely achieved target concentrations in plasma that have been shown to be pharmacologically active in mdx myocyte cultures and in mdx mice. METHODS: Healthy volunteers (18-30 years of age) were enrolled in 2 stages. In Stage 1, 24 subjects were treated with an oral suspension of PTC124 at doses of 10, 20, 30, or 50 mg/kg BID with meals for 7 consecutive days; in Stage 2, 6 additional subjects were treated with 50 mg/kg BID for 14 days. During the study all subjects were followed with clinical observations, safety laboratory testing, palatability evaluations, and plasma sampling for PK analysis was also carried out. White blood cell were collected from subjects enrolled in Stage 2 of the study for Western blot evaluation of the hypothetical potential for protein elongation due to nonspecific normal stop codon readthrough. RESULTS: No drug-related adverse events were evident at any dose level. Reversible, asymptomatic elevations in AST and ALT to <2 times the upper limit of normal were observed among 9 of the 30 subjects enrolled in the study. PTC124 administration safely achieved target trough plasma concentrations exceeding the 2- to 10-µg/mL values that were associated with activity in preclinical models. Western blots evaluating for elongation of reference proteins (cystatin C, C-reative protein and â2 microglobulin) in PBMCs collected from subjects treated at 50 mg/kg BID for 14 days revealed no nonspecific readthrough of normal stop codons. CONCLUSIONS: These data support evaluation of PTC124 as a treatment for patients with DMD in a planned Phase 2 study.

IDIOPATHIC ORBITAL MYOSITIS RESPONSIVE TO IG EV

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Idiopathic orbital myositis is defined as a benign, noninfective clinical syndrome characterized by variable clinical features ranging from diffuse to a very focal orbital inflammation, ptosis, chemosis, motility dysfunction and optic neuropathy. Unilateral presentation is typical. The pathogenesis is still unknown , but an autoimmune process has been suggested. The most used treatment is corticosteroid. Patients who do not respond to corticosteroids have been successfully treated with radiation therapy and immunosuppressive drugs such as cyclosporine and cyclophosphamide. In only one case has Ig ev treatment been successfully used. Here we present the case of a 55 years old woman with a three-year history of ptosis, right eye

ophtalmoparesis and diplopia. Orbit MRI showed oedema in the periorbital tissues and ocular muscles. She was given steroids and antibiotics with no remission of the symptoms. She treated with Ig ev 0,4mg/kg for five days. Five days after the therapy she showed a regression of both diplopia and ptosis. Symptoms and signs continued to improve with periodic Ig ev therapy. This case of idiopathic orbital myositis refractory to steroid therapy, but responding to Ig ev treatment suggests that the latter therapy is a good alternative to steroids.

STATIN RELATED MYOPATHY AND CO Q 10 DEFICIENCY

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Statins (HMG CoA reductase) reduce cholesterol by reducing the synthesis of mevalonate, a intermediary in the cholesterol pathway. This therapy can be associated with a variety of skeletal muscle related complaints CoQ 10 plays a role as antioxidant and as mitochondrial membrane stabilizer, and it is synthetized from mevalonate. Some AA postulated that statins myopathy is related to apoptosis.To better understand statin induced myopathy and to investigate the role of Co Q 10 in the pathogenesis of this condition we studied muscle biopsies of 20 patients with statin related myopathy. In the muscle biopsies we looked for apoptosis using TUNEL reaction. Also we dose either the Co Q10 level and the Complex III activity in muscle tissues. We found that in our patients the muscle biopsy was normal. No TUNEL positivity was found in muscle section of all patients. The Co Q10 level was mildly reduce in 8/20 of patients and in 2 of them was half of normal. The complex III activity was normal. We can suppose that a secondary Co Q10 deficiency present in our patients may be correlated with the clinical presentations of statin related myopathy.

MUSCLE MRI FINDINGS IN LGMD PATIENTS WITH BIOCHEMICAL DEFECTS OF CALPAIN3.

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Introduction: Recently mRNA mutations of CAPN3 gene transcript have been detected in Limb Girdle Muscle Dystrophy 2A (LGMD2A) patients without DNA mutation. Muscle Magnetic Resonance Imaging (MRI) has been performed in LGMD2A patients, showing a typical and well-defined phenotype, with selective posterior thigh and leg muscles involvement. Among our LGMD2 patients, four had absent or reduced calpain-3 at muscle Western Blot (WB), but no mutations were detected in CAPN3 gene by DHPLC.

Objectives: Our objective was to verify if MRI muscle involvement pattern could help in distinguishing between patients with primary or secondary calpain-3 deficits. Methods: Four patients, two siblings (1M, 1F) and two unrelated females [mean age 23.6 years (range 13-31), mean illness duration 12.6 years (range 8-20), median clinical score 3 (range 2-5; Fanin et al, Human Mutation 2004)], underwent to lower limb muscles MRI. We studied as MRI control one female patient with LGMD2A genetically confirmed diagnosis, aged 19, disease duration 12 years, clinical grade 3. Results: Muscle pattern of involvement in two patients was very similar to the one described in literature and observed in our confirmed LGMD2A patient. We observed a selective fatty substitution in posterior thigh muscles with relative sparing of quadriceps and gracilis muscles, and posterior calf muscles involvement with sparing of antero-lateral leg muscles. The other two patients had a different and less selective pattern of muscle involvement. Conclusions: In two patients the MRI pattern was suggestive of LGMD2A, pointing out the possibility of hidden mutations in CAPN3. mRNA analysis is ongoing. Muscle MRI seems helpful as an additional diagnostic tool to select patients for more sofisticated genetic analysis.

PERSISTENT HYPERCKEMIA IN ATHLETES: A NEW PROBLEM IN SPORT PRE-PARTECIPATION SCREENING

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Introduction Risks related to sport activities in asymptomatic athletes with persistent hyperckemia are difficult to evaluate and usually the problem is underestimated in the course of pre-participation screenings. Objective To stress the meaning of high serum CK levels found at rest in athletes without predisposing factors, considering that repeated intense prolonged exercises may produce negative effects on the muscles. Materials and Methods: Twelve athletes with hyperckemia at rest underwent stress test till exaustion, upon muscle examination. Serum values of CK and LDH, their isoenzymes and lactic acidemia were assessed before the stress and after 6, 12, 24, 48 and 72 hours. Seven of

them showing an abnormal response were adressed to the Cardiomyology and Medical Genetics Center for a specialised evaluation. Results and Discussion: Stress tests, performed till exaustion at high load, induced physiological heart rate and blood pressure increments; lactic acidemia increased 5'after the stress (8.5 mMoli/l) and lightly decreased 30' after stress (5.1 mMoli/l). CK values persisted elevated till 48h after stress (155.4 ± 38.5 U/l). Total LDH was always above the normal limits; the maximum increase of LDH4 and LDH5 was 48h after the stress (LDH4 = 11%; LDH5 = 21%). The most part of the athletes had symptoms of muscle weakness after intensive training or showed relative hypotrophy in some muscles. Myological evaluation releaved clear signs of muscle involvement to be defined by muscle biopsy and/or genetic analysis. Conclusions Persistent levels of serum hyperCKemia in athletes need further investigation to identify underliing muscle pathologies. Once a diagnosis has been made, physical activity should be tailored to the physical and sporting needs of the individual.

