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CONTENTS

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INVITED REVIEW

Genetic basis of limb-girdle muscular dystrophies: the 2014 update

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Limb-girdle muscular dystrophies (LGMD) are a highly heterogeneous group of muscle disorders, which first affect the voluntary muscles of the hip and shoulder areas. The definition is highly descriptive and less ambiguous by exclusion: non-X-linked, non-FSH, non-myotonic, non-distal, nonsyndromic, and non-congenital. At present, the genetic classification is becoming too complex, since the acronym LGMD has also been used for a number of other myopathic disorders with overlapping phenotypes. Today, the list of genes to be screened is too large for the gene-by-gene approach and it is well suited for targeted next generation sequencing (NGS) panels that should include any gene that has been so far associated with a clinical picture of LGMD. The present review has the aim of recapitulating the genetic basis of LGMD ordering and of proposing a nomenclature for the orphan forms. This is useful given the pace of new discoveries. Thity-one loci have been identified so far, eight autosomal dominant and 23 autosomal recessive. The dominant forms (LGMD1) are: LGMD1A (myotilin), LGMD1B (lamin A/C), LGMD1C (caveolin 3), LGMD1D (DNAJB6), LGMD1E (desmin), LGMD1F (transportin 3), LGMD1G (HNRPDL), LGMD1H (chr. 3). The autosomal recessive forms (LGMD2) are: LGMD2A (calpain 3), LGMD2B (dysferlin), LGMD2C (γ sarcoglycan), LGMD2D (α sarcoglycan), LGMD2E (β sarcoglycan), LGMD2F (δ sarcoglycan), LGMD2G (telethonin), LGMD2H (TRIM32), LGMD2I (FKRP), LGMD2J (titin), LGMD2K (POMT1), LGMD2L (anoctamin 5), LGMD2M (fukutin), LGMD2N (POMT2), LGMD2O (POMTnG1), LGMD2P (dystroglycan), LGMD2Q (plectin), LGMD2R (desmin), LGMD2S (TRAPPC11), LGMD2T (GMPPB), LGMD2U (ISPD), LGMD2V (Glucosidase, alpha), LGMD2W (PINCH2).

Key words: Limb-girdle muscular dystrophies, LGMD, NGS

Introduction

The term limb-girdle muscular dystrophy refers to a long list of Mendelian disorders characterized by a progressive deterioration of proximal limb muscles. Very often, other muscles are affected, together with the heart and the respiratory muscles. The clinical course and the expressivity may be variable, ranging from severe forms with rapid onset and progression to very mild forms allowing affected people to have fairly normal life spans and activity levels (1). The term LGMD is becoming descriptive and also comprises clinical pictures of different diseases. The original definition was given as muscular dystrophies milder that DMD and inherited as autosomal traits (2). However, the most severe forms with childhood onset also result in dramatic physical weakness and a shortened life-span. The advent of next generation sequencing approaches has accelerated the pace of discovery of new LGMD genes. Ten years ago the list included 16 loci (3), while today the LGMD loci so far identified are thirty-one, eight autosomal dominant and 23 autosomal recessive.

Autosomal dominant LGMD

The LGMD1, i.e. the autosomal dominant forms, have usually an adult-onset and are milder, because affected parents are usually in quite good health at reproductive age. They are relatively rare representing less than 10% of all LGMD. Sometimes, they correspond to particular cases of mutations in genes involved in other disorders, such as myotilin, lamin A/C or caveolin 3 (Table 1).

LGMD1A - LGMD1A may be caused by mutations in the myotilin (*MYOT*) gene at chr. 5q31.2. The cDNA is of 2.2 kb and contains 10 exons. Myotilin is a Z-disk-associated protein. LGMD1A may be considered as an occasional form of LGMD (4). The first clinical report was in 1994 (5). The gene was identified in 2000 (6), but myotilin mutations have been rather associated with myofibrillar myopathy. LGMD1A is characterized by late

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Table 1. Autosomal dominant limb girdle muscular dystrophy.

Gene					Clinical phenotype				
Disease	Locus	Name	Exons	Protein (protein function)	Typical onset	Progression	Cardiomiopathy	sCK	Allelic disorders (OMIM, #)
LGMD1A	5q31.2	TTID	10	myotilin (structural; Z disc)	Adulthood	Slow	Not observed	3-4X	Myopathy, myofibrillar, 3 (609200)
									Myopathy, spheroid body (182920)
LGMD1B	1q22	LMNA	12	lamin A/C (structural; fibrous nuclear lamina)	Variable (4-38y)	Slow	Frequent	1-6X	Cardiomyopathy, dilated, 1A(115200)
									Charcot-Marie-Tooth disease, type 2B1(605588)
									Emery-Dreifuss muscular dystrophy 2, AD(181350)
									Emery-Dreifuss muscular dystrophy 3, AR(181350)
									Heart-hand syndrome, Slovenian type(610140)
									Hutchinson-Gilford progeria(176670)
									Lipodystrophy, familial partial, 2(151660)
									Malouf syndrome(212112)
									Mandibuloacral dysplasia(248370)
									Muscular dystrophy, congenital(613205)
									Restrictive dermopathy, lethal(275210)
LGMD1C	3p25.3	CAV3	2	caveolin 3 (scaffolding protein within caveolar membranes)	Childhood	Slow/ moderate	Frequent	10X	Cardiomyopathy, familial hypertrophic(192600)
									Creatine phosphokinase, elevated serum(123320)
									Long QT syndrome 9(611818)
									Myopathy, distal, Tateyama type(614321)
									Rippling muscle disease(606072)
LGMD1D	7q36	DNAJB6	10	DnaJ/Hsp40 homolog, subfamily B, member 6 (chaperone)	Variable (25-50y)	Slow	Not observed	1-10X	-
LGMD1E	2q35	DES	9	desmin (structural; intermediate filament)	Adulthood	Slow	Frequent	5-10X	Muscular dystrophy, limb-girdle, type 2R(615325)
									Cardiomyopathy, dilated, 1I(604765)
									Myopathy, myofibrillar, 1(601419)
									Scapuloperoneal syndrome, neurogenic, Kaeser type(181400)
LGMD1F	7q32	TNPO3	23	transportin 3 (nuclear importin)	Variable (1-58y)	Slow/ moderate	Not observed	1-3X	-
LGMD1G	4q21	HNRPDL	9	Heterogeneous nuclear ribonucleoprotein D-like protein (ribonucleoprotein, RNA-processing pathways)	Variable (13-53y)	Slow	Not observed	1-9X	-
LGMD1H	3p23-p25	-	-	-	Variable (10-50y)	Slow	Not observed	1-10X	-

onset proximal weakness with a subsequent distal weakness. Some patients show nasal and dysarthric speech. Serum CK is normal or mildly elevated. Muscle pathology shows rimmed vacuoles with or without inclusions. Electron microscopy shows prominent Z-line streaming. Cardiac and respiratory involvement occasionally occurs.

LGMD1B - LGMD1B is also an occasional LGMD form caused by lamin A/C (*LMNA*) gene mutations at chr. 1q22 (7). The reference cDNA is of 3 kb and contains 12 exons. The *LMNA* gene gives rise to at least three splicing isoforms (lamin A, C, lamin AΔ10). The two main isoforms, lamin A and C, are constitutive components of the fibrous nuclear lamina and have different roles, ranging from mechanical nuclear membrane maintenance to gene regulation. The 'laminopathies' comprise different well-characterized phenotypes, some of which are confined to the skeletal muscles or skin, while others are multi-systemic, such as lipodystrophy, Charcot-Marie Tooth disease, progeroid syndromes, dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy (EDMD). The LGMD1B is characterized by a symmetric proximal weakness starting from the legs, associated with atrioventricular conduction disturbances and dysrhythmias. CK is normal to moderately elevated. Most patients develop proximal leg weakness, followed by cardiac arrhythmias and dilated cardiomyopathy, with sudden death 20-30 years later. However, there is a continuity between LGMD1B and EDMD (8). Usually the more severe forms of EDMD with a childhood onset have missense mutations, whereas the milder LGMD1B is associated with heterozygous truncating mutations: this may arise through a loss of LMNA function secondary to haploinsufficiency, whereas dominant-negative or toxic gain-of-function mechanisms may underlie the EDMD phenotypes.

LGMD1C - LGMD1C is caused by mutations in the caveolin 3 gene (*CAV3*) at chr. 3p25.3. The *CAV3* gene encodes a 1.4kb mRNA composed of only two exons. Caveolin-3 is a muscle-specific membrane protein and the principal component of caveolae membrane in muscle cells in vivo: at present this is the only gene in which mutations cause caveolinopathies (9). LGMD1C is characterized by an onset usually in the first decade, a mild-to-moderate proximal muscle weakness, calf hypertrophy, positive Gower sign, and variable muscle cramps after exercise.

LGMD1D - Autosomal dominant LGMD mapped to 7q36 has been classified as LGMD1E in OMIM, but as LGMD1D in the Human Gene Nomenclature Committee Database. In the literature there is another LGMD1D/E erroneously mapped to 6q, but we will use the acronym LGMD1D for the 7q-disease and LGMD1E for the 6q-form. LGMD1D is caused by heterozygous missense mutations in the *DNAJB6* gene at chr. 7q36.3 (10). The reference cDNA sequence is 2.5kb-long, contains 10 exons and encodes DnaJ homolog, subfamily B, member 6. DNAJ family members are characterized by a highly conserved amino acid stretch (2) called the 'J-domain'. They exemplify a molecular chaperone functioning in a wide range of cellular events, such as protein folding and oligomeric protein complex assembly (11). Missense heterozygous mutations of *DNAJB6* (p.Phe89Ile, p.Phe93Leu and p.Pro96Arg) are all located in the Gly/Phe-rich domain of DNAJB6 leading to insufficient clearance of misfolded proteins. Functional testing *in vivo* have shown that the mutations have a dominant toxic effect mediated specifically by the cytoplasmic isoform of DNAJB6. *In vitro* studies have demonstrated that the mutations increase the half-life of DNAJB6, extending this effect to the wild-type protein, and reduce its protective anti-aggregation effect.

DNAJB6 is located in the Z line and interacts with BAG3. Mutations in BAG3 are known to cause myofibrillar myopathy (12). A characteristic pathological finding of LGMD1D is the presence of autophagic vacuoles and protein aggregation. These protein aggregations contain DNAJB6 together with its known ligands MLF1 and HSAP1, and also desmin, αB-crystallin, myotilin, and filamin C, which are known to aggregate in myofibrillar myopathy. These results suggest that the phenotype of LGMD1D also overlaps with that of myofibrillar myopathy.

LGMD1D patients show mildly elevated serum CK levels. The lower limbs are more affected, particularly the soleus, adductor magnus, semimembranosus and biceps femoris. In contrast, the rectus femoralis, gracilis and sartorius and the anterolateral lower leg muscles are mostly spared. *DNAJB6* gene mutations may also be associated with distal-predominant myopathy. Symptoms in the upper limbs appear later. Some patients develop calf hypertrophy. Onset ranges from 25 to 50 years, with some patients maintaining ambulation throughout life. No cardiac or respiratory involvement has been reported so far. The pattern of differential involvement could be identified at different stages of the disease process.

LGMD1E - For the limb girdle muscular dystrophy originally linked to chr. 6q23 (13) we will use the name LGMD1E, even if it should be considered, more correctly, as a form of autosomal dominant desminopathy or myofibrillar myopathy. This form is also known as dilated cardiomyopathy type 1F (CMD1F). One family previously categorized as having LGMD and dilated cardiomyopathy was reported, indeed, to have the splice site mutation IVS3+3A>G in the desmin (*DES*) gene at 2q35 (14).

For desmin see also LGMD2R. As in the desminopathies, LGMD1E family members show dilated cardiomyopathy and conduction defects together with progressive proximal muscle weakness starting in the second or third decade. Some family members had a history of sudden death. Serum creatine kinase is mildly elevated (150-350U/l). Muscle pathology may show dystrophic changes, but later the presence of abundant perinuclear or subsarcolemmal granulofilamentous inclusions have been also observed. The study of these inclusions by laser capture microdissection followed by mass spectrometry analysis, led to the identification of the disease-causing mutations in desmin (14).

LGMD1F - LGMD1F was originally mapped to a 3.68-Mb interval on chromosome 7q32.1-7q32.2 in a very large Italo-Spanish family. We presented the identification of TNPO3 by whole exome sequencing of four affected family members and the complete refining of the region at the WMS 2012. Data were then published (15): a frame-shift mutation in the transportin 3 (*TNPO3*) gene is shared by all affected family members with 94% penetrance. The *TNPO3* gene is composed of 23 exons and encodes a 923-amino acid protein, also expressed in skeletal muscle. The frame-shifted TNPO3 protein is larger than the wt, since it lacks the predicted stop codon and is found around the nucleus, but not inside. Patients with an onset in the early teens, show a more severe phenotype with a rapid disease course, while adult onset patients present a slower course. They have a prominent atrophy of lower limb muscles, involving especially the vastus lateralis and the ileopsoas muscle (16). Interestingly, some patients present with dysphagia, arachnodactyly and respiratory insufficiency. CK range is 1-3x. No cardiac involvement has been reported.

LGMD1G - LGMD1G has been mapped to chr. 4q21. Very recently, the defect in the RNA processing protein HNRPDL has been identified (17) in two different families by whole exome sequencing. The *HNRPDL* gene contains 8 exons and is ubiquiously expressed. The gene product is a heterogeneous ribonucleoprotein family member, which participates in mRNA biogenesis and metabolism. The reduced hnrpdl in zebrafish prodeces a myopathic phenotype. Patients show late-onset LGMD associated with progressive fingers and toes flexion limitation (18).

LGMD1H - By studying a large pedigree from Southern Italy, a novel LGMD locus has been mapped on chromosome 3p23-p25.1 (19). Most of patients present with a slowly progressive proximal muscle weakness, in both upper and lower limbs, with onset during the fourth-fifth decade of life.

Autosomal recessive LGMD

The autosomal recessive forms (LGMD2) are much more common, having a cumulative prevalence of 1:15,000 (2) with some differences among countries, depending on the carrier distribution and the degree of consanguinity.

There are recessive genes in which the loss-of-function mutations on both alleles tipically result in a LGMD phenotype (ordinary LGMD genes): they correspond to the first 8 forms of LGMD2 (LGMD2A-2H) plus LGMD2L. On the contrary, other genes (occasional LGMD genes) show a phenotypic divergence with some mutations associated with LGMD and other ones determining a more complex disorder. Specific variations in occasional LGMD genes cause the other forms (LGMD2I-2U). The best examples come from dystroglycanopathies in which the LGMD presentation is associated with milder alleles of genes mutated in congenital forms with brain involvement (Table 2).

LGMD2A - LGMD2A is caused by Calpain 3 (*CAPN3*) gene mutations and represents the most frequent LGMD worldwide (20, 21). The CAPN3 gene spans 53kb of genomic sequence at chromosome 15q15.2 and the transcript is composed of 24 exons encoding a 94kDa muscle-specific protein. There is a number of heterozygotes (1:100), carrying many different CAPN3 pathogenic changes. Calpains are intracellular nonlysosomal cysteine proteases modulated by calcium ions. A typical calpain is a heterodimer composed of two distinct subunits, one large (> 80 kDa) and the other small (30 kDa). While only one gene encoding the small subunit has been demonstrated, there are many genes for the large one. CAPN3 is similar to ubiquitous Calpain 1 and 2 (m-calpain and micro-calpain), but contains specific insertion sequences (NS, IS1 and IS2). Calpains cleave target proteins to modify their properties, rather than “break down” the substrates.

The phenotypic spectrum of calpainopathies is very broad, but they are true LGMD. For the clinical course, see also (1).

LGMD2B - It is caused by missense or null alleles of the dysferlin (*DYSF*) gene (22). The *DYSF* gene spans 233kb of genomic sequence at chr. 2p13.2 and the major transcript is composed of 6,911 nt containing 57 exons in the HGVS recommended cDNA Reference Sequence. Dysferlin is an ubiquitous 230-KDa transmembrane protein involved in calcium-mediated sarcolemma resealing. LGMD2B is the second most frequent LGMD2 form (15-25%) in numerous countries, but not everywhere (23). Muscle inflammation is recognized in dysferlinopathy and dysferlin is expressed in the immune cells.

Table 2. Autosomal recessive limb girdle muscular dystrophy.

Gene					Clinical phenotype					
Disease	Locus	Name	Exons	Protein product	LGMD phenotype	Typical onset	Progression	Cardiomiopathy	sCK	Allelic disorders (OMIM, #)
LGMD2A	15q15	CAPN3	24	Calpain 3	ordinary	Adolescence	Moderate/ rapid	Rarely observed	3–20X	
LGMD2B	2p13.2	DYSF	56	Dysferlin	ordinary	Young adulthood	Slow	Possible	5-40X	Miyoshi muscular dystrophy 1 (254130) Myopathy, distal, with anterior tibial onset (606768)
LGMD2C	13q12	SGCG	8	γ-Sarcoglycan	ordinary	Early childhood	Rapid	Often severe	10–70X	
LGMD2D	17q21.33	SGCA	10	α-Sarcoglycan	ordinary	Early childhood	Rapid	Often severe	10–70X	
LGMD2E	4q12	SGCB	6	β-Sarcoglycan	ordinary	Early childhood	Rapid	Often severe	10–70X	
LGMD2F	5q33	SGCD	9	δ-Sarcoglycan	ordinary	Early childhood	Rapid	Rarely observed	10–70X	Cardiomyopathy, dilated, 1L (606685)
LGMD2G	17q12	TCAP	2	Telethonin	ordinary	Adolescence	Slow	Possible	10X	Cardiomyopathy, dilated, 1N (607487)
LGMD2H	9q33.1	TRIM32	2	Tripartite motif containing 32	ordinary	Adulthood	Slow	Not observed	10X	Bardet-Biedl syndrome 11 (209900)
LGMD2I	19q13.3	FKRP	4	Fukutin related protein	ordinary	Late childhood	Moderate	Possible	10-20X	
LGMD2J	2q24.3	TTN	312 or more	Titin	occasional	Young adulthood	Severe	Not observed	10-40X	Cardiomyopathy, dilated, 1G (604145) Cardiomyopathy, familial hypertrophic, 9 (613765) Myopathy, early-onset, with fatal cardiomyopathy (611705) Myopathy, proximal, with early respiratory muscle involvement (603689) Tibial muscular dystrophy, tardive (600334)
LGMD2K	9q34.1	POMT1	20	Protein-O-mannosyl transferase 1	occasional	Childhood	Slow	Not observed	10-40X	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 1 (236670) Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 1 (613155) Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 1 (609308)
LGMD2L	11p13-p12	ANO5	22	Anoctamin 5	ordinary	Variable (young to late adulthood)	Slow	Not observed	1-15X	Gnathodiaphyseal dysplasia (166260) Miyoshi muscular dystrophy 3 (613319)
LGMD2M	9q31	FKTN	11	Fukutin	occasional	Early childhood	Moderate	Possible	10-70X	Cardiomyopathy, dilated, 1X (611615) Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 4 (253800) Muscular dystrophy-dystroglycanopathy (congenital without mental retardation), type B, 4 (613152)

(*continues*)

Table 2. (*follows*).

Gene					Clinical phenotype					
Disease	Locus	Name	Exons	Protein product	LGMD phenotype	Typical onset	Progression	Cardiomiopathy	sCK	Allelic disorders (OMIM, #)
LGMD2N	14q24	POMT2	21	Protein-O-mannosyl transferase 2	occasional	Early childhood	Slow	Rarely observed	5-15X	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 2 (613150)
										Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 2 (613156)
LGMD2O	1p34.1	POMGnT1	22	Protein O-linked mannose beta1,2-N-acetylglucosaminyl transferase	occasional	Late childhood	Moderate	Not observed	2-10X	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 3 (253280)
										Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 3 (613151)
										Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 3 (613157)
LGMD2P	3p21	DAG1	3	Dystroglycan	singular	Early childhood	Moderate	Not observed	20X	
LGMD2Q	8q24	PLEC1	32	Plectin	singular	Early childhood	Slow	Not observed	10-50X	Epidermolysis bullosa simplex with pyloric atresia (612138)
										Epidermolysis bullosa simplex, Ogna type (131950)
										Muscular dystrophy with epidermolysis bullosa simplex (226670)
LGMD2R	2q35	DES	9	Desmin (structural; intermediate filament)	occasional	Young adulthood		A-V conduction block	1X	Muscular dystrophy, limb-girdle, type 2R(615325)
										Cardiomyopathy, dilated, 1I(604765)
										Myopathy, myofibrillar, 1(601419)
										Scapuloperoneal syndrome, neurogenic, Kaeser type(181400)
LGMD2S	4q35	TRAPPC11	30	Transport protein particle complex 11	occasional	Young adulthood	Slow	Not observed	9-16X	
LGMD2T	3p21	GMPPB	8	GDP-mannose pyrophosphorylase B	occasional	Early childhood-Young adulthood		Possible		Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 14 (615350)
										Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 14 (615351)
LGMD2U	7p21	ISPD	10	Isoprenoid synthase domain containing	occasional	Early / Late	Rapid/ Moderate	Possible	6-50X	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 7 (614643)
LGMD2V	17q25.3	GAA	20	Alpha-1,4-glucosidase	occasional	Variable	Variable (Rapid to slow)	Possible	1-20X	Glycogen storage disease II (232300)
LGMD2W	2q14	LIMS2	7	Lim and senescent cell antigen-like domains 2	?	Childhood	-	Possible	-	

The "dysferlinopathies" include limb-girdle muscular dystrophy type 2B (LGMD2B) and the allelic forms Miyoshi myopathy (MM), which is an adult-onset distal form, and distal myopathy with anterior tibialis onset (DMAT), but varied phenotypes are observed. LGMD2B affects earlier the proximal muscles of the arms whereas MM affects the posterior muscles of the leg.

DYSF gene mutations are associated with heterogeneous clinical pictures ranging from severe functional disability to mild late-onset forms (24). About 25% of cases are clinically misdiagnosed as having polymyositis (25). This classification into separate phenotypes does not reveal true disease differences (26) and the allelic forms are not due to different mutations. Additional factors (e.g., additional mutations in neuromuscular disease genes or sport activities that include maximal eccentric contractions) may worsen the disease expression of causative mutations in dysferlinopathies (27).

WB analysis is very useful and specific (28) when < 20% level of Dysferlin has been identified, although Dysferlin can also be increased or secondarily reduced. NGS-based testing is preferred due to the huge number of exons to be screened and the lack of mutational hot-spots. mRNA analysis also works from blood, albeit with some splice differences (29).

LGMD2C-D-E-F

Loss-of-function mutations in any of the genes encoding the four members of the skeletal muscle sarcoglycan complex, alpha, beta, gamma and delta-sarcoglycan cause LGMD2D, 2E, 2C and 2F, respectively (30-33). Sarcoglycans are components of the dystrophin-complex. They are all N-glycosylated transmembrane proteins with a short intra-cellular domain, a single transmembrane region and a large extra-cellular domain containing a cluster of conserved cysteines.

Sarcoglycanopathies have a childhood onset, similar to intermediate form of Duchenne/Becker dystrophies, and involve both cardiac and respiratory functions. We consider the possibility to classify these forms apart from the other LGMD.

LGMD2C - The gamma-sarcoglycan gene spans 144kb of genomic sequence at chromosome 13q12.12 and the transcript is composed of 8 exons. LGMD2C is common in the Maghreb and India (34) for the high allele frequency of 525delT and in gypsies for the C283Y allele. LGMD2C patients may show the absence of ψ-sarcoglycan together with traces of the other non-mutated sarcoglycans.

LGMD2D - The alpha-sarcoglycan gene spans 10kb of genomic sequence at chromosome 17q21.33 and the major transcript is composed of 10 exons. The protein product of 387 amino acids and 50kDa was originally named adhalin and contains a "dystroglycan-type" cadherin-like domain that is present in metazoan dystroglycans (35).

LGMD2E - The beta-sarcoglycan gene spans 15kb of genomic sequence at chromosome 4q11 and the major transcript is composed of 6 exons. The protein contains of 318 amino acids and weighs 43kDa.

LGMD2F - Delta-sarcoglycan is by far the largest LGMD gene, spanning 433kb of genomic sequence at chromosome 5q33.3 and the major transcript is composed of 9 exons. Intron 2 alone spans 164kb, one the largest of the human genome. Delta and gamma sarcoglycan are homologous and of identical size (35kDa).

LGMD2G - Mutations in titin cap (Tcap)/Telethonin cause LGMD2G, one of the rarest forms of LGMD (36). Tcap provides links to the N-terminus of titin and other Z-disc proteins. Patients show adolescence-onset weakness initially affecting the proximal pelvic muscles and then the distal legs with calf hypertrophy. A homozygous nonsense mutation in the *TCAP* gene has been described in patient a congenital muscular dystrophy. The *TCAP* gene has also been associated with cardiomyopathy (37), while common variants may play a role in genetic susceptibility to dilated cardiomyopathy. Immunofluorescence and Western blot assays may show a Telethonin deficiency. Full sequencing testing may be cost-effective in all cases, because the gene is composed only of two small exons.

The telethonin gene *(TCAP)* spans 1.2kb of genomic sequence at chromosome 17q12 and the transcript is composed of 2 exons. The protein product is a 19kDa protein found in striated and cardiac muscle. It binds to the titin Z1-Z2 domains and is a substrate of titin kinase, interactions thought to be critical for sarcomere assembly. Only two different mutations have been described in the *TCAP* gene in Brazilian patients (36). A mutation (R87Q) was found in a patient with dilated cardiomyopathy (37). Moreover, a human muscle LIM protein (MLP) mutation (W4R) associated with dilated cardiomyopathy (DCM) results in a marked defect in Telethonin interaction/localization (38).

LGMD2H - The Tripartite-motif-containing gene 32 *(TRIM32)* gene spans 14kb of genomic sequence at chromosome 9q33.1 and the transcript is composed of 2 exons, with the first noncoding and the second encoding a 673 aa protein of 72kDa. TRIM32 is a ubiquitous E3 ubiquitin ligase that belongs to a protein family comprising at least 70 human members sharing the tripartite motif (TRIM). The TRIM motif includes three zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region. The protein localizes to cytoplasmic bodies. Although the function of TRIM32 is unknown, analysis of the domain structure of this protein suggests that it may be an E3-ubiquitin ligase (39).

LGMD2H is usually a late-onset condition characterized by proximal weakness, atrophy, and moderately raised levels of creatine kinase. Until 2008, the only LGMD2H mutation was Asp487Asn found in Hutterite families (40). Different *TRIM32* mutations were then identified in Italian LGMD patients (41) that accounts for about 3% of LGMD2. The D487N mutation of TRIM32 causes the more severe sarcotubular myopathy (STM). Recently, two other LGMD2H patients have been described associated with STM morphotype (42).

LGMD2I, LGMD2K, LGMD2M, LGMD2N, LGMD2O, and LGMD2P

The name dystroglycanopathy has been given to defects due to mutations in six genes (*POMT1, POMT2, POMGnT1, FKTN, FKRP* and *DAG1*) (43). These variations reduce dystroglycan glycosylation and cause a wide range of phenotypes ranging from mild congenital muscular dystrophies to dramatic conditions, including brain and eye anomalies (muscle–eye–brain disease or Walker–Warburg syndrome).

LGMD2I - The fukutin-related protein gene spans 12kb of genomic sequence at chromosome 19q13.32 and the transcript is composed of 4 exons, with the first three noncoding. The extracellular part of the dystrophin/utrophin-associated complex is also involved in congenital muscular dystrophies, as well as in LGMD2I. Fukuyama-type congenital muscular dystrophy (FCMD), is one of the most common autosomal recessive disorders in Japan characterized by a congenital muscular dystrophy associated with brain malformation (micropolygria) due to a defect in the migration of neurons caused by mutation in the fukutin gene at 9q31 (44). Mutations in the fukutin-related protein gene *(FKRP)* at 19q13 cause a form of congenital muscular dystrophy with secondary laminin alpha2 deficiency and abnormal glycosylation of alpha-dystroglycan (45). The same gene is also involved in LGMD2I (15).

All of these diseases are associated with changes in alpha-dystroglycan expression due to a glycosylation defect of alpha-dystroglycan. Dystroglycan is normally expressed and recognized by polyclonal antibodies, but it is abnormally glycosylated and not recognized by monoclonal antibodies directed against certain epitopes. FKRP is resident in the Golgi apparatus. The P448L mutation, that results in CMD1C, causes a complete mislocalization of the protein and the alpha-dystroglycan is not processed, while LGMD2I mutations affect the putative active site of the protein or cause inefficient Golgi localization (46).

LGMD2I mutations appear to be a relatively common cause of LGMD, accounting for at least 10% of all LGMD with either severe or mild phenotypes (47, 48).

LGMD2J - *TTN* is one of the most complex human genes. The titin gene spans 294,442 bp of genomic sequence at chromosome 2q31 and the major transcript is composed of 363 exons. It encodes the largest protein of the human genome composed of 38,138 amino acids with a physical length of 2 microns. An 11-bp indel mutation in the last titin exon causes tibial muscular dystrophy and Gerull et al. (49) showed that a 2-bp insertion in exon 326 of the *TTN* gene causes autosomal dominant dilated cardiomyopathy (CMD1G; 604145). A homozygous mutation in the C terminus of titin (FINmaj 11bp deletion/insertion) causes LGMD2J (50). Titin is the giant sarcomeric protein that forms a continuous filament system in the myofibrils of striated muscle, with single molecules spanning from the sarcomeric Z-disc to the M-band (51). Other "titinopathic" clinical phenotypes are tibial muscular dystrophy (TMD, Udd myopathy) (52) or more severe cardiac and muscular phenotypes (53).

CAPN3 binds M-band titin at is7 within the region affected by the LGMD2J mutations and shows a secondary deficiency in the LGMD2J muscle (54). Interactions with titin may protect CAPN3 from autolytic activation and removal of the CAPN3 protease reverses the titin myopathology (55).

The French nonsense mutation (Q33396X) located in Mex6, seems to cause a milder phenotype than the typical FINmaj mutation (51). Due to the huge gene size, NGS sequencing is the only possible way to study this gene. However, the high number of variants and polymorphisms may have a confounding effect on the diagnosis.

LGMD2K - LGMD2K is caused by hypomorphic missense mutations in the *POMT1* gene at 9q34, containing 20 exons and spanning about 20 kb. Mutations allowing a residual enzyme activity are linked to mild forms. Different *POMT1* alleles, cause congenital muscular dystrophies due to defects of the dystroglycan glycosylation (MDDGC1) and including severe forms with brain and eye anomalies or mental retardation (56-58).

LGMD2L - LGMD2L is caused by mutations in the anoctamin-5 (*ANO5*) gene at 11p14.3 (59). The *ANO5* gene spans 90,192 bp and contains 22 exons; the coding sequence is 2.7kb for 913 amino acids. Alternative gene names are *TMEM16E* and *GDD1*. Anoctamins are a family of calcium-activated chloride channels (60). This form of LGMD2 is one of the most frequent in Northern Europe encompassing 10%-20% of cases (61). The penetrance is probably incomplete, since females are less frequently affected than males. The most common mutation in Northern Europe is c.191 dupA in exon 5 (62). Patients are usually ambulant and the onset is in adulthood. They show asymmetric muscle involvement with prevalent quadriceps atrophy and pain following exercise. CK levels are 5-20x. There is no evidence for contractures,

cardiomyopathy or respiratory involvement. LGMD2L is allelic with the AD gnathodiaphyseal dysphasia (63) and with AR distal myopathy (MMD3) (64).

LGMD2M - This is associated with mutations in the fukutin gene (*FKTN*) at chr. 9q31.2 (65). The *FKTN* gene spans 82,989 bp and contains 10 coding exons, the main transcript is 7.4kb encoding a protein of 413 amino acids. Also in this case LGMD2M is a milder form caused by at least one hypomorphic missense mutation in a gene that, with both non-functional alleles, is associated with more severe phenotypes (66): WWS, MEB or congenital muscular dystrophies (67). In LGMD2M the CNS is not affected and the intelligence is normal. Patients are hypotonic, may be ambulant and the onset is in early childhood. They show symmetric and diffuse muscle involvement that deteriorates with acute febrile illness. Improvement is seen with steroids. CK levels are 10-50x. There is also evidence for spinal rigidity, contractures and cardiomyopathy and respiratory involvement.

LGMD2N - Mutations in the *POMT2* gene, containing 21 exons, at chr. 14q24 cause LGMD2N (68). POMT2 is a second *O*-mannosyltransferase overlapping with POMT1 expression. *POMT2* mutations usually have a dramatic effect: they cause Walker-Warburg syndrome or muscle-eye-brain-like (69), but rarely are associated with LGMD (70). This may occur when the α-dystroglycan glycosylation is only slightly reduced. In these cases the mutations are usually missense and the phenotype is characterized by LGMD without brain involvement, very high serum CK.