EFFECTS OF CA2+-BINDING AGENT EGTA ON FIBER CONTRACTILITY AND SARCO-ENDOPLASMATIC RETICULUM CA-ATPASE ISOFORM DISTRIBUTION IN HINDLIMB SUSPENDED RATS

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Excessive intracellular calcium accumulation is believed to trigger on the development of functional and structural changes of muscle fibers under microgravity conditions [Ingalls et al, 1999, 2001, Clarke et al, 2001, Litvinova et al, 2003]. And we suggested that myoplasmic Ca2+ excess might contribute to the altered myosin heavy chain (MHC) and sarco-endoplasmic reticulum Ca-ATPase (SERCA) isoform distribution in an unloaded muscle.The hypothesis was testified in the 14 day hindlimb suspension study with application of Ca2+ -binding agent (10% EGTA). 24 rats were divided into 4 groups: hindlimb-suspended rats administered with saline i.p. injections (6), hindlimb-suspended rats with EGTA treatment (6). The third and the fourth groups consisted of vivarium controls that were i.p. injected with the saline or EGTA. In the previous study [Duan et al, 1990], intraperitoneal injections of EGTA decreased the elevated basal Ca2+ level in fibers. The diameter of muscle fibers of unloaded rat soleus muscle decreased by 18% in comparison with that of control group and no significant differences between rats with injections of EGTA and without them was revealed. The decrease of maximal tension was pronounced. Values of absolute tension in rats treated with physiological saline were less than in the control group by 45%, and in EGTA-treated rats – by 28%. This discrepancy resulted in decrease of maximal specific tension. Interestingly, the decrease of maximal specific tension of muscle fibers after exposure to unloading gives evidence that the decrease of the fiber contractile properties should be in part explained by the mechanisms other than fiber atrophy. And injections of EGTA prevented effects of those mechanisms, which induce the decline of tension of muscle fibers, but are not linked with reduction of fiber size. The Ca/tension curve in hindlimb suspended saline-treated rats shifted to the right and the pCa thresholds from 7.02±0.05 in cage controls to 6.69±0.02 ($p<0.05$) that means the less Ca sensitivity of myofibrils of unloaded soleus muscle. At the same time pCa threshold in EGTA-treated hindlimb suspended rats was 7.10±0.05. It is concluded that chronic binding of excess Ca results in increase of Ca sensitivity indices. In saline administered suspended animals the percentage of MHC 1 fibers was 69±2% vs 78±1% in saline administered controls ($p<0.05$). The MHC2A fiber percentage was respectively increased in suspended rats with almost no changes in hybrid fibers. The suspended animals administered with EGTA had 73±3% MHC 1 fibers, which didn't exhibit significant differences from either vivarium control or saline suspended rats. Thus the unloading brought about the decline in slow-twitch fiber percentage with the respective increase in fast-twitch fiber percent. The EGTA administration slightly attenuated these changes. Analysis of sections stained with monoclonal antibodies against SERCA showed quite another evolution of fiber distribution. The hindlimb suspension induced the increased number of fibers stained positively for both isoforms (15% increase for so-called "fast" SERCA1 and 10% increase for so-called "slow" SERCA2) with the huge amount of fibers expressing both isoforms. In EGTA treated unloaded animals the increase of SERCA1 positive fibers was completely inhibited. It is concluded that intra-fiber calcium excess contributes to unloading-induced alterations in MHC and SERCA isoform patterns but distribution of these proteins is regulated independently.

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MUSCLE TISSUE ENGINEERING: STRATEGIES FOR REPAIR AND REGENERATION IN HUMAN DEGENERATIVE MUSCLE DISEASES

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Enzymatically isolated and in vitro cultured myoblasts provide an almost unlimited source for muscle reconstruction. Experimental myoblast transplantation has been performed for the treatment of various pathophysiologically very different diseases such as: (i) neurodegenerative diseases; (ii) Duchenne's muscular dystrophy. Besides, this technique may also be suitable for treating some genetic disorders or hormone deficiencies via gene transfer using myoblasts as a shuttle vehicle.

To date myoblast transplantations have been predominantly performed by injection of myoblast cell suspensions into mature skeletal muscle. These single cells have been shown to

fuse with the host myofibers but at low efficiency. For this reason, different scaffold materials have been proposed in cell reimplantation. These materials should fulfill several preconditions, such as: (i) high levels of biocompatibility and biodegradability, (ii) low degree of cytotoxicity and (iii) high affinity to biological surfaces. Furthermore, biodegradable biomaterials should provide (iv) mechanical stability to the construct and may additionally serve as a (v) temporary guide for three-dimensional tissue regeneration. The aim of our study was to design a new scaffold as delivery vehicle for the implantation of skeletal muscle cells. In particular, laminin-coated scaffolds were studied as an alternative to uncoated ones to promote cell attachment and differentiation, as previously reported for monolayer cultures of cardiac myocytes. Our study clearly demonstrates that myoblasts seeded on laminin-coated biodegradable biomaterials can be successfully transplanted into a preformed capsule. As expected, the implanted myoblasts were able to migrate into the surrounding tissue. The formation of multinucleated myotubes demonstrates the suitability of these materials and suggests that this technique may be suitable for future skeletal muscle engineering applications.