LGMD2O - It is associated with milder mutations in the *POMGnT1* gene at chr. 1p32 (71). Usually mutations in the *POMGnT1* gene are associated with more severe phenotypes than LGMD, such as Walker-Warburg syndrome or MEB. A homozygous hypomorphic allele of the *POMGnT1* gene was found as a 9-bp promotor duplication (72).

LGMD2P - LGMD2P is caused by specific changes of the dystroglycan *(DAG1)* gene itself. Recently, Campbell has reported a missense mutation in the dystroglycan gene in an LGMD patient with cognitive impairment (73). This substitution interferes with LARGE-dependent maturation of phosphorylated O-mannosyl glycans on α-dystroglycan affecting its binding to laminin. As a rule the dystroglycanopathies are due to mutations in genes involved in the glycosylation pathway of dystroglycan, but the dystroglycan gene is normal.

LGMD2Q - This form of LGMD is mutation-specific since other mutations in the Plectin (*PLEC1*) gene at chrom. 8q24.3 cause epidermolysis bullosa simplex (74). LGMD2Q has been identified as a homozygous 9-bp deletion in consanguineous Turkish families (75). The deletion affects an AUG that is only present in a muscle-specific transcript Plectin 1f), while there are many other alternative first exons that are spliced to a common exon 2. These patients produce normal skin plectin and do not show skin pathology. LGMD2Q patients show early-onset non-progressive or slowly progressive LGMD.

LGMD2R - Desmin is the muscle-specific member of the intermediate filament (IF) protein family (76). The desmin (*DES*) gene at 2q35 contains 9 exons and spans about 8.4 kb. It encodes a 468-amino acid protein. Autosomal dominant mutations in the *DES* gene are associated with myofibrillar myopathy (14). The overlap with the *DES* gene has also been claimed for LGMD1E (77). A homozygous splice site mutation has been identified in two Turkish sibs, born of consanguineous parents, in intron 7 of the *DES* gene (c.1289-2A>G), resulting in the addition of 16 amino acids from residue 428. Since then, other mutations have been identified. The patients have onset in their teens or twenties of progressive proximal muscle weakness and non-specific atrophy affecting both the upper and lower limbs. The serum Ck is normal. LGMD2R patients usually show A-V conduction blocks but no cardiomyopathy.

LGMD2S - This is caused by mutation in the transport protein particle complex 11 (*TRAPPC11*) gene that spans 54,328 bp at chr. 4q35, the mRNA is 4.5kb and contains 30 exons.

Recently, mutations in *TRAPPC11* have been identified in a consanguineous Syrian family with an uncharacterized form of LGMD and in five Hutterite individuals presenting with myopathy, ID, hyperkinetic movements and ataxia (78).

TRAPPC11 is a transport protein particle component involved in anterograde membrane transport from the endoplasmic reticulum (ER) to the ER-to-Golgi intermediate compartment (ERGIC) in mammals (79). Mutations identified so far (c.2938G>A/ p.Gly980Arg and c.1287+5G>A) cause modifications in TRAPP complex composition, in Golgi morphology and in cell trafficking. The LGMD2S pathogenic mechanism is similar to that causing Danon disease, an X-linked myopathy due to LAMP2 mutations and affecting the secretory pathway (80).

The LGMD2S phenotype ranges from a slowly progressive LGMD with childhood onset and high CK to a syndrome characterized by myopathy but also neurological involvement (ID and ataxia).

LGMD2T - LGMD2T is caused by milder mutations in the GDP-mannose pyrophosphorylase B (*GMPPB*) gene (81). The *GMPPB* gene is a small gene of 2,453bp at chr. 3p21. The mRNA is 1.7kb and contains 8 exons. Mutations in the *GMPPB* gene have been associated with congenital muscular dystrophies with hypoglycosylation of α-dystroglycan and also with LGMD only in three un-

related patients so far reported. The patients from Indian and Egyptian descent presented with microcephaly and intellectual delay. All 3 patients had increased serum creatine kinase and dystrophic findings on muscle biopsy. Muscle biopsy showed hypoglycosylation of DAG1. The English LGMD patient was a 6-year-old boy with exercise intolerance and CK = 3,000 UI. Two missense mutations were identified: pAsp27His and p.Val330Ile.

LGMD2U - This is the form caused by some particular alleles of the isoprenoid synthase domain containing *(ISPD)* gene. The *ISPD* gene spans 333kb at chromosome 7p21 and contains 10 exons. *ISPD* mutations disrupt dystroglycan mannosylation and cause of Walker-Warburg syndrome (82, 83). Mutations in *ISPD* as well as *TMEM5* genes have been associated with severe cobblestone lissencephaly (84). Null alleles of *ISPD* produce Walker Warburg or cobblestone lissencephaly with brain vascular anomalies, but at least one milder mutation in one allele has been found in LGMD (68 69). We named this forms as LGMD2U. The association between mutations in the *ISPD* gene and LGMD was, however, older than that of forms 2P-2T, but to avoid discordant definitions among the LGMD2U should be considered as that caused by some alleles of *ISPD*. LGMD2U is progressive, with most cases with LGMD losing ambulation in their early teenage years, thus following a DMD-like path. In several patients, there is muscle pseudohypertrophy, including the tongue. Respiratory and cardiac functions also decline, resembling other dystroglycanopathies.

LGMD2V - This is a proposal to name as LGMD2V an occasional LGMD form that derives from mild mutations of the acid alpha-glucosidase *(GAA)* gene (85). The *GAA* gene maps at chr 17q25.3 and comprises 20 exons with a protein product of 953 aa. Defects in *GAA* are the cause of glycogen storage disease type 2 (GSD2, MIM: 232300). GSD2 is a metabolic disorder with a broad clinical spectrum. The severe infantile form, or Pompe disease, presents at birth with massive accumulation of glycogen in muscle, heart and liver. Late-onset Pompe disease may present from the second to as late as the seventh decade of life with progressive proximal muscle weakness primarily affecting the lower limbs, as in a limb-girdle muscular dystrophy. Final outcome depends on respiratory muscle failure.

LGMD2W - This caused by mutations in the LIM and senescent cell antigen-like-containing domain protein 2 *(LIMS2/ PINCH2)* gene at chromosome 2q14. The gene comprises 7 coding exons. It encodes a 341-aa member of a small family of focal adhesion proteins. The encoded protein has five LIM domains, each domain forming two zinc fingers, which permit interactions which regulate cell shape and migration. Patients show a childhood onset LGMD with macroglossia and calf enlargement. They also developed decreased ejection fraction with global left ventricular dysfunction in their 3rd decade, severe quadriparesis and relative sparing of the face, and characteristically a broad based triangular tongue**.** This form has been presented in a poster session at the ASHG 2013.

The classification of LGMD is becoming too complex. We tried to reorganize the different genes so far described following the traditional nomenclature. However for the autosomal recessive forms there are few letters available. The next forms will be LGMD2X, LGMD2Y and LGMD2Z. We propose, after the LGMD2Z form, the acronyms LGMD2AA, LGMD2AB, LGMD2AC, etc. to avoid renaming consolidated definitions thereby generating even higher confusion.

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Acta Myologica • 2014; XXXIII: p. 13-18

ORIGINAL ARTICLE

Evaluation of neural damage in Duchenne muscular dystrophy patients

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The presence of non-progressive cognitive impairment is recognized as a common feature in a substantial proportion of patients with Duchenne muscular dystrophy (DMD). Concurrently, the amyloid beta peptide ($A\beta_{42}$) protein has been associated with changes in memory and cognitive functions. Also, it has been shown that different subtypes of neural stem/progenitor cells (CD 34, CD 45, nestin) are involved in the innate repair of plasticity mechanisms by the injured brain, in which Nerve Growth Factor (NGF) acts as chemotactic agents to recruit such cells. Accordingly, the present study investigated levels of CD 34, CD 45, nestin and NGF in an attempt to investigate makers of neural regeneration in DMD. Neural damage was assayed in terms of $A\beta_{42}$. Results showed that $A\beta_{42}$ (21.9 ± 6.7 vs. 12.13 ± 4.5) was significantly increased among DMD patients compared to controls. NGF (165.8 ± 72 vs. 89.8 ± 35.9) and mononuclear cells expressing nestin (18.9 ± 6 vs. 9 ± 4), CD 45 (64 ± 5.4 vs. 53.3 ± 5.2) and CD34 (75 ± 6.2 vs. 60 ± 4.8) were significantly increased among DMD patients compared to controls. In conclusion cognitive function decline in DMD patients is associated with increased levels of $A\beta_{42}$, which is suggested to be the cause of brain damage in such patients. The significant increase plasma NFG and in the number of mononuclear cells bearing $CD_{34, CD45}$ and nestin indicates that regeneration is an ongoing process in these patients. However, this regeneration cannot counterbalance the damage induced by dystrophine mutation and increased $A\beta_{42}$.

Key words: Duchenne muscular dystrophy, neural damage, cognitive function

Introduction

Duchenne muscular dystrophy (DMD) represents an X-linked recessive disorder related to mutations in the dystrophin gene which is located on chromosome Xp21.1 (1). It is one of the most common and severe form of dystrophinopathies, characterized by progressive and disabling muscle weakness affecting approximately 1 in 3000 to 4000 male births (2). The disease is characterized by ongoing degeneration and regeneration of skeletal muscle that leads to replacement of muscle by connective tissue and fat (3).

In addition to the profound skeletal muscle lesions, DMD is associated with mild to severe cognitive deficits and poor academic achievement, which are independent from the muscular handicap or clinical environment (4). Full-scale intelligence quotient (IQ) scores of DMD patients are distributed in accordance with the assumption that the cognitive defect results from the same mutations that cause myopathy (5). In fact, about one third of DMD boys have IQ scores below 70 and display mental retardation. Deficits affect both receptive and expressive language skills, with alterations in auditory comprehension, phonological knowledge and language, and delayed acquisition of reading, which has been partly attributed to a form of developmental dyslexia, that is, dysphonetic dyslexia (5). Impaired short- and long-term memory performances are consistently reported and include defective recall, working memory, memory span, and visuo-spatial skills (5, 6-8).

Amyloid beta peptide (Aβ) is a proteolytically processed fragment of the amyloid precursor protein (APP) (9). It occurs in different length variants with peptides of 40 amino acid residues ($A\beta_{40}$) and 42 amino acid residues ($A\beta_{42}$), the latter is the most prevalent. The accumulation Aβ plaques is a key feature in the brains of Alzheimer Disease (AD) patients and is implicated in the disruption of normal cellular processes leading to neurodegeneration (10). Aβ is secreted into the extracellular space allowing its detection in the CSF and plasma (11). Functional studies have demonstrated that oligomeric Aβ species can impair long-term potentiation (LTP) and synaptic function in mature neurons (12). The magnitude of amyloid plaque deposition in the brain correlates poorly

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with cognitive decline, and emerging evidence suggests that Aβ oligomers may be the major culprits in this regard (13).

NGF is a neurotrophin, shown to support the survival and differentiation of neurons during brain development (14), and reduces neural degeneration (15) and promotes peripheral nerve regeneration in rats (16). Lately, it has been shown that different subtypes of neural stem/progenitor cells respond differently to traumatic brain injury, which induces their activation reflecting the induction of innate repair and plasticity mechanisms by the injured brain (17, 18), where during such process nestin and CD34 expression increases and is serum level dependent (19, 20). It was reported that CD34 cells are present in DMD patients for tissue regeneration (21). It has been demonstrated that CD45 subset comprise juvenile protective factors for the maintenance of brain microvascular health (22).

During the last two decades, the role of dystrophin in the CNS has been investigated in DMD boys and the dystrophin deficient mdx mouse (model of DMD), and have demonstrated a range of abnormalities in CNS function, from behavioral and cognitive dysfunction to alterations in the clustering of ion channels in single identified neurons (23). Accordingly, this study was conducted in order investigate markers related to neural damage and repair in DMD patients. The study investigated levels of CD 34, CD 45 and nestin in an attempt to investigate markers of regeneration in blood of DMD patients and degeneration in terms of $A\beta_{42}$ in relation to IQ.

Subjects and methods

Subjects were 60 boys diagnosed clinically and at the molecular level as having DMD (mean of age (8.1 ± 1.9), versus 20 age and socioeconomic matching healthy boys (mean of age 8.2 ± 2.2). Patients and controls were chosen to be free from any infection and receiving no therapeutic treatment known to increase the oxidative stress. Blood samples were drawn after their parents' consent.

Biochemical Investigations

$A\beta_{42}$

This was carried out using Amyloid Beta (Aβ) ELISA Kit (Millipore catalog number EZHS42 (24).

CD45, CD34 and Nestin Quantification

To quantify EPCs in circulation, peripheral mononuclear cells were first isolated from the blood samples (0.5 mM EDTA). The isolated cells were labeled with the phycoenythrin (PE)-conjugated monoclonal nestin antibody and Fluorescein isothiocyanate (FITC) conjugated CD34 (Macs). The stained cells were washed with phosphate buffered saline and /BSA and then analyzed by flow cytometry at the Faculty of Medicine, Cairo University (25).

Nerve Growth Factor

This is an enzyme-Linked immunosorbent assay, which employs an antibody specific for human for ß-NGF coated on 96 well plate (26).

IQ

This was carried out using the Wechsler Intelligence Scale for Children third edition (WISC III): It provides scores for Verbal IQ, Performance IQ and Full Scale IQ (27).

Results

Results showed that $A\beta_{42}$ (21.9 ± 6.7 vs. 12.13 ± 4.5) was significantly increased among DMD patients compared to controls (Table 1) and that it has a significant negative relation with IQ of the patients (Fig. 1). NGF

Table 1. Markers of neural damage among DMD compared to controls.

	DMD	Controls	t	P
Amyloid Beta Peptide 42	21.9 ± 6.7	12.13 ± 4.5	4.3	P < 0.001
Mean of IQ	74.8 ± 9.3	95.4 ± 10	10.9	P < 0.00001

Table 2. Markers of neural regeneration among DMD compared to controls.

	DMD	Controls	t	p
Nestin	18.9 ± 6	9 ± 4	12.3	P < 0.0001
CD34	75 ± 6.2	60 ± 4.8	9.1	P < 0.0001
CD45	64 ± 5.4	53.3 ± 5.2		P < 0.001
-NGF (pg/ml)	165.8 ± 72	89.8 ± 35.9	4.6	P < 0.001

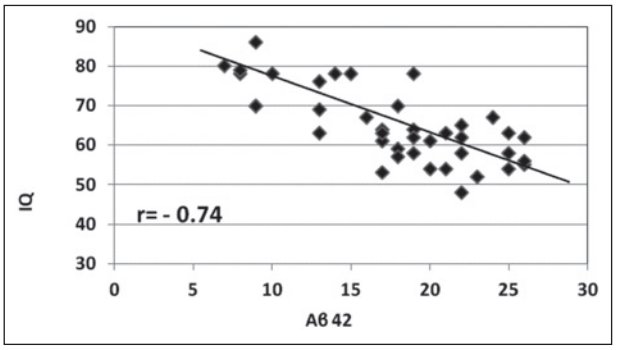

Figure 1. Correlation between $A\beta_{42}$ and IQ among DMD patients.

(165.8 ± 72 vs. 89.8 ± 35.9) and mononuclear cells expressing nestin (18.9 ± 6 vs. 9 ± 4), CD 45 (64 ± 5.4 vs. 53.3 ± 5.2) and CD34 (75 ± 6.2 vs. 60 ± 4.8) were significantly increased among DMD patients (Table 2).

Discussion

Results of the present study showed that $A\beta_{42}$ was significantly higher among DMD patients compared to controls and that a significant negative correlation exist between $A\beta_{42}$ and IQ of such patients. Data regarding levels of $A\beta_{42}$ in DMD are null. However, it has been shown that in patients carrying mutations predicted to affect dystrophin isoforms expressed in the brain, are associated with higher risk of cognitive impairment (28) and since $A\beta_{42}$ has been shown to be associated with cognitive function impairment, the present study assumed that $A\beta_{42}$ levels might be increased in DMD patients compared to controls. Supporting this assumption is that: a direct relation between the deposition of insoluble $A\beta_{42}$ after traumatic brain injury and the changes in brain interstitial fluid Aβ levels has been reported, where the disruption of the blood brain barrier has been shown to play an important role in the pathogenesis of epilepsy (29). Partial or generalized epilepsy has been reported in DMD (30). Also the mdx mice were shown to be susceptible to seizure among administration of convulsing drugs (31) and brain edema and severe alterations of the glial and endothelial cells have recently been demonstrated in such mice (32).