EFFECTS OF NF-kB BLOCKADE ON MUSCLE GENE EXPRESSION IN MDX MICE

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Nuclear Factor Kappa-B (NF-kB) is a major transcription factor modulating the cellular immune, inflammatory and proliferative responses. Previous studies provided evidences of a pathogenic role played by activation of NF-kB in mdx mice and Duchenne muscular dystrophy (DMD). We have recently demonstrated that treatment with IRFI 042, a vitamin E analogue inhibiting NF-kB, ameliorates muscle function, decreases serum CK levels and muscle necrosis and enhances regeneration in mdx micę. The aim of the study was to identify the genes differentially expressed in skeletal muscle of IRFI 042-treated mdx mice, searching for a molecular hallmark responsible of the treatment positive effect. Microarray experiments were performed on leg muscles using a GeneChips microarray slide with a whole mouse genome of 44K genes (Agilent Technologies, Italy). Selective chemokine upregulation was studied by RT-PCR and immunoblot. Results in vehicle-treated animals provided evidence for coordinated activity of numerous components of a chronic inflammatory response, including cytochine and chemochine signaling, leukocyte adhesion and complement system activation. Moreover genes of extracellular matrix and those involved in regeneration process were up-regulated. After treatment with IRFI 042 we noticed a decreased expression of chronic inflammatory response components and an enhanced expression of genes involved in repair processes. These data better clarify the mechanisms underlying the NF-kB blockade-induced benefit in mdx mice and suggest new treatment avenues in DMD.

Keywords: mdx mice, nuclear factor kappa-B, IRFI 042, microarrays, therapy.

ATOMIC FORCE MICROSCOPY INVESTIGATION IN DUCHENNE MUSCULAR DYSTROPHY AND POLYMYOSITIS MUSCLES

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The lack of dystrophin in the mdx mouse has ben shown to severely compromise the mechanical integriy of myofibers and may cause the muscle fibers to be more vulnerable to mechanical stress, hypo-osmotic shock, and contraction-induced damages, leading to membrane microdamages and initiating necrotic changes. Atomic force microscopy (AFM) is a well known method used to image the surface of various inorganic and organic samples. The technique is able to give informations about the mechanical properties of the sample surface such as adhesion force and elasticity. We investigated cryostat sections of muscle samples from 5 patients each with Duchenne muscular dystrophy (DMD) and polymyositis (PM) and 5 normal controls by AFM. Increased cytoscheletal adhesion and visco-elasticity of muscle fibers were found in DMD and PM samples, when compared to normal controls. They appared higher in PM than in DMD. Increased adhesion and visco-elasticity in diseased muscles may be due to abnormalities of cytoscheletal architecture, charatherizing degeneration and necrosis in both diseases. Higher values in PM could be explained by the role of metalloproteinases played in inflammatory myopathies. More studies are needed to detail the usefulness of AFM in studying pathophysiological mechanisms in myopathies.

RHABDOMYOLYSIS AS PRESENTING SYMPTOM OF GLYCOGEN DEBRANCHER ENZYME DEFICIENCY

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Deficiency of amylo-1,6-glucosidase, 4a glucanotransferase enzyme (AGL) is responsible of glycogen storage disorder (GDS) type III, inherited with an autosomal recessive trait. GDS type III recognizes a clinical and biochemical heterogeneity. The classic clinical form is characterized by hepatomegaly, recurrent hypoglycemia, hyperlipidemia, short stature and more frequently cardiomyopathy and myopathy. The muscle involvement usually is predominant in the adult form with slowly progressive distal muscle weakness. We describe a 29 years old man who was admitted to our department because of a recent episode of dark urines and muscle pain after intense physical exercise. Since childhood he complained of exercise intolerance with muscle weakness. His parents were first cousins. Neurological examination showed a mild bilateral proximal weakness at upper limbs and decresead reflexes. CK was 8236 IU/l. Forearm ischemic test showed one-fold increase of lactate after ischemia. EMG revealed a myopathic pattern with high frequency bizarre discharges. Echocardiographic study revealed a mild hypertrophic cardiomyopathy. Muscle biopsy evidenced fiber size variability, cytoplasmic vacuoles and marked glycogen storage. Biochemical investigations identified a severe reduction of debrancher enzyme activity (0.5 nmol/ min/mg/prot. – n.v. 3.4 ± 1), and higher acid maltase activity (248 nmol/min/mg prot - n.v. 91 ± 30.6). Rhabdomyolysis is very rarely described in GDS type III. Our case suggests that GDS type III should be considered in patients with recurrent myoglobinuria and cardiomyopathy in absence of hypoglicemia and hepatic involvement.

TOWARDS THE MOLECULAR DIAGNOSIS OF ALL DMD/BMD CASES

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In the majority of cases (60%), the dystrophin gene is broken by huge deletions that span hundreds of thousands of DNA nucleotides, encompassing one or more exons. In the remaining of cases, elusive mutations have been claimed to be responsible for the DMD/BMD phenotype. In the last few years, a number of these missing mutations have been detected with a laborious use of quantitative analyses or DNA scanning techniques. The identification of these mutations has a pivotal importance in the reliable definition of the carrier status in females and for prenatal diagnosis. It is therefore unbelievable that one third of DMD/BMD patients do not obtain any molecular information about their status. Our aim is to help all requesting Italian centres that recruit DMD/BMD patients, by offering a complete and free of charge high−throughput testing. We perform molecular diagnosis on DNA samples including: 1) quantitative DNA analyses of all the 79 exons and promoters of the gene to identify deletion/duplications in patients and deletions/duplications in carriers; 2) small mutation screening of all the promoters, the muscle exons and exon−flanking introns.

RED YEAST RICE (MONASCUS PURPUREUS) AS HYPERCHOLESTEROLEMIC DRUG: A CASE REPORT OF MUSCLE DAMAGE

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We report the case of a 74-year-old patient with diabetes who was treated with statins (simvastatin and then atorvastatin) for hypercholesterolemia. After 4 years of continuous therapy the drug was discontinued because of generalized muscle weakness. Laboratory investigations revealed significant increase of creatine kinase (CK) peaking at 3000 U/L. Five months after statin withdrawal his symptoms partially improved but CK was still 3700 U/L. Clinical examination was normal and open muscle biopsy was performed. Size variability with several atrophic fibers and prevalence of type II fibers were observed. Immuno-histochemistry and Western blot demonstrated normal distribution of sarcolemmal proteins. Inflammatory markers were negative. Mitochondrial enzyme activities and CoQ levels were studied. With further questioning the patient admitted that after statin therapy he had begun to assume a natural product prepared from rice fermented with red yeast (Monascus purpureus), used in Chinese cuisine and as a medicinal food to promote blood circulation for centuries. The product contains a compound (monacolin) identical to lovastatin with the same inhibitory effect on HMG-CoA reductase enzyme and favorably impacts lipid profiles of hypercholesterolemic individuals. Three months after its discontinuation in our patient, CK was 1300 U/L. In the literature two cases of adverse muscle effects due to red yeast rice are reported. Since the population receiving hypocholesterolemic agents is large and growing, doctors and

patients should be alert not only about side effects of statins but also about natural products often used as an alternative treatment.

FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY AND HEART ARRHYTHMIA: DATA FROM A MULTICENTER STUDY AND REVIEW OF THE LITERATURE

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Subjects with FacioScapuloHumeral Muscular Dystrophy (FSHD) do not usually suffer from significant cardiac symptoms; however, in the few controversial studies reported to date, heart alterations have been shown to be possible. In 1990, in a group of 30 FSHD cases, Stevenson et al. reported a significant presence of arrhythmia or susceptibility to arrhythmia (27%), while in 1992 de Visser et al. detected no alterations in the ECG-Holter monitoring 31 FSHD patients. In 1998, in a study of 100 cases, Laforet et al. found symptomatic arrhythmia and conduction defects in 5 subjects, aged 14 to 59 years. Currently, a series of 105 FSHD patients, with the characteristic 4q35 deletion, are under cardiac investigation by our multicenter study by clinical examination, surface ECG, 24-hour ECG and echocardiography. The present analysis concerns data on 76 patients, 42 males and 34 females with a mean age of 47 years (range 14-79). Overt cardiac involvement was evident in 10 cases, mainly represented by paroxysmal tachycardia and palpitations. Subclinical signs of conduction delay or arrhythmia were detected by 24-hour ECG in other 12 cases. Altogether, arrhythmic symptoms or signs were found in 29% of the patients. However, considering only cases aged under 60 years,with no cardiovascular risk factors, symptomatic or asymptomatic alterations were present in 18% (10/57). On the whole, considering our data and those available in the literature, arrhythmic alterations appears to be detected more frequently than expected in FSHD patients, even if clear cardiac symptoms are seldom referred. Further studies are needed to establish definite occurrence of heart abnormalities in FSHD.

AMYLOID MYOPATHY PRESENTING WITH RHABDOMYOLYSIS

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Amyloid myopathy is a rare disorder and its diagnosis is often overlooked, even on muscle byopsy. A man experienced an episode of acute rhabdomyolysis (CK:100,000 U/l) at age of 57. In that occasion he was admitted to another hospital and received a diagnosis of polymyositis. Deflazacort (6mg/day) was started with poor response. Laboratory investigation reveled IgG monoclonal component and MGUS was also diagnosed. Ecoardiography evidenced interatrial septum hypertrophy. Furtherly the patient developed a persistent proximal muscle weakness and CK value remained constantly increased (1000-2000 U/l).

When he came to our department, at the age of 66 years, a proximal myopathy with major impairment at lower limbs was clearly evident. CK level was 1415 U/l. EMG showed a myogenic pattern without at rest activity. Muscle biopsy revealed a "dystrophic" pattern with sclero-adipose substitution and slight increase of perymisial connective tissue, atrophic, degenerating and regenerating fibers, internal nuclei. No infiltrating cells were detectable. Focal areas with Congo-red positive materials, consistent with amyloid deposition, were identifiable in numerous vessel walls, in the endomisium surrounding some muscle fibers and even at sarcolemmal level. Immunocytochemistry for C5b9 complement fragment showed a similar distribution. Hematological studies led to the diagnosis of l light chain secreting myeloma. Rhabdomyolisis is a rare complication of haematological diseases, and in our case it was the presenting symptom of systemic amyloidosis. Amyloid myopathy should be suspected in adults with progressive muscular weakness of uncertain cause. The presence of a monoclonal protein in serum is an important diagnostic clue in amyloid myopathy.

UNUSUAL OUTCOME IN A MILD DYSTROPHINOPATHY

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Becker muscular dystrophy (BMD), like Duchenne muscular dystrophy (DMD), is caused by mutation in the dystrophin gene, located at Xp21, which affects the sarcolemmal protein dystrophin. This protein is absent in patients with Duchenne muscular dystrophy, but is reduced in amount or abnormal in size in with Becker muscular dystrophy. BMD can be distinguished from DMD on the basis of clinical severity and rate of progression, increased of CK levels and muscle biopsy changes. We described a patient with an unusual phenotype of Becker muscular dystrophy. He was referred at 4 years of age for motor milestones delay. Serum CK was mildly elevated. EMG showed myogen pattern. Muscle biopsy performed at the onset showed a mild dystrophic pattern and a reduction of the dystrophin immunostaining. In the following years, clinical aspects improved: at the age of 12 years the neurological examination, CK serum level, and EMG were normal. Second muscle biopsy was performed; it did not show any dystrophic change, while immunostaining showed a mild redution of dystrophin and sarcoglycans. Dystrophin immunoblotting showed an higher molecular weight than expected with normal amount of protein. DNA analysis of the dystrophin gene showed a duplication of exon 16. At the present time, he is 16-year-old and he is clinically asymptomatic. His serum CK is normal. To our knowledge, isolated duplication of exon 16 of dystrophin gene has been never described before. The atypical clinical history of this patient amplifies our knowledge of clinical phenotypes of BMD, in particular confirming that normal CK may be associated with a mutation in dystrophin gene.

EXPRESSION PROFILING IN EARLY PHASE DMD

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Microarray based gene expression profiling can be used to monitor global "gene expression changes" between normal and diseased tissues, highlighting adaptive changes and signal transduction pathways specifically induced or suppressed. Duchenne Muscular Dystrophy (DMD) becomes clinically evident in childhood but histological abnormalities are already evident in skeletal muscle during fetal life and in preclinical stage of the disease.

To identify gene expression changes occurring in preclinical DMD, we studied individual expression profiles of 20 DMD patients of age ranging from 1.5 to 20 months and 12 age-matched controls, using the U133A Affymetrix Genechips. By comparing the two populations (unequal variance, two tail T-test; $p<0.001$, FDR 0.05) we identified in DMD patients 272 genes upregulated and 38 downregulated. Main biological processes found altered in DMD are the deposition and remodelling of extracellular matrix, infiltration of immune system effector cells, expression of developmental isoforms of sarcomeric proteins and depression of energy metabolism. Among all the components of the Dystrophin Associated Protein Complex (DAPC), only Dystrophin and a1-Syntrophin mRNA showed a significant downregulation in DMD patients. In conclusion, modification of muscle tissue expression profile occurs early in DMD, and precedes the appearance of clinical signs. We believe that knowledge of global gene expression changes associated to this disease, may allow the identification of pharmacological targets and the development of new therapeutic strategies.

LEFT VENTRICULAR DIASTOLIC FUNCTION IN MYOTONIC DYSTROPHY

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Myotonic dystrophy (MD) is the most frequent autosomal dominant myopathy in adults, with an incidence of 1: 8000 births. We studied 80 patients (age range 38±22 years), 65% of them males and 35% females. We recorded the echograms by a trans-thoracic approach, according to the criteria recommended by the American Society of Echocardiography. The following parameters were evaluated, by transmitralic Doppler echocardiography: E wave, A wave, E/A ratio, isovolumetric relaxation time (IVRT), deceleration time (DT), DTI on mitral ring and Doppler of pulmunar veins.

A mild mitralic regurgitation was present in 90% of patients; 4 of them showed a mitral prolaps (LAM). Cardiac dimensions (ventricular and atrial chambers, septum and posterior free wall ticknesses) were within the normal limits. The most part of patients, older than 50 years, showed a hypokinesia involving both left free wall and/or septum or apex, or just the apex. All the examined patients showed a diastolic dysfunction, although in a different stage. Furthermore, we compare the trans-mitralic flow pattern in a subset of 23 sibships from 17 not related families in subsequent generations. We found that younger patients showed a more severe pattern of diastolic dysfunction, when compared with their parents. The data here shown suggest that

diastolic dysfunction is a primary feature of cardiac involvement in Myotonic Dystrophy.

MYOPATHY AND MITOCHONDRIAL IMPAIRMENT IN PATIENTS WITH HYPERFERRITINEMIA OF DIFFERENT ORIGIN

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Ferritin is a multimeric protein composed of 24 subunits where iron can be stored in a readily available but non-toxic form. Regulation of ferritin synthesis involves an interaction between an iron regulatory protein and part of the ferritin mRNA as iron regulatory element. An excess of ferritin synthesis occurs in presence of iron overload and as a consequence of primary or secondary (inflammation, cancer, chronic blood infusion, dietetic iron overload, alcoholic liver disease) hemochromatosis. We report three male patients (34, 54 and 33 yr-old) with increased serum ferritin and CK and with muscle symptoms such as cramps and fatigue. Deltoid biopsy showed increased variability coefficient of myofiber diameter and COX hyporeactive fibers with subsarcolemmal rims. One patient presented ragged blue fibers and, at the molecular analysis, was carrier of H63D mutation for hereditary hemochromatosis. In the other two patients an acquired iron metabolism alteration was present, at an extent to do bleedings. Perls' method failed to identify iron muscle deposits. These results indicate that hyperferritinemia and alterations in iron metabolism can be responsible of myopathy with mitochondrial abnormalities at skeletal muscle level. Mitochondrial Fe/S-protein clusters, indispensable for oxidative phosphorylation and ATP production as well as for reactive oxygen species balance, could be the putative targets of such a condition.

CONGENITAL MUSCULAR DYSTROPHY WITH MENTAL RETARDATION AND CEREBELLAR CYSTS: REPORT OF A CASE UNRELATED TO FKRP GENE.

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About 50% of patients with congenital muscular dystrophy (CMD) show brain abnormalities revealed in vivo by MRI (Barkowich 1998; Patel and Barkowich 2002). The pattern of cerebral involvement is a very helpful component of clinical-instrumental characterisation of this heterogeneous condition and may suggest appropriate immunocitochemical and genetic tests to complete diagnosis. A part of LAMA2 gene, an increasing number of genes encoding for putative or demonstrated glycosyltransferases are in fact being associated with different types of CMD with brain involvement. Cerebellar cortical dysplasia including subcortical cysts is relatively frequent in CMD with abnormal glycosilation such as Fukuyama type CMD and Walker Warburg syndrome, is characteristic of CMD with mental retardation due to fukutin-related protein (FKRP) gene mutations (Talim et al, 2000; Triki et al, 2003; Topaloglu et al, 2003), while it is not reported in LAMA2 CMD. Despite new knowledges and improvement of genotype-phenotype correlation, a proportion of cases affected by CMD with brain involvement does not fall into a well defined clinical, immunohistochemical and genetic phenotype. We report a case of CMD with subcortical cerebellar cysts of difficult classification. A Tunisian male boy aged 3 years presented congenital motor delay, hypotonia, mental retardation with no language development and contractures. He was able to sit, eyes were normal and CK was markedly high(more than 1000 U/L). Brain MRI showed multiple cerebellar subcortical cysts and normal cerebral white matter and cortical gyration. Needle muscle biopsy showed a marked dystrophic pattern. Despite this case closely resemble CMD with structural brain involvement related to FKRP gene mutations, alfa-dystroglycan was normal and no pathogenetic mutations in FKRP gene were found. Laminin alpha2 expression was severely reduced as in LAMA2 CMD. This case document the clinical and immunocitochemical overlap between different CMD forms with brain involvement.

HISTOPATHOLOGICAL AND MORPHOMETRIC ANALYSIS HELP TO DIFFERENTIATE MYOTONIC DYSTROPHY TYPE 1 AND TYPE 2

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Myotonic dystrophy (DM) has been characterized on genetic ground through the association with two different loci identifying DM type 1 (DM1) and DM type 2 (DM2). Molecular analysis for genotyping DM2 is presently available only in a few centers. Since the clinical features of DM1 and DM2 may present striking similarity, muscle biopsy studies might be needed for differential diagnosis. Although the histopathological changes of DM2 muscles have been generally described as relatively mild and non-specific, detailed studies are lacking. Therefore, we performed a morphological and morphometric analysis on muscle biopsies from 10 DM2 and 7 DM1 patients. In DM1 cases, we observed preferential type 1 fiber atrophy, as previously

described, and a higher prevalence of central nucleation among type 1 fibers in all cases. In DM2 muscle biopsies, atrophy of type 1 fibers was absent, while a high rate of hypertrophic fibers of either type was detected in most cases. Moreover, as opposed to DM1, all cases showed preferential central nucleation in type 2 fibers. These data contribute to better define specific histopathological patterns for DM1 and DM2, and may help clinicians to differentiate between these two forms of myotonic dystrophy and to correctly address genetic studies.

MULTICENTER STUDY TO EVALUATE THE ARRHYTMIC RISK IN MYOTONIC DYSTROPHY TYPE 1: RESULTS OF ONE-YEAR COMMON PROTOCOL

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Myotonic Dystrophy type 1 (DM1) is the most frequent adult age muscular dystrophy, with an incidence of 1:8000 births. Cardiac involvement is characterised by atrio-ventricular conduction defects evolving to a complete heart block or ventricular tachy-arrhythmias, both causing sudden death.