Our recent finding that Aβ 42 was significantly higher among Down syndrome (DS) patients compared to controls (20 ± 5.1 vs. 11.9 ± 3.4) (33) provides further proof that mental retardation is associated with increased levels of Aβ 42 in blood and gives clue that DMD mental retardation is associated with increased levels of Aβ 42. Previous studies have shown that individuals with DS have increased levels of Aβ40 and Aβ42 peptides in plasma together with increased risk for Alzheimer's disease (AD), neuropathology and clinical dementia (34-38).

In recent years there has been a substantial increase in the understanding of the role of dystrophin in the CNS. These studies have been largely carried out on DMD boys and the dystrophin deficient mdx mouse and have demonstrated a range of abnormalities in CNS function, from behavioral and cognitive dysfunction to alterations in the clustering of ion channels in single identified neurons (39). Dystrophin is considered the central component of a scaffold of proteins expressed in a variety of tissues including the brain, where it is involved in the clustering of several membrane receptors and ion channels and in the modulation of cellular signal integration and synaptic plasticity (30). Normally, in the cerebellum, dystrophin appears to play a role in normal neuronal function or development. Two carboxy-terminal dystrophin proteins (Dp), Dp71 and Dp140, are both expressed in the brain, in addition to full-length central nervous system dystrophins, and are initiated between exons 62 and 63, and upstream from exon 44, respectively (40-42). Rearrangements in the second part of the dystrophin gene tend to be more commonly associated with cognitive impairment, and several reports described mutations in the Dp71 coding region as a factor that contributes to the severity of mental retardation (42-44). It is suggested that a lack of the Dp140 isoform is thought to play a significant role in cognitive performances in Duchenne muscular dystrophy (45, 46) and mutations involving the Dp71 region are often associated with severe cognitive impairment (47, 48).

Putative alterations of the brain vascular permeability have been suggested by some studies, which may also participate to behavioral deficits in mdx mice (31). Initial observations of mdx brains revealed severe alterations of endothelial cells with open tight junctions surrounded by swollen glial processes and enhanced vascular permeability suggesting brain blood barrier (BBB) breakdown (48). Follow-up studies suggested that this results partially from hypoxic condition leading to the activation of hypoxia inducible factor-1α contributing to both BBB opening and compensatory angiogenesis, along with changes in expression of matrix metalloproteinases, nerve and vascular growth factors (32). Hence, the hypothesis that a progressive decline in respiratory function due to muscle degeneration, could worsen the brain and cognitive impairments in advanced DMD patients through a reduction in cerebral oxygenation and BBB disruption (49).

NFG was significantly higher in blood of DMD compared to controls in the present study. Although, NFG in blood of DMD studies are scarce, a previous study has shown by means of immunohistochemistry, that regenerating muscle fibers from DMD patients consistently

express NGF, as do myofibroblasts and mast cells (50). By contrast, rest fibers from dystrophic patients, as well as muscle fibers from healthy, control patients and even regenerative muscle fibers in polymyositis do not show NGF immunoreactivity (51, 52). Supporting this finding is a study carried out on mdx dystrophic mouse that demonstrated, by western blotting and real time polymerase chain reaction (RT-PCR), a higher expression of NGF and its receptor mRNA and protein in mdx brain as compared to controls (53). NFG was markedly elevated in the male mdx mouse at 8 and 11 weeks of age (54).

In the present study the numbers of mononuclear cells bearing CD 45, CD34 and nestin markers were significantly increased compared to controls, indicating that regeneration is an ongoing process in DMD patients. It can be expected that CD34 cells are present in DMD patients for tissue regeneration (21), but their capacity for muscle regeneration is hindered. CD34 is also important for vascular repair, and in rat model for traumatic brain injury (TBI). CD34 has been shown to be mobilized from the bone marrow to peripheral blood and brain tissue, a process critical for vascular repair (55). The recruitment of hematopoietic progenitor cells from the bone marrow into the peripheral blood after acute ischemic stroke when no thrombolytic treatment was given was identified in human studies, suggesting that increased progenitor cell recruitment might be caused by so far unknown signaling stimuli of the ischemic brain for stem cell mobilization (19).

Results of the present study showed that mean of mononuclear cell expressing nestin surface marker was significantly higher among DMD patients compared to control. An in vitro previous study, reported that nestin was found specifically in myopathic muscle fibers in Duchenne/Becker muscular dystrophy and myositis but was absent in controls (56). Nestin-Cre/DG null mice have been shown to exhibit earlier and more widespread disruptions of neuronal migration and developed hydrocephalus (57). Nestin has been also shown to play an important role in remodeling and repairing in the postnatal and adult central nervous system in rat models (58) and that a subset of neural progenitors bearing nestin becomes active after injury and can compensate for the injury-induced loss of granular neurons (59). The present study cannot confirm, whether the increased expressing nestin surface marker in circulating blood is due to muscle damage or brain damage in DMD.

Mononuclear cells expressing CD45 was significantly increased among DMD patients compared to controls. Cells expressing CD45 are regarded as muscle regenerating cells (60, 61). Their number increases in the presence of muscle damage (61, 62). It has been demonstrated that CD45 subset comprise juvenile protective factors whose quantitative and qualitative normalization can attenuate the progression of ischemic-hemorrhagic stroke pathogenesis in rat model likely through the maintenance of brain microvascular health (22). It has been demonstrated that genetic loss of CD45 (1) accelerates cerebral amyloidosis (2), causes brain accumulation of soluble oligomeric Aβ species and reduction in plasma-soluble Aβ (3), promotes proinflammatory and anti-Aβ phagocytic microglial activation (4), and leads to mitochondrial dysfunction and neuronal loss in *mice model of Alzheimer Disease* (63).

In conclusion cognitive function decline in DMD patients is associated with increased levels in $A\beta_{42}$, which is suggested to be the cause of brain damage in such patients. The significant increase plasma NFG and in the number of mononuclear cells bearing $CD_{34, CD45}$ and nestin indicates that regeneration is an ongoing process in these patients. However, this regeneration cannot counterbalance the damage induced by dystrophine mutation

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Acta Myologica • 2014; XXXIII: p. 19-21

CASE REPORT

Limb girdle muscular dystrophy type 2L presenting as necrotizing myopathy

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Recessive mutations in the ANO5 gene, encoding anoctamin 5, cause proximal limb girdle muscular dystrophy (LGMD2L), Miyoshi-type distal myopathy (MM3) and asymptomatic hyperCKemia.

We report a woman with exertion-induced myalgia and weakness in the hip girdle manifesting at the age of 40. Creatine kinase (CK) was increased 20-fold. Histologically the dominating feature was necrotizing myopathy, but long-term immunosuppressive therapy did not change CK level or myopathic symptoms. Molecular genetic investigation led to the finding of the homozygous ANO5 c.191dupA mutation. This is a report of a muscular dystrophy due to ANO5 mutation presenting histologically as necrotizing myopathy. For this reason our finding extends the histological spectrum of myopathies due to ANO5 mutations as well as the possible differential diagnoses for necrotizing myopathy.

Key words: Anoctamin 5, limb girdle muscular dystrophy 2L, necrotizing myopathy

Case report

Recessive mutations in the ANO5 gene (ANO5, MIM 6086629) are associated with limb girdle muscular dystrophy (LGMD) 2L; known to be the third most common LGMD in Northern and Central Europe (1-3) but also with a distal non-dysferlin Miyoshi type dystrophy (MM3) or with asymptomatic hyperCKemia (4, 5). We present here a patient homozygous for the ANO5 mutation c.191dupA with necrotizing myopathy as the dominating histological feature.

A 40-year-old athletic Caucasian woman started to complain about exertion-induced weakness and myalgia, especially in thighs and buttocks. At the time she had been weight training and mountain biking several times a week. Creatine kinase (CK) was 20-fold increased. A muscle biopsy from the gastrocnemius muscle presented as necrotizing myopathy (Fig. 1). Due to MHC upregulation myositis therapy with prednisolone and methotrexate (MTX) was initiated which diminished myalgia but the CK remained constantly raised (10- to 20-fold, maximum 35-fold) over several years. Investigations for myotoxic medication, potential malignancies and antibodies against signal recognition particle were negative. Magnetic resonance imaging (MRI) revealed asymmetric fatty atrophy of both thighs with accentuation of the posterior compartment (Fig. 2). First presentation of the patient in our clinic was five years after disease onset. She then complained of ongoing exercise intolerance with myalgia of the upper legs and hip girdle, difficulties in climbing stairs and rising from sitting or squatting position. Examination revealed mildly asymmetric weakness of the hip flexors and foot plantarflexors with restricted monopedal jumping and tiptoe walking. The CK was elevated 15-fold. Some fibrillations in the gastrocnemius muscle were obvious. The permanent elevation of CK despite immunosuppression, and the clinical presentation with prevalent proximal leg weakness as well as the charcteristic MRI-findings prompted us to search for causative mutations in ANO5. Amplification and sequencing of exon 5 of the ANO5 gene revealed the homozygous mutation c.191dupA (4) a founder mutation frequently identified in northern Europe patients with ANO5 myopathy (2-4, 6, 7). Therefore we diagnosed a necrotizing myopathy characterized by necrotic fibres and the absence of inflammatory and dystrophic signs, accompanied by an otherwise typical clinical presentation of ANO5 myopathy with LGMD phenotype.

Necrotizing myopathy has been associated with a variety of neoplasms and with autoimmune processes, e.g. as in the anti-signal recognition particle (anti-SRP)

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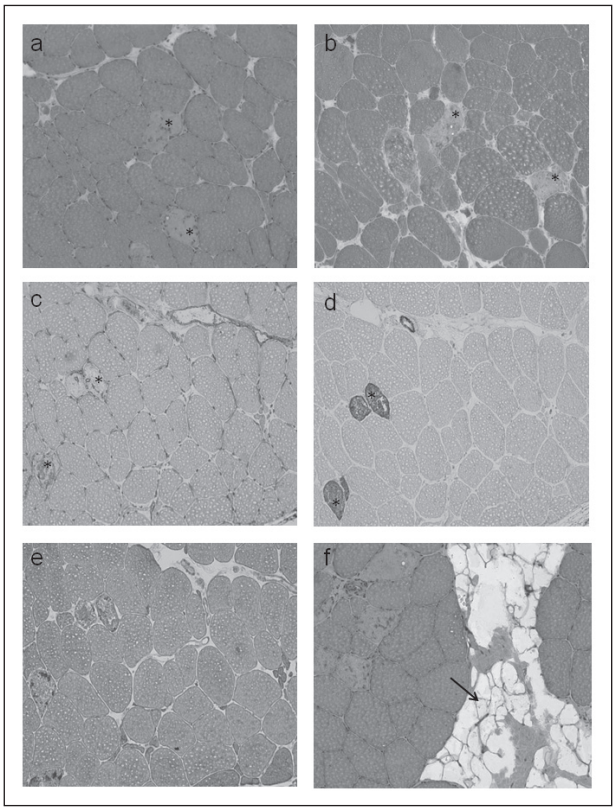

Figure 1. (a-d). Muscle biopsy from the gastrocnemic muscle. (a) H&E, (b) Gomori Trichrome. Occurence of 8% disseminated necrotic muscle fibers *, positive for (c) major histocompatibility complex (MHC I) and (d) complement (C5b9). Some smaller muscle fibres (25 µm) contained internalized caveolin and (e) dysferlin, indicating regenerating fibres. No inflammatory infiltrates, fibrosis, fatty degeneration, or further myopathic changes were detected. (f) H&E. Some lipocytes (arrow) were visible in the perifascicular connective tissue. Magnification x 10.

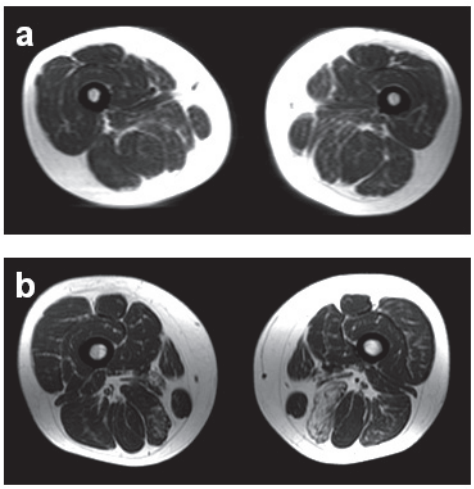

Figure 2. Magnetic resonance imaging (MRI, T1 fat suppressed) of the upper legs of the patient at 42 years old, with very mild asymmetric fatty replacement in the posterior compartment, accentuated in the left semimembranous muscle (a). Further progression at follow-up after one year (b).

syndrome. However, we did not find any evidence of such etiological causes in our patient. Interestingly most recently Claeys at al. presented a patient with immune mediated necrotizing myopathy with antibodies against 3-hydoxy-3-methylglutaryl-coenzyme-A-reductase without previous statin exposure in which 2 pathogenetic mutations of ANO5 (c.191dupA, exon 5; c.1627dupA, exon 15) were identified (8). Since we did not test for other antibodies, we cannot completely rule out the possibility of myositis or immune mediated necrotizing myopathy, although it is very unlikely because immunosuppressive therapy had no beneficial effect in our patient. There was also no hint of other underlying causes for myocyte necrosis; e.g. rhabdomyolysis in metabolic myopathies, necrosis as a sequelae of inflammation, toxin and drug-induced causes.

Few necrotic fibers can also occur as part of the dystrophic pattern in ANO5 myopathy (2, 9, 10), but in contrast to necotizing myopathy they are invariably associated with fatty and fibrotic remodelling.

Deficiency in Anoctamin 5, a putative calcium-activated chloride channel in skeletal muscle, is associated with multifocal loss of the costameres and gaps in the sarcolemmal membrane. Therefore a defective membrane repair might result in a higher vulnerability of muscle fibres, causing ongoing hyperCKemia and necrosis even in early (histological) stages of ANO 5 myopathy.

ANO5 myopathy can present as necrotizing myopathy extending the histological spectrum of myopathies due to ANO5 mutations as well as the possible differential diagnoses for necrotizing myopathy.

Acknowledgments

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A gating model for wildtype and R1448H Nav1.4 channels in paramyotonia

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We studied the consequences of the Nav1.4 mutation R1448H that is situated in the fourth voltage sensor of the channel and causes paramyotonia, a cold-induced myotonia followed by weakness. Previous work showed that the mutation uncouples inactivation from activation. We measured whole-cell Na^+ currents at 10, 15, 20, and 25°C using HEK293 cells stably transfected with wildtype (WT) and R1448H Na^+ channels. A Markov model was developed the parameters of which reproduced the data measured on WT and R1448H channels in the whole voltage and temperature range. It required an additional transient inactivated state and an additional closed-state inactivation transition not previously described. The model was used to predict single-channel properties, free energy barriers and temperature dependence of rates. It allowed us to draw the following conclusions: i) open-state inactivation results from a two-step process; ii) the channel re-openings that cause paramyotonia originate from enhanced deactivation/reactivation and not from destabilized inactivation; iii) the closed-state inactivation of R1448H is strikingly enhanced. We assume that latter explains the episodic weakness following cold-induced myotonia.

Key words: Paramyotonia, markov model, sodium channel, closed-state inactivation, channelopathy, skeletal muscle

Introduction

Paramyotonia congenita (PC) is characterized by muscle stiffness provoked by exposure to cold and particularly by exercise in cold environment (1). During deep cooling the myotonia disappears and gives way to flaccid paralysis which may last several hours. Causative mutations are in the skeletal muscle sodium channel Nav1.4. Investigations of the biophysical alterations in channel gating due to PC mutations has revealed several gating defects consistent with membrane hyperexcitability. Mutant channels inactivate more slowly and with less voltage dependence than WT channels, deactivate more slowly, and exhibit a more rapid rate of recovery from fast inactivation (2). The very frequently occurring R1448H mutation which affects the outermost amino acid of the transmembrane segment S4 of domain DIV has been attributed to an uncoupling of fast inactivation from activation (3).

Voltage-gated Na^+ channels are essential for the generation of action potentials. They consist of four homologous domains (DI to DIV) which each contain six transmembrane segments (S1 to S6). At depolarization, the S4 segments, which contain several positive amino acid residues and therefore function as voltage sensors, can move outwardly and thereby alter channel confirmation and function. Different charge contents of the various S4 segments suggest that the charges have domain-specific functions. While S4 of DI and DII are thought to play a prominent role in Na^+ channel activation, S4 of DIII and DIV regulate fast inactivation (4). Finally, the pore with its selectivity filter is lined by the loops between S5 and S6 and the S5 and S6 segments itself.

Na^+ channel activation is a multi-step process which is usually implemented as a series of closed states leading to one or more open states. Generally, the distributions of single-channel open times follow a single exponential (5). Inactivation is coupled to activation (6). The voltage dependence of inactivation is supported by a significant gating current during fast inactivation (7) and can be used to describe voltage dependence of channel reopening and mean open times (8). The time course of fast inactivation is reported to be single-exponential as well as double-exponential which is either implemented as two open states (9) or by a two-step inactivation process (10). After reaching the fast inactivated state, Na^+ channels do not go immediately back to the closed states, repolarization of the membrane is necessary to initiate recovery.

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To account for recovery from fast inactivation, which is not occurring by re-entering the open state (11), models were expanded with transitions between inactivated and closed states (12). As inactivation occurs from open as well as from closed states, and recovery from fast inactivation develops with a delay (13), multiple inactivation states are assumed.

Since low temperature is the trigger for paramyotonia, temperature effects have been studied and shown to affect both the kinetic and steady-state parameters of Nav1.4 WT and R1448H channels. This is not surprising, given that each of the voltage-dependent gating steps is likely to involve different conformational changes in the channel and so require the breaking and/or forming of chemical bonds with different energies. However, data obtained at room temperature cannot be extrapolated to physiological temperatures using a single temperature scaling factor. Therefore measurements in a wide temperature range and a suitable gating model which is valid in a large potential and temperature range are required to study R1448H. In the present study, we characterized the gating of Nav1.4 WT and R1448H mutant channels with the whole-cell configuration of the patch-clamp technique between 5 and 30 °C. Also, we determined parameters of a Markov model which was able to fit the measurements at all potentials and temperatures. The model was then used to predict gating currents and single-channel properties.

Materials and methods

Na^+ channel expression

WT and mutant (R1448H) α-subunit constructs of human skeletal muscle Na^+ channels were assembled in the mammalian expression vector pRC/C MV and transfected into human embryonic kidney cells (HEK 293) by the calcium phosphate precipitation method. Since transient expression was low (< 10%) stable cell lines were obtained by antibiotic selection as previously described (14).

Recording techniques

Whole-cell currents were recorded using an Axopatch 200A patch-clamp amplifier (Molecular Devices, USA). Signal acquisition and processing was done using the DigiData card (1200) and pCLAMP (V6) software (Molecular Devices, USA). Whole-cell currents were filtered at 10 kHz, and digitized at 10 or 20 μs. Patch pipettes were pulled on a Zeitz Puller (Zeitz Instruments, Martinsried, Germany). Pipette resistance ranged from 0.8 to 1.2 MΩ. The extracellular recording solution was (in mM): 150 NaCl, 2 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES, titrated to pH 7.4 with NaOH. The pipette solution was (in mM): 105 CsF, 35 NaCl, 10 EGTA and 10 HEPES, titrated to pH 7.4 with CsOH. After achieving the whole-cell configuration, cells were held for 10 min at -120 mV to ensure proper diffusion of the pipette solution into the cell and to stabilize of the Na^+ current amplitude. Recording temperature was maintained between 5 and 30°C by a Peltier device and a HC-100A temperature controller (Dagan, USA). To avoid evaporation at high temperatures and dilution by condensation at low temperatures, the bath solution was continuously exchanged by a gravity driven perfusion system.

Electrophysiological protocols and data analysis

Voltage dependence of activation was obtained by a series of 50 ms depolarizing pulses from a holding potential of -140 mV ranging from -85 to 55 mV and steady-state fast inactivation was obtained by 200 ms conditioning pulses from -150 to -45 mV from a holding potential of -140 mV followed by a test pulse to -15 mV. Activation and steady-state fast inactivation curves were fit with standard Boltzmann function as previously described (15). Time constants of fast inactivation were obtained by fitting double-exponential functions to the decaying part of the current traces obtained with the activation protocol. Because the fast component accounted for > 90% of the current amplitude, macroscopic inactivation of the Na^+ current was quantified by the fast component only. Time course of entry into fast inactivation (closed-inactivation) was obtained by a double pulse protocol. From a holding potential of -140 mV a conditioning pulse V_{cond} (-100, -90, -80, -70 mV) for increasing durations (from 0.1 to 300 ms) was applied in order to inactivate Na^+ channels without opening. The conditioning pulse was followed by a test pulse to -15 mV to determine the fraction of non-inactivated channels. Time course of entry into fast inactivation was obtained by fitting a single exponential function to the normalized curve. Recovery from fast inactivation was determined by a double pulse protocol. A 150 ms pulse to -15 mV was used to inactivate all Na^+ channels. A test pulse to -15 mV followed after an increasing interval (from 0.025 to 250 ms) at the recovery potential (-140, -120 and -100 mV). Time course of recovery from fast inactivation was obtained by fitting single/double exponential function to the normalized curve.

Curve fits and data analysis were performed with pCLAMP 8.0 (Molecular Devices), Excel (Microsoft, Inc. Redmond, WA), and Origin (MICROCAL Software, Inc., Northhampton, MA). Differences from WT and mutant were considered as significant at $p < 0.05$ (Student's t-test). Grouped data are presented as mean ± SEM. SEM is represented in graphs as bars when in exceeds the size of the symbol.

Sodium channel gating model

Recordings from activation, steady-state fast inactivation, entry into closed-state inactivation and recovery from fast inactivation were simultaneously fit to a gating model using an advanced version of IonFit software (16). Model parameters were optimized using the least squares method. Ionic currents were simulated by solving master equations of a continuous-time Markov process,

$$\frac{d}{dt}P_i(t) = \sum_j r_{ji}(V)P_j(t) - r_{ij}(V)P_i(t) \quad (1),$$

whereby $P_i(t)$ denotes the population of state i at a given time t, and $r_{ij}(V)$ denotes the rate constant for the transition from state i to j. The voltage dependent forward $r_{ij}(V)$ and backward $r_{ji}(V)$ transition rates between state i and j were assumed to be single-exponential functions of voltage (17),

$$r_{ij}(V) = r'_{ij} \cdot exp\left(\frac{zxr_{ij} \cdot FV}{RT}\right) \quad (2),$$

$$r_{ji}(V) = r'_{ji} \cdot \exp\left(\frac{-zxr_{ji} \cdot FV}{RT}\right) \quad (3)$$

whereby zxr_{ij} and zxr_{ji} represent the effective charge moving from an original state to the barrier peak, as a product of the total charge moved and the fraction of the electric field where the barrier peak was located. r'_{ij} and r'_{ji} represent the rate constants at 0 mV, including enthalpic and entropic factors. F represents the Faraday constant, R the ideal gas constant, V the membrane potential and T the absolute temperature. The initial state populations were determined as a steady-state solution of Eq. 1 at a holding potential V_{hold} with $dP_i(t)/dt=0$. For steady-state fast inactivation curve, recovery from fast inactivation and entry into fast inaction, currents were simulated according to the pulse protocols and the respective current peak amplitudes were determined. Data sets used to determine model parameters consisted of six current traces for test pulses of -40 to 10 mV, the steady state inactivation curve between -160 and -45 mV, time course of entry into fast inactivation at four different prepulse potentials (-100 to -70 mV) and time course of recovery from fast inactivation at three different recovery potentials (-140 to -100 mV).

To describe the energy profile, the rate constants in Eq. 2 and Eq. 3 were written with explicit entropic ΔS and enthalpic ΔH terms. The voltage independent parts are equal to the pre-factors r'_{ij} and r'_{ji},

$$r'_{ij}(T) = \frac{k_B T}{h} \cdot exp\left(\frac{-\Delta H_{r_{ij}} + T\Delta S_{r_{ij}}}{RT}\right) \quad (4)$$

$$r'_{ji}(T) = \frac{k_B T}{h} \cdot exp\left(\frac{-\Delta H_{r_{ji}} + T\Delta S_{r_{ji}}}{RT}\right) \quad (5)$$

and can be used to determine ΔH and ΔS.

Rate constants were used to calculate single channel properties. If a channel opens, the number of openings before inactivation follows a geometric distribution (18), the mean of which may be calculated from the model's rate constants

$$N = \frac{1}{1 - \left(\frac{\alpha_2}{\alpha_3 + \alpha_2 + \beta_1}\right) \cdot \left(\frac{\beta_2}{\alpha_6 + \beta_2}\right)} \quad (6)$$

The mean open time τ_O of single channels of the model was estimated by the reciprocal sum of the rates leaving the open state

$$\tau_O = \frac{1}{\alpha_6 + \beta_2} \quad (7)$$

To test the hypothesis of an increased probability of $O \rightarrow C_4 \rightarrow I_2$ transitions, the steady-state probability was calculated by

$$P(O \rightarrow C_4 \rightarrow I_2) = \frac{\beta_2}{\beta_2 + \alpha_6} \cdot \frac{\alpha_3}{\alpha_3 + \alpha_2 + \beta_1} \quad (8)$$

It is very likely that there are variations in basic properties of channel population from cell to cell, and this variation may mimic the real variation seen in native preparations. For this reason all fits and simulations were done by using data of individual cells and results were pooled afterwards.

Results

Whole-cell currents

At all temperatures activation kinetics and sodium currents decay were slower for R1448H than for WT (Fig. 1A). Cooling from 30°C to 10°C slowed kinetics ~10-fold and reduced peak current amplitudes to a quarter for both WT and mutant channels (Fig. 1B). In contrast, cooling increased the total sodium influx into the cell by different amounts: at 10°C in relation to 30°C, the area under the curve was multiplied by a factor of two for WT and by a factor of four for R1448H (Fig. 1C).

Steady-state activation curves were almost identical for WT and R1448H regardless of temperature (Fig. 2A, Table 1). Cooling decreased activation slope factor from ~-7mV to ~-10mV and potentials at half maximal activation were shifted by ~+8 mV to the right for WT

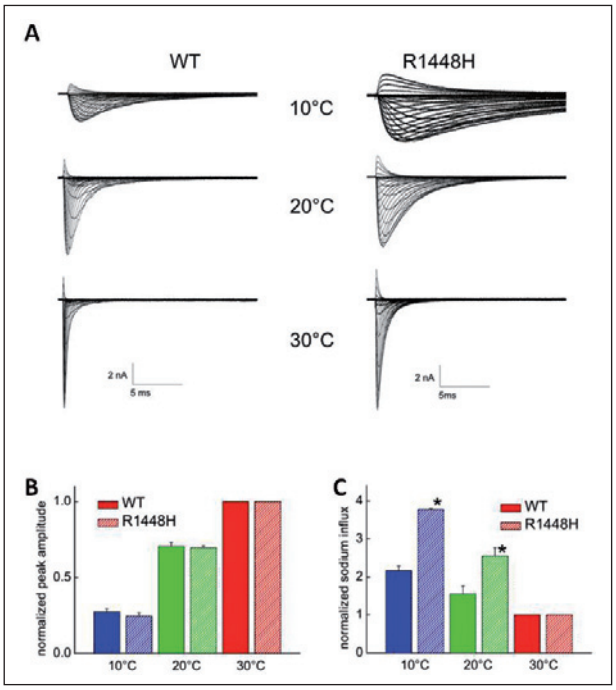

Figure 1. A Raw data. Representative whole-cell current traces recorded at different temperatures from HEK293 cells stably expressing either WT (left) or R1448H (right) mutant channels: 10°C (top), 20°C (middle) and 30°C (bottom). Note the slowed inactivation of the mutant. B Temperature effect on amplitude. Temperature dependency of currents through WT or R1448H Nav1.4 channels normalized to values at 30°C. C Temperature effect on flux. Temperature dependency of Na^+ influx through WT or R1448H Nav1.4 channels. For B and C, values are mean ± SEM (n = 6). SEM is shown as bars and * indicates a significant difference between WT and R1448H ($p < 0:05$). Note that while the amplitude shows similar temperature dependence, the flux of the mutant is increased due to the slowed inactivation.

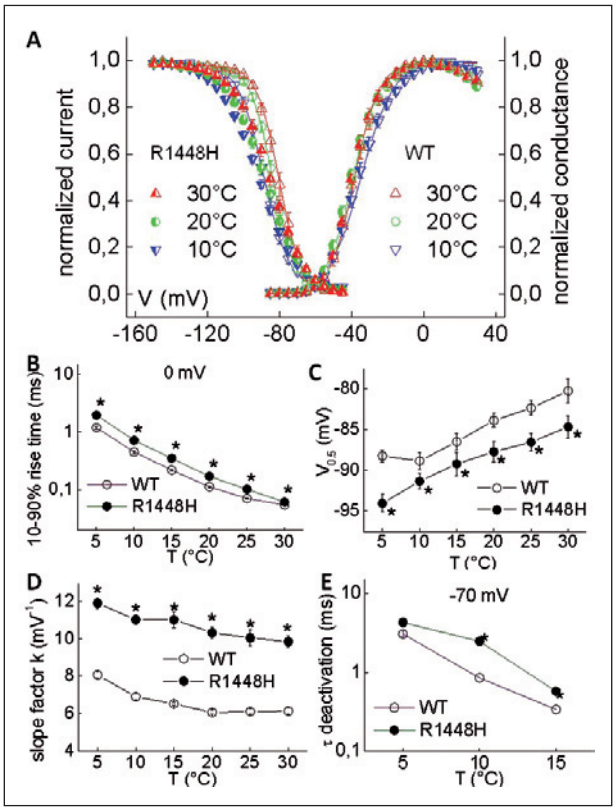

Figure 2. A Activation and steady-state fast inactivation. Activation and steady-state fast inactivation curves for WT and R1448H. Voltage dependence of activation was determined by 50 ms depolarizing pulses to the indicated potentials from a holding potential of -140 mV. Steady-state inactivation curve was determined with a 200 ms prepulse to the indicated potentials prior to a 10 ms test pulse to -15 mV (-140 mV holding potential). Solid lines represent fits to standard Boltzmann functions. Note that activation is similar but inactivation is changed for the mutant. B Rise time. 10-90% rise time as a measure for activation WT and R1448H. Note the slower kinetics of the mutant. C and D parameters of inactivation. Temperature dependence of Boltzmann parameters of WT and R1448H for steady-state fast inactivation. Note the left-shift (more negative $V_{0.5}$) and decreased steepness (larger k value) of the mutant. E Deactivation time constant. Comparison of deactivation time constants at -70 mV, the only potential with a significant difference. Note the accelerated kinetics of the mutant. For A to E, all values are mean ± SEM (n = 8-30). SEM is shown as bars when it exceeds the size of the symbol and * indicates a significant difference between WT and R1448H ($p < 0:05$).

and R1448H alike. Rise time of activation at 0 mV and higher was significantly increased in R1448H compared to WT ($p \leq 0.05$, Fig. 2B). Steady-state inactivation differed significantly ($p = 0.05$) for the mutant as well: R1448H curves were significantly shifted to the left by ~6 mV and revealed an increase of slope factor by ~4 mV (Fig. 2A, C, D, Table 1). Since deactivation cannot be measured at room temperature, we cooled to 15°C, 10°C and 5°C to resolve sufficient data points for a fit. Deactivation time course was almost indistinguishable for mutant and WT except for the near-threshold voltage of -70 mV (Fig. 2E).

For threshold-near potentials, the time constants of fast inactivation T_h form the open state were smaller for R1448H than WT while at more depolarized potentials, they were larger than for WT (Fig. 3: OSI). The difference in time constants was especially prominent in the voltage range of -60 to -30 mV and markedly increased with cooling. Cooling slowed fast inactivation of WT and R1448H at all voltages tested and shifted the point of intersection of WT and R1448H curves to more negative potentials.

Table 1. Boltzmann parameters of G(V) and SSFI curves.

°C		5	10	15	20	25	30
WT *G*(V)	$V_{0.5}$(mV) k(mV) n	-31.7 ± 1.6 -9.7 ± 0.2 8	-35.7 ± 1.4† -8.3 ± 0.2† 13	-36.8 ± 1.0 -7.8 ± 0.1 20	-38.8 ± 0.7 -7.2 ± 0.1 30	-40.4 ± 1.0 -6.8 ± 0.2 19	-37.6 ± 1.2 -6.6 ± 0.3† 14
R1448H *G*(V)	$V_{0.5}$(mV) k(mV) n	-31.3 ± 1.3 -9.6 ± 0.2 13	-37.0 ± 1.0† -8.3 ± 0.2† 18	-38.3 ± 0.7 -8.0 ± 0.1 12	-40.3 ± 0.7 -7.3 ± 0.1 19	-41.5 ± 1.1 -6.9 ± 0.3 10	-39.4 ± 0.5 -7.0 ± 0.1 14
WT SSFI	$V_{0.5}$(mV) k(mV) n	-88.2 ± 0.7 8.06 ± 0.2 8	-87.3 ± 0.7† 6.8 ± 0.2† 13	-84.8 ± 0.4 6.4 ± 0.1 19	-83.0 ± 0.5 6.1 ± 0.1 30	-82.4 ± 0.9 6.1 ± 0.1 19	-79.9 ± 1.0† 6.2 ± 0.1 14
R1448H SSFI	$V_{0.5}$(mV) k(mV) n	-93.7 ± 1.1* 12.0 ± 0.3* 13	-91.0 ± 0.8*† 10.7 ± 0.2*† 18	-88.7 ± 1.2* 10.8 ± 0.3* 12	-87.0 ± 1.1* 10.1 ± 0.2* 18	-86.7 ± 1.1* 9.9 ± 0.4* 10	-85.0 ± 1.3* 9.5 ± 0.3* 14

* indicates a significant difference between WT and R1448H at the same temperature, † indicates a significant difference between 10, 20 and 30°C, (p < 0:05).