Aim of the study, is to evaluate the arrhytmic risk in DM1 patients by adopting a common protocol of 6-month interval examinations. Two-hundred-fifteen patients (121 males and 94 females) affected DM1, have been consecutively enrolled from January 2003. The diagnosis was made according to the family history, clinical features and genetic analysis. All the patients underwent neurological and cardiological examinations. Electrophysiological study was performed in 74 patients. Standard and/or Holter ECG documented sinus bradycardia in 107 pts, sinus pausae > 3 sec in 2, atrial fibrillation/flutter in 5, couples and/or runs of ventricular tachycardia in 15 pts. First-degree AV block was present in 62 pts, second-degree AV block in 6 pts. The analysis of heart rate variability showed a significant depression of autonomic nervous system when compared with the controls. Echocardiography showed a reduced ejection fraction in few patients. EF study showed sinus nodal dysfunction in 10% of patients, anomalous AV conduction in 18%, HV interval > 70 msec in 37%. Pacemaker was implanted in 28 (2 of them subsequently showing a progression of AV block), automated defibrillator in 15 (3 of them receiving appropriate DC shock) and a loop recorder in 13 patients (1 of them subsequently necessitating of pacemaker implant). The study shows the usefulness of a systematic follow-up in DM1 patients to avoid the occurrence of fatal events.

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GENETIC HETEROGENEITY OF LAMIN A/C ASSOCIATED PHENOTYPES

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Mutations in the nuclear envelope protein lamin A/C have been associated to a variety of pathologies, denominated laminopathies, affecting mainly muscular, nervous and adipous tissues. More than 100 mutations scattered along the LMNA gene have been described to date and, although no clear genotype-phenotype correlation could be established, some clustering has been recognized.

We have analyzed 150 consecutive patients with possible mutation in the LMNA gene. Patients underwent LMNA gene evaluation when displaying 1) clear myopathy with scapuloperoneal or limb-girdle distribution and the exclusion of the abnormal expression of dystrophin, calpain3, dyspherlin, caveolin3, sarcoglycans and emerin; 2) association of myopathy and cardiomyopathy; 3) family history of myopathy plus cardiomyopathy or sudden death. We identified 22 mutations (14%), 14 of which had not been previously described, among which a conservative substitution leading to the activation of a cryptic splice site in exon 6. Most of the patients showed weakness with scapuloperoneal distribution, 40% of them presented cardiomyopathy and in one case the association of myopathy and neuropathy. Moreover, five relatives of LMNA mutated patients were asymptomathic although carrying the same genetic alteration. Our analysis shows that lamin A/C is not only associated to a variety of clinical presentations but also to a diverseness of genetic variants and underlines that the interpretation of molecular analysis results is not always straightforward.

MR INVESTIGATION IN INFLAMMATORY MYOPATHIES

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Introduction Muscle MR examination is emerging as a powerful tool in muscle diseases.

Methods We studied 22 patients with inflammatory myopathy, 11 affected by Polymyositis (PM) and 11 by Inclusion Body Myositis (IBM). Muscle examination was performed on a 1.5-Tesla MR scanner, with T1-W SE images (TR/TE=500/35 msec) and T2-W STIR images (T1=1 50 msec). Axial slices were obtained from psoas to distal foot muscles.

Results In IBM, T1-W sequences showed a prevalent involvement of the quadriceps, with an increasing gradient of severity from the proximal to the distal portion of the thigh, while the posterior compartment was always involved less severely than the anterior. Moreover, MR examination was in keeping with the level of functional severity, although MR sensitivity was higher than clinical examination in detecting the extent and severity of muscle involvement. In contrast, in PM patients, especially during the acute phases of disease, MR T1-W sequences underestimated the severity of muscle involvement, as it resulted from clinical evaluation.

Focal areas of hyperintensity on T2-W STIR images, indicating muscle "oedema", were observed in both IBM and PM patients; interestingly, in the follow-up of some PM patients, they persisted during the recovery phase and correlated with the degree of serum CK.

Conclusions Muscle MR examination is a useful tool in the diagnosis and follow-up of inflammatory myopathies.

DYSTROPHIN RE-FRAMING BY ANTISENSE OLIGONUCLEOTIDES: SPECIFIC EXON SKIPPING IN IN VITRO SYSTEMS

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By using antisense oligonucleotides (AONs) it is possible to induce specific exon skipping and to reframe dystrophin, restoring the synthesis of a shorter though functional dystrophin. It is theoretically conceivable that this approach may correct the reading frame, therefore obtaining a milder BMD from a DMD phenotype. We analysed by in vitro cell-free splicing assays two peculiar mutations. The first consists of an out-of-frame 37 nucleotides insertion into exon 34 (in frame) causing a DMD. The second mutation we analysed is represented by a dystrophin intron 11 deletion creating a novel out of frame exon (alu-exon) and causing XLDC. By using minigenes containing the mutated exons we reproduced the incorporation of both aberrant exons into the transcript.

We also identified an exonic splicing enhancer motif within the alu-exon responsible for its inclusion into the dystrophin transcript. As the skipping of these two aberrant exons will be able to restore the dystrophin synthesis, AONs will be designed both on the alu-exon ESE and on exonic purine rich exon 34 regions. We also aim at targeting with AONs the identified ESE motif in myoblast cultures from a female carrier of the alu-exon mutation. The restored dystrophin synthesis will be analysed both by immuno-cytochemical analysis and Western blotting.

AUTOIMMUNE JUVENILE LIMB-GIRDLE MYASTHENIA

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Myasthenia gravis is an uncommon disease in children and may be misdiagnosed. Limb-girdle myasthenia is a rare disorder which includes familial and autoimmune forms. Patients present proximal muscle weakness and wasting without daily fluctuations and cranial nerves involvement. In our experience, myasthenic patients with an onset of disturbances before 20 years represent 14.2 % of the whole myasthenic population observed in a 17-year study. We investigated five cases of autoimmune juvenile limb-girdle myasthenia starting before the age of 15 years with attention to clinical diagnostic difficulties, evolution, laboratory, instrumental data and response to treatment. Symptoms were represented by proximal muscle weakness; deltoid weakness was present in each case and in two patients an impairment of tibial muscles was clearly evident. Antibodies against acetylcoline receptors were present in three cases. Treatment with acetylcholinesterase inhibitors improved symptomatology in all. Four patients underwent thymectomy.

To date, there are no reports about the occurrence of limb-girdle myasthenia during childhood. Our study evidences that there is a small group of subjects with juvenile myasthenia, which constitute 2.3% of such population, presenting clinical features of chronic limb-girdle myasthenia. In view of the predominant proximal weakness and of the age at onset, many patients may carry the diagnosis of limb-girdle muscular dystrophy, polymyositis or other myopathies. Limb-girdle myasthenia requires a strong index of suspicion. We suggest to suspect this form in children with unclassifiable myopathy, mostly affecting deltoid muscles and lower extremities.