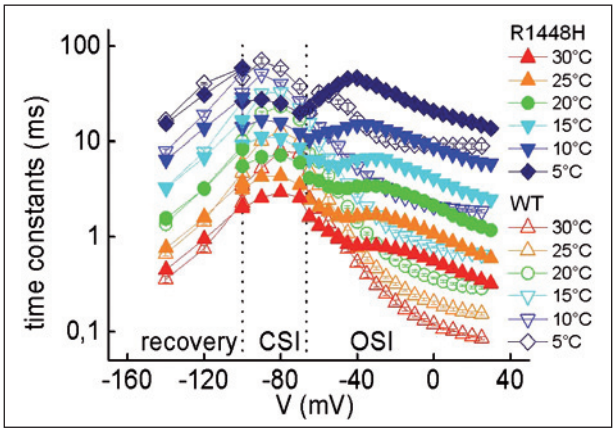

Figure 3. Time constants. Time constants from and into the fast inactivated-state were plotted against the corresponding membrane potentials. Recovery, entry (Closed-state inactivation, CSI) and inactivation from the open-state (OSI) were determined for WT and R1448H between 5 and 30°C. Values are mean ± SEM (n = 5-30). SEM is shown as bars when it exceeds the size of the symbol and * indicates a significant difference between WT and R1448H (p < 0:05). Note the accelerated CSI and slowed OSI of the mutant at all temperatures, while the recovery is indistinguishable.

Additionally, R1448H reduced voltage dependence of T_h for all temperatures tested.

R1448H accelerated entry into closed-state inactivation (CSI) by about two-fold on average (Fig. 3: CSI, Table 2). The left-shift of the steady-state inactivation curve may explain this enhanced closed-state inactivation. The mutation reduced its voltage dependence, possibly by the removed S4 charge, and slowed the open-state inactivation. Finally, R1448H showed a tendency to recover more rapidly without reaching significant levels (Fig. 3: recovery, Table 3). However, neither the delay in onset to recovery nor its voltage and temperature dependence were altered by R1448H.

Gating model

The gating model used in the present study consisted of a series of four closed states C_1-C_4, one open state O and four inactivated states I_1-I_3 and I_T (Fig. 4). By convention, all transitions towards O have positive valences because they are favored by depolarization, while those away from O have negative valences because they are favored by repolarization. Rate constants between the closed states and rate constants between C_3-C_4-O and I_1-I_2-I_3 were assumed to be equal to reduce the number of free parameters. The model is based on previous models of Vandenberg and Bezanilla (19). However, to properly describe gating of WT and R1448H and their temperature dependence, two modifications were made. First, we introduced a transient inactivated state I_T as previously suggested by Kahlig et al. (10) to account for the biphasic inactivation especially at low temperatures. Second, we introduced a transition between C_4 and I_2. This transition was essential to reproduce inactivation from closed states especially in the voltage range around threshold of Na^+ channels.

The model was able to reproduce all measurements including the strong voltage dependence of channel activation, open and closed state inactivation, recovery and temperature dependence (Fig. 5). The model resulted in rate constants of WT and R1448H which were similar for most transitions (Tables 4 and 5). However the rate constants alpha3, beta3 and alpha6 markedly differed

Table 2. Time constants of entry into fast inactivation.

°C		5	10	15	20	25	30
WT	$\tau_{entry-100}$(ms)	47.0 ± 7.4	32.5 ± 3.8†	16.1 ± 1.6	10.1 ± 1.3	5.9 ± 0.8	3.4 ± 0.7†
	n	8	15	18	22	15	8
	$\tau_{entry-90}$(ms)	70.8 ± 4.8	51.3 ± 2.7†	31.9 ± 1.9	19.7 ± 0.9	10.3 ± 0.5	5.2 ± 0.7†
	n	7	15	20	24	15	9
	$\tau_{entry-80}$(ms)	58.7 ± 4.0	40.4 ± 2.4†	32.9 ± 2.1	23.5 ± 0.9	12.9 ± 0.7	8.0 ± 0.5†
	n	7	15	20	24	15	9
	$\tau_{entry-70}$(ms)	38.1 ± 2.6	26.2 ± 1.8†	22.1 ± 1.4	17.1 ± 1.0	10.0 ± 0.8	7.6 ± 0.8†
	n	7	13	20	22	14	9
R1448H	$\tau_{entry-100}$(ms)	26.4 ± 1.2*	14.1 ± 0.7*†	9.2 ± 0.5*	5.5 ± 0.3*	3.2 ± 0.2*	2.0 ± 0.2†
	n	10	18	12	17	10	9
	$\tau_{entry-90}$(ms)	27.8 ± 0.9*	17.0 ± 0.7*†	11.2 ± 0.5*	6.9 ± 0.3*	4.3 ± 0.2*	2.6 ± 0.1*†
	n	10	18	12	16	9	9
	$\tau_{entry-80}$(ms)	25.3 ± 0.7*	15.8 ± 0.6*†	10.9 ± 0.5*	7.2 ± 0.2*	4.4 ± 0.1*	2.9 ± 0.2*†
	n	10	18	12	17	9	9
	$\tau_{entry-70}$(ms)	20.1 ± 0.7*	12.3 ± 0.5*†	8.7 ± 0.4*	6.0 ± 0.2*	3.5 ± 0.1*	2.6 ± 0.2*†
	n	10	18	12	17	8	9

* indicates a significant difference between WT and R1448H at the same temperature, † indicates a significant difference between 10, 20 and 30°C, ($p < 0:05$).

Table 3. Time constants of recovery from fast inactivation.

°C		5	10	15	20	25	30
WT(-140 mV)	τ_{rec_1}(ms)	16.61 ± 0.49	7.94 ± 0.42†	3.28 ± 0.11	1.37 ± 0.04	0.66 ± 0.03	0.35 ± 0.03†
	τ_{rec_2}(ms)	-	-	34.6 ± 3.8	24.5 ± 2.2	19.1 ± 1.4	16.6 ± 0.8
	delay (ms)	1.54 ± 0.03	0.81 ± 0.04†	0.57 ± 0.01	0.31 ± 0.01	0.18 ± 0.01	0.1 ± 0.003†
	n	6	14	17	19	10	9
R1448H(-140 mV)	τ_{rec_1}(ms)	15.50 ± 1.79	6.45 ± 0.28†	3.23 ± 0.14	1.55 ± 0.08	0.76 ± 0.04	0.45 ± 0.04†
	τ_{rec_2}(ms)	-	-	39.3 ± 4.1	32.8 ± 3.6	21.1 ± 1.3	21.5 ± 1.9
	delay (ms)	1.79 ± 0.25	0.84 ± 0.03†	0.58 ± 0.02	0.3 ± 0.01	0.19 ± 0.02	0.099 ± 0.003†
	n	9	17	12	18	7	7
WT(-120 mV)	τ_{rec_1}(ms)	40.92 ± 1.09	19.14 ± 1.16†	7.81 ± 0.36	3.21 ± 0.11	1.45 ± 0.09	0.75 ± 0.06†
	τ_{rec_2}(ms)	-	-	63.1 ± 6.7	50.2 ± 5.6	38.2 ± 3.2	36.0 ± 3.0
	delay (ms)	3.26 ± 0.07	1.51 ± 0.10†	1.03 ± 0.02	0.55 ± 0.02	0.29 ± 0.01	0.15 ± 0.01†
	n	6	14	18	19	10	9
R1448H(-120 mV)	τ_{rec_1}(ms)	31.44 ± 3.26*	13.86 ± 0.66*†	6.88 ± 0.28	3.18 ± 0.18	1.60 ± 0.16	0.95 ± 0.07*†
	τ_{rec_2}(ms)	-	-	79.5 ± 15.9	51.8 ± 6.8	39.4 ± 3.4	47.8 ± 3.5
	delay (ms)	3.34 ± 0.48	1.45 ± 0.05†	0.97 ± 0.04	0.57 ± 0.02	0.30 ± 0.01	0.14 ± 0.01†
	n	9	17	10	18	6	7
WT(-100 mV)	$\tau_{rec\_}$ (ms)	59.97 ± 2.89	52.82 ± 2.90†	26.21 ± 1.40	9.08 ± 0.35	4.72 ± 0.34	2.27 ± 0.24†
	τ_{rec_2}(ms)	-	-	-	58.2 ± 5.8	98.6 ± 26.2	96.0 ± 18.5
	delay (ms)	4.20 ± 0.15	3.31 ± 0.23†	1.93 ± 0.07	1.10 ± 0.04	0.56 ± 0.03	0.33 ± 0.04†
	n	5	13	19	16	8	10
R1448H(-100 mV)	τ_{rec_1}(ms)	59.30 ± 3.32	29.17 ± 1.43*†	16.85 ± 0.86*	8.36 ± 0.43	3.75 ± 0.30	2.10 ± 0.11†
	τ_{rec_2}(ms)	-	-	-	-	53.2 ± 4.4	110.8 ± 19.5
	delay (ms)	6.01 ± 0.78	3.14 ± 0.19†	1.79 ± 0.14	0.96 ± 0.06	0.58 ± 0.05	0.27 ± 0.03†
	n	8	17	12	17	6	8

* indicates a significant difference between WT and R1448H at the same temperature, † indicates a significant difference between 10, 20 and 30°C, ($p < 0:05$).

between WT and R1448H. The smaller alpha6 explains the impaired fast inactivation of R1448H. The most striking difference between WT and mutant related to alpha3 and beta3, the transition between C4 and I2. In contrast to the WT for which the calculated rate constants suggest that this transition does not occur, the mu-

tant performed this closed-state inactivation transition with a high likelihood.

Effective charge movement increased with temperature for all transitions in both WT and R1448H by about 30%. For example the equivalent gating charge for opening the channels from rest was 5.31 to 6.78 e0 for WT and 4.39 to 5.56 e0 for mutant, obtained by summing the charges for each of the four transitions from C1 to O (values dependent on voltage, Table S6). Most of the charge movement in the activation pathway was concentrated in the last transition (C4-O) 2.58 ± 0.06 to 3.06 ± 0.04 e0 for WT and 2.53 ± 0.05 to 2.98 ± 0.09 e0 for mutant. We interpret this finding so that this transition may represent several steps in one the final of which may really be voltage-independent. In general, effective charge movement for transitions from inactivated to closed states during recovery were notably larger compared to their respective forward rates during closed-state inactivation accounting for the strong voltage dependence of recovery from inactivation. For the mutant the equivalent gating charge movement during recovery was smaller than for WT leading to reduced voltage dependence. About 50% of total gating charge of WT and 40% of charge for the mutant was immobilized by fast inactivation.

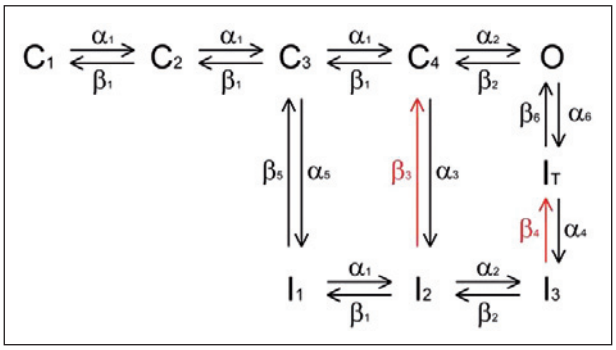

Figure 4. Gating model. Gating model used for kinetic simulations, consists of 4 closed-states C1–C4, one open- state O, four inactivated-states I1–I3 and IT. Arrows between states indicate possible transitions and _i and _i (i=1..6) represent the voltage-dependent rate constants. Rate constants _3 and _4 were calculated based on microscopic reversibility. Note the additional transient inactivated state IT and the transition C4-I2 both of which have not been previously used.

Free energy barriers

The energy changes involved in the transitions between the closed-states (C1–C2–C3–C4) and the paral-

Table 4. Model parameters for WT.

Transition	Parameter	10°C	15°C	20°C	25°C
C – C and I_1 – I_2	zxα1	0.1957 ± 0.0020	0.1882 ± 0.0132	0.1716 ± 0.0153	0.1678 ± 0.0125
	α1	7.75e03 ± 3.10e02	1.25e04 ± 1.96e02	2.27e04 ± 3.30e02	3.36e04 ± 1.18e03
	zxβ1	-0.7134 ± 0.0144	-0.8312 ± 0.0205	-0.9831 ± 0.0166	-1.0712 ± 0.0167
	β	1.61e03 ± 6.90e01	1.88e03 ± 1.09e02	1.87e03 ± 1.09e02	2.19e03 ± 6.99e01
C_4 – O and I_2 – I_3	zx α2	1.9079 ± 0.0422	1.9649 ± 0.0323	1.9775 ± 0.0164	1.8978 ± 0.0341
	α2	2.80e04 ± 1.46e03	6.37e04 ± 3.65e03	1.43e05 ± 8.41e03	3.50e05 ± 1.30e04
	zxβ2	-0.6711 ± 0.0374	-0.8309 ± 0.0596	-0.9167 ± 0.0307	-1.1666 ± 0.0289
	β2	5.93e01 ± 5.17e00	1.88e02 ± 4.02e01	5.70e02 ± 2.44e01	2.03e03 ± 3.29e01
C_4 – I_2	zxα3	0.0001 ± 0.0001	0.0153 ± 0.0122	0.0552 ± 0.0475	0.0310 ± 0.0201
	α3	5.40e-07 ± 1.61e-07	5.56e-06 ± 1.53e-06	3.14e-05 ± 1.57e-05	5.14e-04 ± 1.39e-04
	zxβ3	-1.0982 ± 0.0034	-1.2190 ± 0.0254	-1.3437 ± 0.0406	-1.5462 ± 0.0251
	β3	3.47e-10 ± 1.09e-10	3.81e-09 ± 1.16e-09	2.22e-08 ± 1.35e-08	2.74e-07 ± 6.99e-08
I_T – I_3	zxα4	0.0004 ± 0.0002	0.0140 ± 0.0123	0.0207 ± 0.0160	0.0004 ± 0.0002
	α4	5.36e01 ± 5.63e00	1.19e02 ± 8.12e00	2.48e02 ± 1.06e01	5.46e02 ± 1.71e01
	zxβ4	-0.8464 ± 0.0113	-0.8826 ± 0.0349	-0.9534 ± 0.0291	-1.0731 ± 0.0304
	β4	8.56e-01 ± 1.76e-01	3.30e00 ± 4.11e-01	1.45e01 ± 2.02e00	4.79e01 ± 4.51e00
C_3 – I_1	zxα5	0.0016 ± 0.0006	0.0040 ± 0.0026	0.0113 ± 0.0076	0.0123 ± 0.0107
	α5	3.78e02 ± 1.76e01	4.74e02 ± 2.30e01	4.92e02 ± 2.26e01	6.89e02 ± 2.06e01
	zxβ5	-1.0967 ± 0.0032	-1.2302 ± 0.0258	-1.3877 ± 0.0310	-1.5650 ± 0.0088
	β5	2.40e-01 ± 1.70e-02	3.06e-01 ± 3.96e-02	3.19e-01 ± 4.81e-02	3.73e-01 ± 1.60e-02
O – I_T	zxα6	0.2514 ± 0.0098	0.3349 ± 0.0148	0.4063 ± 0.0258	0.4692 ± 0.0131
	α6	5.29e02 ± 2.24e01	1.42e03 ± 5.73e01	3.08e03 ± 1.06e02	6.05e03 ± 3.12e01
	zxβ6	-0.0002 ± 0.0001	-0.0027 ± 0.0016	-0.0185 ± 0.0164	-0.0345 ± 0.0164
	β6	2.40e01 ± 2.24e00	3.27e01 ± 1.47e00	3.37e01 ± 1.40e00	3.90e01 ± 2.65e00
	n	7	8	6	9

Model parameters αi and β i (i = 1..6) are the corresponding forward and backward transition rate constants at 0 mV. Parameters zxαi and zxβi are the valences of the corresponding transitions and reflect the voltage dependence of transition rates.* indicates a significant difference between WT and mutant (p < 0:05).