ELECTROPHYSIOLOGY SHOWS THAT LONG-TERM DENERVATED RAT MYOFIBERS MAINTAIN RESTING MEMBRANE POTENTIAL AND EXCITABILITY EVEN AFTER ALL MYOFIBRILLAR PROTEINS ARE LOST

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Six-nine months after spinal motoneuron lesion, rat leg muscles show histological characteristics of long-term permanent denervation, i.e., severely atrophic myofibers, with peculiar nuclear groupings, are dispersed among adipocytes and connective tissue (denervated degenerated muscle, DDM). Though at later stages myofibrills are almost absent, electron microscopy confirms the presence of the majority of the myofibers in DDM muscles. On the other hand on isolated muscles in vitro electrophysiology shows that a resting membrane potential is reduced, but present, and that the myofibers under proper high current stimulation are able to sustain tetanic contractions. Altogether, these observations extend the perspectives of denervated muscles managements by reinnervation and/or functional electrical stimulation.

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DETECTION OF MITOCHONDRIAL DEFECTS IN COLLAGEN VI DEFICIENT MUSCLE CULTURES FROM UCMD PATIENTS AND COL6A1 KNOCKOUT MICE: AN ULTRASTRUCTURAL STUDY

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Collagen type VI is an extracellular matrix protein expressed in several tissues including skeletal muscle. Dominant and recessive mutations in collagen VI genes cause two inherited muscle disorders, Bethlem myopathy and Ullrich congenital muscular dystrophy. In mice, Col6a1 gene inactivation induces a myopathic phenotype associated with ultrastructural alterations of mitochondria, mitochondrial dysfunction and apoptosis of muscle fibers. These alterations are caused by increased opening of the mitochondrial permeability transition pore (PTP) and they are prevented in vivo and in vitro by treatment with the PTP inhibitor cyclosporin A. Here we report an ultrastructural study on cultured muscle cells from Col6a1 knockout mice and three patients affected by Ullrich congenital muscular dystrophy with collagen VI deficiency. In Col6a1 knockout and UCMD cells, swollen mitochondria with extracted matrix and disorganized cristae were detected. Groups of clustered mitochondria were also found. After treatment with oligomycin, a selective inhibitor of F1F0-ATPase, the number of altered mitochondria appeared increased in Col6a1 knockout and UCMD cells, suggesting a latent mitochondrial disfunction in collagen VI deficient muscle cells. The altered phenotype of mitochondria was rescued by addition of cyclosporin A to oligomycin-treated samples. These data support the hypothesis that mitochondrial defects underlie the pathogenetic mechanism in collagen VI human pathologies as previously demonstrated in Col6a1 knockout mice.

SLEEP APNEA DETECTION IN MYOTONIC DYSTROPHY USING NASAL PRESSURE HOLTER RECORDING

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Background: Polysomnography, the gold-standard for the diagnosis of sleep apne syndrome (SAS), frequent and uderdiagnosed in myotonic dystrophy type 1 (DM1), is often badly tolerated by the patients and available in specialized

sleep study centers only. Aims: To verify whether nasal pressure holter recording detects sleep hyponea or apnea in patients with myotonic dystrophies without or with minimal respiratory complaints. Methods: 4 patients (2 DM1 and 2 DM2,mean age 44.3 ± 2.3, mean BMI 35.3 ± 4.6) with no respiratory or cardiac complaints were subjected to nocturnal nasal pressure Holter recording associated with cardiac frequency and RR interval variability monitoring.

Results: In 3 of 4 patients nasal pressure Holter monitoring revealed a pathological apnea index and in one DM1 patient with known obstructive SAS, the apnea index, revealed by the nasal pressure Holter monitoring, was consistent with that indicated by polysomnographic recording. Cardiac conduction profiles (RR variability, low frequency and high frequency ratios during the night and day) before and during the sleep apnea/hypopnea episodes were obtained.

Conclusions: Although preliminary our data indicate that nasal pressure Holter monitoring associated with cardiac monitoring is a simple, quick, non-invasive and well-tolerated technique to screen patients with DM1 and DM2 at high risk for SAS and to refer patients for further diagnostic work-up.

THE FIRST RECESSIVE MUTATION OF ANT1 IS ASSOCIATED WITH EARLY ONSET HYPERTROPHIC CARDIOMYOPATHY AND SKELETAL MYOPATHY

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Multiple mitochondrial DNA deletions are associated with clinically heterogeneous disorders transmitted as mendelian traits. Dominant missense mutations were found in the gene encoding the heart and skeletal muscle-specific isoform of the adenine nucleotide translocator (ANT1) in 5 families with autosomal dominant PEO and 1 sporadic patient. We herein report a sporadic patient who presented with hypertrophic cardiomyopathy, mild myopathy with exercise intolerance and lactic acidosis, but no ophthalmoplegia. A muscle biopsy showed the presence of numerous ragged red fibers, and Southern blot analysis disclosed multiple deletions of muscle mitochondrial DNA. Molecular analysis revealed a novel C to A homozygous mutation at nucleotide 368 of the ANT1 gene. The mutation converted a highly conserved alanin into an aspartic acid at codon 123 and could no be detected in more then 100 normal control. The expression of the equivalent mutations in AAC2, the yeast orthologue of human ANT1, results in a very drastic phenotype characterized by a marked growth defect on non-fermentable carbon sources and hardly any ATP/ADP translocation activity in mitochondria. This is the first report of a recessive mutation in the ANT1 gene. The clinical and biochemical features are different from those found in dominant ANT1 mutations, resembling those described in ANT1 KO mice.

PREMATURE TRUNCATION OF CAVEOLIN-3 SUGGESTS HAPLOINSUFFICIENCY IN AUTOSOMAL RECESSIVE RIPPLING MUSCLE DISEASE

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The caveolin-3 (CAV3) gene is organized in two exons spanning 12kb of genomic DNA and encodes for the human caveolin-3, a 150 amino acids protein with a molecular weight of ~22kDa. Caveolin-3 is the principal structural protein of caveolar membrane domains in skeletal muscle and in the heart. The alteration of caveolin-3 have been associated with four overlapping neuromuscular phenotypes: limb-girdle muscular dystrophy (LGMD1C), rippling muscle disease (RMD), distal myopathy (DM) and hyperCKemia. We report a 36-year-old woman with hyperCKemia (500-1000 UI/l), who showed, since childhood, generalized muscle weakness, calf cramps and myalgia, calf and quadriceps hypertrophy, and pes cavus. Percussion induced rapid muscle contractions (PIRC) and mounding (PIMM) were present. Electromyography showed absence of action potentials while nerve conduction velocity was normal. Muscle biopsy showed aspecific myopathic lesions and the complete absence of caveolin-3 both by immunohistochemistry and immunoblotting. Family history was negative for neuromuscolar disorders. However the father showed calf hypertrophy and slightly elevated levels of CK (250UI/l). CK levels were normal in her mother and brother.