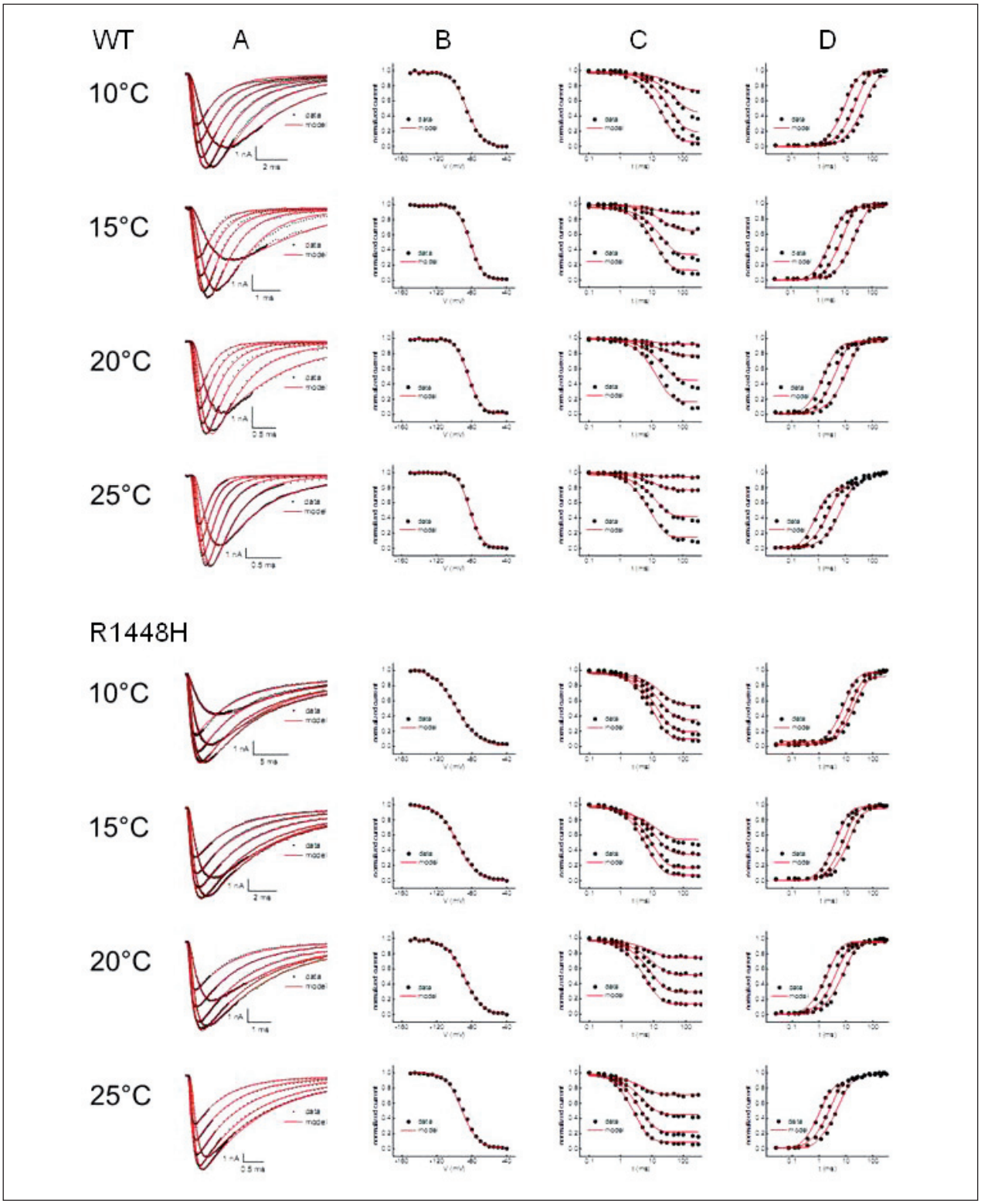

Figure 5. Representative current traces and fits. Original data at 10 to 25°C (black dots) are superimposed with fits of the model (red lines) obtained by simultaneous fitting of A) a series of 6 current traces elicited by pulses from -40 to 10 mV, (B) the steady-state fast inactivation curve determined in the range between -150 and -40 mV, (C) the time course of entry into fast inactivation at prepulse potentials of -100, -90, -80 -70 mV and (D) the time course of recovery from fast inactivation at recovery potentials of -140, -120, -100 mV for WT (top) and R1448H (bottom). Note the quality of the fits.

Table 5. Model parameters for R1448H.

Transition	Parameter	10°C	15°C	20°C	25°C
C – C and I_1 – I_2	zxα1	0.1339 ± 0.0145*	0.1122 ± 0.0083*	0.1264 ± 0.0129*	0.1166 ± 0.0165*
	α1	7.18e03 ± 1.61e02	1.27e04 ± 4.12e02	2.26e04 ± 7.90e02	3.62e04 ± 1.60e03
	zxβ1	-0.4894 ± 0.0168*	-0.6171 ± 0.0192*	-0.6763 ± 0.0177*	-0.7396 ± 0.0245*
	β	3.06e03 ± 1.14e02*	4.06e03 ± 1.77e02*	4.97e03 ± 9.29e01*	6.09e03 ± 5.45e02*
C_4 – O and I_2 – I_3	zx α2	1.8584 ± 0.0441	1.8970 ± 0.0513	1.9001 ± 0.0469	1.8041 ± 0.0828
	α2	2.93e04 ± 1.60e03	7.08e04 ± 6.47e03	1.52e05 ± 7.35e03	4.27e05 ± 3.24e04*
	zxβ2	-0.6685 ± 0.0310	-0.7235 ± 0.0472	-0.8106 ± 0.0200*	-1.1766 ± 0.0449
	β2	2.46e01 ± 2.65e00*	1.09e02 ± 5.04e00*	3.73e02 ± 2.25e01*	1.06e03 ± 1.70e02*
C_4 – I_2	zxα3	0.0027 ± 0.0010	0.0291 ± 0.0278	0.0009 ± 0.0003	0.0036 ± 0.0020
	α3	7.50e02 ± 3.55e01*	8.37e02 ± 5.29e01*	8.07e02 ± 4.37e01*	1.07e03 ± 4.22e01*
	zxβ3	-0.9188 ± 0.0187*	-0.9477 ± 0.0324*	-1.0421 ± 0.0270*	-1.2099 ± 0.0254*
	β3	1.19e00 ± 1.17e-01*	1.28e00 ± 1.65e-01*	1.39e00 ± 1.69e-01*	1.92e00 ± 2.54e-01*
I_T – I_3	zxα4	0.0051 ± 0.0024	0.0022 ± 0.0012	0.0135 ± 0.0124	0.0009 ± 0.0003
	α4	3.53e01 ± 3.88e00*	5.53e01 ± 2.73e00*	1.39e02 ± 1.91e01*	1.87e02 ± 8.12e00*
	zxβ4	-0.6289 ± 0.0176*	-0.6326 ± 0.0283*	-0.6043 ± 0.0322*	-0.7623 ± 0.0335*
	β4	4.28e-01 ± 5.99e-02*	1.12e00 ± 1.35e-01*	5.91e00 ± 1.21e00*	1.38e01 ± 2.93e00*
C_3 – I_1	zxα5	0.0036 ± 0.0024	0.0020 ± 0.0011	0.0001 ± 0.0001	0.0013 ± 0.0008
	α5	5.34e02 ± 7.97e01	7.25e02 ± 6.43e01*	8.75e02 ± 3.03e01*	1.08e03 ± 5.57e01*
	zxβ5	-0.9179 ± 0.0183*	-0.9749 ± 0.0147*	-1.0429 ± 0.0270*	-1.2121 ± 0.0247*
	β5	8.03e-01 ± 8.72e-02*	1.06e00 ± 8.29e-02*	1.52e00 ± 1.94e-01*	1.89e00 ± 1.89e-01*
O – I_T	zxα6	0.2861 ± 0.0071*	0.3328 ± 0.0086	0.4250 ± 0.0110	0.4500 ± 0.0129
	α6	1.16e02 ± 7.78e00*	2.61e02 ± 1.04e01*	5.56e02 ± 1.47e01*	1.03e03 ± 3.54e01*
	zxβ6	-0.0015 ± 0.0012	0.0093 ± 0.0092	-0.0002 ± 0.0001	-0.0001 ± 0.0001
	β6	1.61e01 ± 2.11e00*	2.01e01 ± 1.54e00*	2.39e01 ± 2.96e00*	2.75e01 ± 3.06e00*
	n	8	8	8	6

Model parameters αi and β i (i = 1..6) are the corresponding forward and backward transition rate constants at 0 mV. Parameters zxαi and zxβi are the valences of the corresponding transitions and reflect the voltage dependence of transition rates.* indicates a significant difference between WT and mutant (p < 0:05).

Table 6. Equivalent gating charges.

WT	Transitions	No	10 °C	15 °C	20 °C	25°C
	C – C and I_1 – I_2	1	0.91 ± 0.01	1.02 ± 0.02	1.15 ± 0.02	1.24 ± 0.02
	C_4 – O and I_2 – I_3	2	2.58 ± 0.06	2.80 ± 0.07	2.89 ± 0.03	3.06 ± 0.04
	C_4 – I_2	3	1.10 ± 0.01	1.23 ± 0.03	1.40 ± 0.06	1.58 ± 0.03
	I_T – I_3	4	0.85 ± 0.01	0.90 ± 0.04	0.97 ± 0.03	1.07 ± 0.03
	C_3 – I_1	5	1.10 ± 0.01	1.23 ± 0.03	1.40 ± 0.03	1.58 ± 0.01
	O – I_T	6	0.25 ± 0.01	0.34 ± 0.01	0.42 ± 0.03	0.50 ± 0.02
R1448H	Transitions	No	10 °C	15 °C	20 °C	25°C
	C – C and I_1 – I_2	1	0.62 ± 0.02	0.73 ± 0.02	0.80 ± 0.02	0.86 ± 0.03
	C_4 – O and I_2 – I_3	2	2.53 ± 0.05	2.62 ± 0.07	2.71 ± 0.05	2.98 ± 0.09
	C_4 – I_2	3	0.92 ± 0.02	0.98 ± 0.04	1.04 ± 0.03	1.21 ± 0.03
	I_T – I_3	4	0.63 ± 0.02	0.63 ± 0.03	0.62 ± 0.03	0.76 ± 0.03
	C_3 – I_1	5	0.92 ± 0.02	0.98 ± 0.01	1.04 ± 0.03	1.21 ± 0.02
	O – I_T	6	0.29 ± 0.01	0.34 ± 0.01	0.43 ± 0.01	0.45 ± 0.01

Equivalent gating charges calculated for model C14. Values are mean ± SEM (n = 6-9).

lel inactivated-states (I1–I2) consist of both entropic and enthalpic changes, suggesting that chemical bonds are reforming and conformational changes of the channel are taking place. For the C4–O transition there is a net decrease in enthalpy along with a net decrease in entropy when the channel goes from the last closed state C4 to the open state O (Table 7). This result suggests that the opening step corresponds to a reorganization of the channel with a decrease in the degrees of freedom of the molecule giving a more ordered system in the open state. While the energy barrier for O–I_T was increased by 5% in the mutant (Fig. 6, left), the one for C4–I2 was reduced down

Table 7. Parameters of the energy barriers.

		ΔH	ΔS	TΔS$_{20}$	ΔG10	ΔG25
C – C/I$_1$ – I$_2$	WT	56 ± 6	211 ± 18	62	-4 ± 8	-7 ± 8
	R1448H	43 ± 3	160 ± 11	47	-2 ± 4	-4 ± 5
C$_4$ – O/I$_2$ – I$_3$	WT	-52 ± 10	-131 ± 27	-38	-15 ± 13	-13 ± 13
	R1448H	-34 ± 15	-66 ± 39	-19	-16 ± 19	-15 ± 19
C$_3$ – I$_1$	WT	8 ± 7	87 ± 23	26	-17 ± 10	-19 ± 10
	R1448H	-8 ± 4	27 ± 12	8	-15 ± 5	-16 ± 5
C$_4$ – I$_2$	WT	42 ± 75	203 ± 131	60	-16 ± 84	-19 ± 85
	R1448H	-6 ± 10	31 ± 31	9	-15 ± 13	-16 ± 13
O – I$_T$	WT	88 ± 8	336 ± 25	99	-7 ± 11	-13 ± 11
	R1448H	74 ± 5	277 ± 9	81	-5 ± 6	-9 ± 6
I$_T$ – I$_3$	WT	-76 ± 9	-235 ± 24	-69	-10 ± 12	-6 ± 12
	R1448H	-81 ± 29	-251 ± 81	-73	-10 ± 37	-7 ± 38

ΔH (kJ /mol) and ΔS (J /Kmol) values were obtained by fitting Eq. 4 and Eq. 5 to α'i and β'i (i=1..6) values. TΔS20 (kJ /mol) was calculated for 20°C. ΔG10 and ΔG25 (kJ /mol) were calculated using ΔG = ΔH – TΔS for 10°C and 25°C respectively. Values are fit values ± SEM.

to 50%, 50 vs. 95 kJ/mol, confirming the facilitated transitions between C4 and I2 due to strikingly increased alpha3, i.e. meaning enhanced closed-state inactivation for R1448H (Fig. 6, right).

Single-channel behavior

Our finding that entry into rapid inactivation of R1448H was faster than for WT at threshold-near potentials (Fig. 3) was interpreted as tendency of R1448H channels to deactivate and inactivate through closed states. To further prove this hypothesis, the probability of transitions from O to I2 was modeled and it is obvious that this transition occurs in R1448H and not in WT (Fig. 7). Cooling shows a clear increase in the probability for this transition as expected from the whole-cell current data at lower temperatures.

The model's rate constants were used to calculate single-channel properties to determine whether the slowing of the current decay observed for R1448H can arise from longer open times or an increased number of openings. The estimated mean open times were up to 4-fold longer for R1448H than for WT. Cooling increased the mean open time of both R1448H and WT channels (Fig. 8 top). The bell-shaped curves showed open-time maxima between -50 and 0 mV. To the left of the maximum, the mean open time was dominated by the rate beta2 and to the right of the maximum by alpha6. This means that Na^+ channels open and close several times before they finally enter the inactivated state. Importantly the calculated number of openings was ~20% greater for R1448H than for WT (Fig. 8 bottom). Cooling reduced the number of re-openings for both WT and R1448H. In summary the slowed decay of whole-cell currents (Fig. 1) is due to an increase in open times which are further increased by cooling. The rate constants and the transition probabilities showed that the increased num-

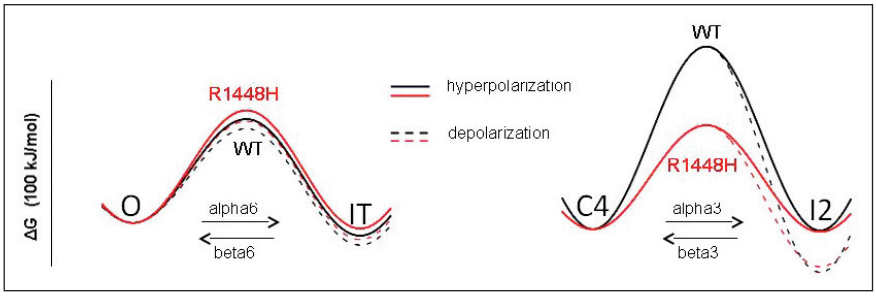

Figure 6. Free energy barriers between states. Total free energy barriers between states were calculated for -160 mV (solid line) and + 50 mV (scattered line) for WT (black) and R1448H (red). The value to the left of the energy barrier was set to 0 to allow direct comparison between curves. Note the very low barrier for the C4-I2 closed-sate inactivation transition (right) for the mutant. In contrast the O-IT open-state inactivation transition barrier is only very mildly elevated for the mutant (left).