CAV3 gene analysis showed that the patient was a compound heterozygous for two novel mutations: a deletion encopassing exon 2 inherited by the father and a 1 bp insertion in exon1 inherited by the mother. Both mutations lead to a truncated CAV3 protein. Our study confirms that RMD can be transmitted as AR trait due to CAV3 mutations. In addition, we reported for the first time CAV3 mutations leading to the premature truncation of the protein, indicating that Caveolin-3 deficiency can be also caused by haploinsufficiency.

THE INFLUENCE OF PARTIAL SPINAL CORD DAMAGE ON PHENOTYPE OF RAT LIMB SKELETAL MUSCLES

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It is known that neurotrophical control of various functions of skeletal muscles is accomplished by motoneurons by axon transport of trophical factors named as neuroregulator substances and also can be mediated through impulse activity. At the same time the association between the neurotrophical control of the various features of skeletal muscles and the function of the upper motoneurons is still unknown. The assessment of this association can be determined in the model utilizing the partial damage of the spinal cord on the different levels. We have established the increase numbers of slow MF in m. soleus (known as slow muscle) and fast MF in the m. plantaris after three weeks of such partial damage. The same effect was not observed after the denervation. Taken together, supraspinal influences together with motoneurons are participating in the neuronal regulation of functional activity of skeletal muscles.

CIRCULATING MUSCLE-SPECIFIC AUTOANTIBODY TO CARBONIC ANHYDRASE III IN A PATIENT WITH MYOFIBRILLAR MYOPATHY

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We report on a 60-year-old woman who developed muscle weakness three years after the removal of a breast cancer. A muscle biopsy, performed two years after the beginning of symptoms, showed necrotic fibers, endomysial and perimysial inflammatory infiltrates and abnormal desmin- and aB-crystallin-positive foci in many muscle fibers.By electron microscope, myofibrillar disruption, streaming of Z disk and abundant granulo-filamentous material, that was diffusely interspersed among the myofibrils, were observed. MHC class I antigen was present on the sarcolemma of a few muscle fibers. Immunoblot analysis confirmed the increased expression of.desmin and aB-crystallin, but no mutations of desmin, aB-crystallin and myotilin genes were found. A circulating muscle-specific autoantibody to carbonic anhydrase III was detected in the patient's serum and the carbonic anhydrase III antigen was expressed by the patient's breast tumor. The possible pathogenetic role of the muscle-specific autoantibody in causing this myopathy is discussed.

STATIN-INDUCED MYOPATHY: EVIDENCE OF REDUCED COQ10 LEVELS IN MUSCLE TISSUE OF 32 PATIENTS

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An increasing number of patients is currently treated with HMG-CoA reductase inhibitors (statins) for their efficacy in preventing cardiovascular diseases. Although these drugs are generally well tolerated, recurrent side effects include hyperCKemia, myalgias, and weakness, in some cases with severe outcome. However, little is known about the mechanisms and the real incidence of muscle and nerve damage. Recently, decreased serum levels of CoQ10 were found in asymptomatic subjects treated with atorvastatin, supporting the hypothesis of an inhibitory effect on CoQ10 synthesis. We studied 53 patients placed in statin therapy (from 3 months to 14 years), complaining of any of the following symptoms: myalgias, cramps, fasciculations, easy fatigability, and weakness, lasting longer than three months after drug withdrawal (3 months to 4 years); patients with occasional finding of persistent asymptomatic hyperCKemia were also observed. We performed muscle biopsy in 32 patients with persistent muscle weakness and/or CK levels constantly higher than 500 U/l. Muscle biopsy revealed from minimal to marked changes in 28 patients; mitochondrial alterations were present in 18 patients (COX negative fibers in 9); marked inflammatory signs were observed in two cases. Biochemical analysis of respiratory chain enzymes and mtDNA Southern blot analysis were normal. Skeletal muscle CoQ10 levels were markedly decreased in 28 out of 32 patients (15 to 4 mg/g muscle tissue, control values 25 ± 4.5). These results show that statins may cause not only acute but also chronic myopathic damage and that they remarkably decrease muscle CoQ10 concentration. Their inhibitory effect on CoQ10 synthesis has a primary but not exclusive role in determining muscle damage.

MORPHOLOGICAL FEATURES OF FSHD MOUSE MODEL

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FSHD is an autosomal dominant muscular dystrophy associated with D4Z4 deletion in chromosome 4q35. This deletion leads to inappropriate over-expression of the 4q35 genes located upstream, namely FRG1, FRG2 and ANT1. To study FSHD pathogenesis, we generated transgenic mice selectively over-expressing these three genes in skeletal muscle. We analyzed muscle tissues from 4 and 16 weeks old transgenic mice using histological histochemical and ultrastructural techniques. FRG1 overexpressing mice show a dystrophic pattern (fiber size variability, central nuclei, increased endo- and perimysial connective tissue, fiber necrosis and regeneration, increased FA activity and NADH altered distribution) whereas FRG2 and ANT1 overexpressing mice are normal. Different muscles display variable histopathological changes. Mice overexpressing low (10-fold), medium (26-fold) and high (42-fold) FRG1 levels show a direct correlation between the amount of transgene over-expression and the severity of dystrophic changes.

Histopathological signs of muscular dystrophy increase with age. Surprisingly, muscles from 4 weeks old mice show diffuse fiber atrophy without dystrophic features. Ultrastructural examination revealed marked proliferation of collagen fibrils only in FRG1-medium and high transgenic mice. Immunohistochemical analysis both at EM and light microscopy revealed nuclear localization of FRG1 suggesting a possible involvement of the protein in RNA processing.

IMMUNOHISTOCHEMICAL CHARACTERISTIC OF THE MAN CHEWING MUSCLES IN THE DYSFUNCTION OF TEMPOROMANDIBULAR JOINT

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Chewing muscles of the man belongs to typically fast muscles with prevalence of oxidative-glycolytic fibers. Using the monoclonal antibodies to the different myosin's the changes of amount slow and fast muscle fibers (MF) during various temporomandibular joint dysfunctions were shown. Moreover the insignificant increasing in content of fast MF was detected. The decreasing of amount of steady against exhaustion oxidative MF by determination of succinate dehydrogenase activity was demonstrated. That well explains clinical displays at such patients. Thus, the chewing muscles are arranged for conditions, which appear during dysfunction of a joint not only by change of synthesis of contractile proteins, but also by type change of a energetic metabolism in some MF.

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