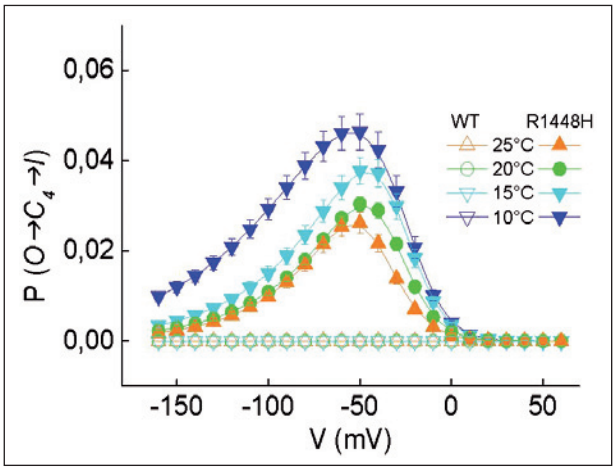

Figure 7. Voltage dependence of closed-sate inactivation probability. The probability for a transition from O → C4 → I2 was calculated according Eq. 8 for WT (open symbols) and R1448H (filled symbols) for 10°C-25°C. Values are mean ± SEM (n = 6-9). SEM is shown as bars when it exceeds the size of the symbol. Note that this transition practically does not occur in WT (P~0), but only in the mutant.

ber of R1448H openings is due to re-openings from the closed state C_4 and not from the inactivated states. As the mutant channel shows the minimum of the energy landscape for I3, the channels reach this state by the C4–I2 pathway instead of by IT. Mutant channels go along the O→C4→I2→I3 pathway.

Discussion

Our whole-cell data confirms previous studies in so far as R1448H slows open-state inactivation and shifts steady-state rapid inactivation to more negative potentials (3, 20-22) and that the seemingly temperature sensitivity in paramyotonia is a result of channel kinetics which are already slowed in the warmth and undergo a normal slowing with cooling (23, 24). Therefore, we assume that the required changes made to our model to best fit the data are not the result of our specific measurement or our set-up but rather reveal generally valid states and transitions.

The required introduction of the transient inactivated state IT into our model suggests that open-state inactivation may result from a two-step process. The two inactivation phases become more obvious at low temperatures whereas they cannot be temporally resolved at higher temperatures. A biphasic inactivation process is actually in agreement with the classical HH model and with previous single channel measurements (3). We interpret the two phases to be linked to deactivation and inactivation.

The required enabling of the C4 - I2 transition (which really occurs in the mutant only and not in WT) is mainly responsible for the channel re-openings of R1448H which are known to cause repetitive action potentials and paramyotonia. The transition rates and energy barriers of our model suggest that the re-openings originate from C4–O transitions and not from O–I transitions since the inactivation on-rate is reduced. This view is further evidenced by the enhanced inactivation from closed states, a slightly accelerated recovery from rapid inactivation, and the absence of a persistent current due to the limited number of re-openings by the increased rate of C_4-I_2 transi-

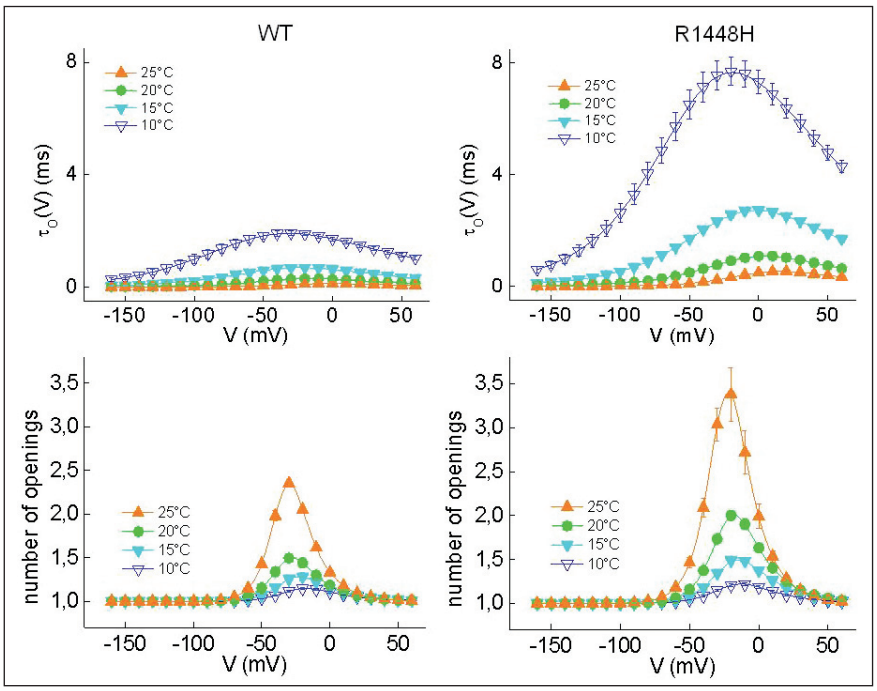

Figure 8. Temperature and voltage dependence of open times and number of openings. Temperature and voltage dependence of the mean open time (top) and the number of openings before inactivation (bottom) was calculated for indicated voltages for WT (left) and R1448H (right) between 10 and 25°C. Values are mean ± SEM (n = 6-9). SEM is shown as bars when it exceeds the size of the symbol. Note the elongated open time and the elevated number of openings in the mutant compared to WT.

tions. The enhanced deactivation has been previously also deduced (3).

As found previously for R1448H but not R1448C (3), closed-state inactivation (CSI) is strikingly enhanced for the R1448H mutation. We assume this is due to a more outward positioned resting-state S4 because of the eliminated positive charge at residue 1448 similar to calcium channel mutations (16). The enhanced CSI can explain the transition from myotonia to flaccid muscle weakness. Since R1448H impairs the movement of the voltage sensor, the receptor for the inactivation gate is more readily available for voltages around the activation threshold and less available for further depolarized voltages. The slowing of the rapid inactivation prolongs the duration of muscle action potentials as measured in vivo (25), whereby the combination of repetitive activity and prolonged duration of each action potential leads to a cold-induced depolarization and thus intracellular Na^+ accumulation that can even be detected by ^{23}Na magnetic resonance tomography in vivo (26, 27).

Acknowledgements

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MEMORIES BY A MYOLOGIST

Vladimir Karlovich Roth (1848-1916): the founder of neuromuscular diseases studies in Russia

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This article shortly examines the biography, scientific activity and scientific work on neuromuscular diseases of the famous Russian neurologist Vladimir Roth who was the founder of neuromuscular disorders study in Russia. In 1876 he was the first in Russia who performed an autopsy and a detailed histological study of a case of progressive muscular atrophy, in which he did not find changes in the nervous system. He called this disease "muscular tabes" i.e. myopathy. In 1884 Vladimir Roth expressed his opinion about the nosological place of the peripheral type of muscular tabes to be considered as a distal myopathy. Dr. Roth became well-known for his monograph of the neuromuscular diseases, published in Moscow in 1895 under the name "Muscular Tabes" in which he described the history of neuromuscular diseases in a very detailed way, analyzing 1014 cases published in the world literature from 1830 to 1893 and 125 personal observations in the period 1874-1894. He performed a thorough analysis of the pattern of muscle involvement using both electrodiagnostic and histological study of muscles and central/peripheral nervous system. We report a short review of this monograph and two cases of peripheral (distal) myopathy.

Key words: Muscular tabes, distal myopathy, peripheral neuromuscular involvement

Vladimir Roth is the founder of neuromuscular diseases studies in Russia. He was a disciple of A. Ya. Kozhevnikov – the founder of Russian neurology. Roth was the great clinician and neuropathologist. He was also identified as a brilliant master on electric diagnostic and electric therapy of the neuromuscular diseases.

Roth's scientific biography

Vladimir Roth (Fig. 1) was born on October 5, 1848 in the city of Orel in a family of a pharmacist, originating from Sweden. After gymnasium he was admitted to the Moscow University which he completed in 1871 with honors and stayed as a resident at the neurology clinic upon recommendation of Professor A. Ya. Kozhevnikov.

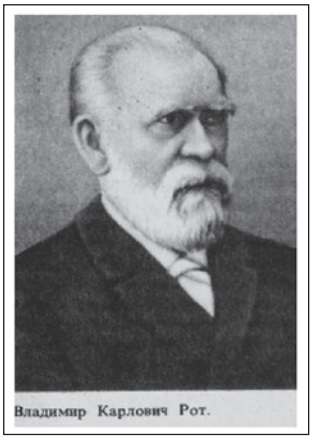

Figure 1. Vladimir Karlovich Roth (1848-1916).

In 1876, after completion of residency, Dr. Roth went to study and work abroad. During 4 years Dr. Roth worked at clinics and laboratories of Paris, Berlin and Vienna with Drs. Vulpian, Charcot, Magnan, Ranvier, Claude Bernard, Broca, Virchow, Leaden, Westphal, Meynert, Obersteiner and Benedikt.

After return to Moscow from 1881 to 1890 he headed a 40-bed Department of Neurology at the Old Catherine's Hospital and delivered lectures in diseases of nervous system and electric therapy. In 1895 he was appointed an Extraordinary Professor of Neurology of the Moscow University and headed out-patient clinic of nervous diseases (1890-1894). In 1899 director of the clinic of nervous diseases (after A. Ya. Kozhevnikov) and from 1902 to 1911 he was Staff Professor of Neurology in the Department of Medicine of the Moscow University.

Roth's scientific activity

Professional, scientific, teaching and social activities of Dr. Roth were associated with the Department of Medicine of the Moscow University, founded in 1755. This

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institution is currently named the First Sechenov State Medical University.

As an invited presenter Dr. Roth participated in many Russian and international scientific meetings: in Geneva (1877), Copenhagen (1884), Magdeburg (1884), Lubeck (1886), Berlin (1890), Bern (1895), Leipzig, Prague, Bordeaux.

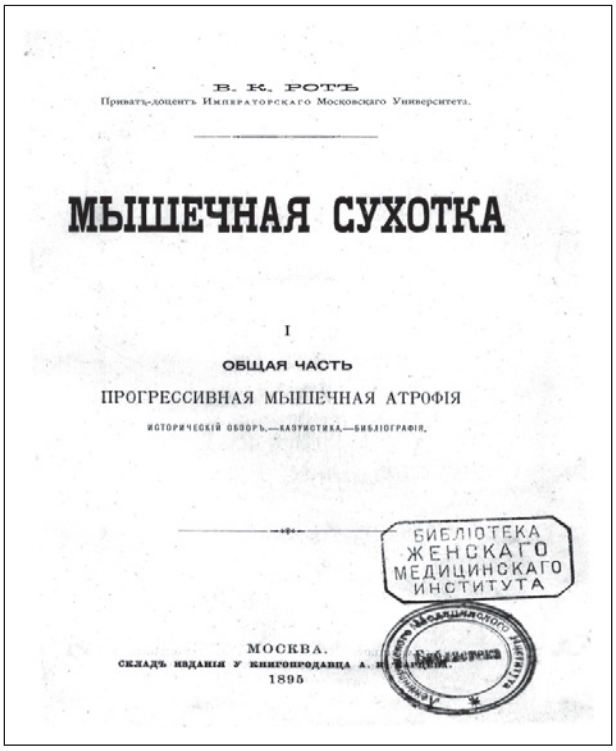
In 1897 Dr. Roth participated in the XII International Meeting of Medical Doctors in Moscow as a lead secretary and organizer. This was the first International medical meeting to be held in Russia. In 1890 Dr. Roth participated in establishing the Moscow Society of Neurologists and Psychiatrists and was elected a chairman of this society (after A. Kozhevnikov). He took part in the work of the "Society of Russian Doctors in the memory of N.I. Pirogov" established in 1881. In 1891 he became the chief editor of the "Korsakov's Journal of Neurology and Psychiatry". This was the first neurological journal to be written and published in Russia. In 1897 Roth edited the first Russian textbook on nervous diseases "Course of Nervous Diseases". He was the founder the Institute of Neurology at the Moscow Imperial University.

Roth's scientific work on neuromuscular diseases including myopathies from 1874 to 1895 and his famous book "Muscular tabes" (Moscow 1895) (Fig. 2).

Dr. Roth published 45 scientific works in Russian and foreign literature, devoted to different aspects of neuromuscular diseases.

In order to better understand the role of Dr. Roth in recognition of some neuromuscular diseases it is necessary to discuss some terminological definition which Dr. Roth used to distinguish these diseases. Dr. Roth described two groups of diseases with atrophy and weakness of muscles (1-3). The first group was called progressive muscular atrophy due to the affection of spinal cord and peripheral nerves which included primary and secondary spinal atrophy, polyneuropathies, neuropathies and amyotrophic lateral sclerosis. The second group was called muscular tabes resulting from disorders of muscle fibers themselves with fatty and connective tissue substitution. In other words the name muscular tabes included myopathies which Dr. Roth divided into basic (central), peripheral and transitional form of muscular tabes in his own material. However, it is necessary to note that when Roth described casuistics from the literature he used the name "peripheral type of muscular tabes" for designation of the neurogenic distal atrophy because according to the pattern of muscle involvement Dr. Roth considered that the cases described by Charcot et Marie, Tooth, Hoffmann, Joffroy, ets. connected with the lesion of muscle themselves but not peripheral nerves or spinal cord (see below and Tab. 1).

In 1873 Dr. Roth started his studies of progressive muscular atrophy, as suggested to him by Prof. Kozhevnikov. In 1874 Dr. Roth gave the information in the Moscow Medical Newspaper (4) about patient K., aged 22 (Fig. 3) with muscle affection similar to Duchenne's progressive muscular atrophy of childhood. After the patient's death of lung tuberculosis in 1876 Roth made a very detailed autopsy and post mortem histological study in which he did not find changes in the nervous system. These results were reported and published later (1, 2, 5). This study showed a pattern of myopathy (the normal and atrophic fibers, the fiber with granular degeneration, capillaries, connective tissue growth and formation of fat cells) while study of the spinal cord, medulla oblongata, sympathetic nerve, nerve roots and peripheral nerves showed no abnormality (description of histology of this case takes 25 pages (pp. 120-144) (6) (Figs. 6, 7). Autopsy and histology results were presented by the author at a meeting of the Society of Russian Medical Doctors and published among the proceedings of this society in 1880 (Moscow) (5). Slides of muscle and nervous tissue (spinal cord and nerves) were presented to Professors Babukhin, Kozhevnikov and Klein in Moscow, as well as to Professors Charcot and Vulpian in Paris, Benedikt in Vienna, who agreed with the author's conclusions.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МЫШЕЧНАЯ СУХОТКА

I

ОБЩАЯ ЧАСТЬ

ПРОГРЕССИВНАЯ МЫШЕЧНАЯ АТРОФІЯ

ИСТОРИЧЕСКІЙ ОБЗОРЪ.—КАЗУИСТИКА.—БИБЛІОГРАФІЯ.

МОСКВА.
СКЛАДЪ ИЗДАНІЯ У КНИГОПРОДАВЦА А. И.
1895

Figure 2. Roth V.K. Muscular tabes. I. General part. Progressive muscular atrophy: historical review, casuistry and references. Kartzev Publisher, Moscow, 1895.

Dr. Roth became well-known especially for his monograph of the neuromuscular diseases published in Mos-

Table 1. Review and grouping of casuistry from literature (1830-1893, author's note) (A total of 1014 cases).

Time of publication	Cases of different neuropaties attributed by the author to progressive muscle atrophy							Spinal amiotrophy								Basic form of muscular tabes									
	Amyotrophic lateral sclerosis	Glyomatosis and syringomielia	Complex spinal and subacute	Spinal pachimeningitis	Nevritis	Nonspecific nevrotic diseases	Origin unknown	Pure, protopathic and type Aran-Duchenne-Charcot	Humeroscapular type	Radial type	Childhood hereditary type	Adult hereditary spinal type	Policencephalomielitis chronica	Peripheral type of muscular tabes	Hypertrophic neuritis Dejerine	Descending form of children and adults	Ascending form of children (pseudohypertrophic)	Ascending form of adults	Typical	Indefinite transitional cases	Total cases of basic muscular tabes	Total spinal forms	Percentage relation (%) of the basic muscular tabes to all amyotrophies collected	Relation of the basic muscular tabes to pure amyotrophies only	Total cases which were analysed
Under 1853	2	1	6	-	2	2	1	-	1	-	-	-	-	2	-	1	12[1]	-	1	-	14	15	45%	14:1	31
From 1854-58	12	1	16	1	4	2	8	8	-	-	-	-	-	2	-	3	1	2	4	-	10	52	16%	5:4	64
,, 1859-63	14	2	5	2	7	2	1	3	1	-	-	-	-	2	-	15[2]	7	-	-	3	25	37	46%	8:1	64
,, 1864-68	6	1	5	-	2	2	1	3	-	-	-	-	-	-	-	4	35[5]	-	-	-	39	20	66%	13:1	59
,, 1869-73	10	5	2	1	4	2	-	4	-	-	-	-	-	17[1]	-	23[3]	24	7	3	6	63	28	58%	18:1	108
,, 1874-78	7	8	10	-	5	1	16	7	1	-	-	-	-	-	-	5	41	4	5	1	56	55	50%	8:1	111
,, 1879-83	5	4	8	-	6	2	-	2	-	1	-	-	1	-	7	84[6]	7	2	4	104	28	79%	52:1	133	
,, 1884-88	5	4	2	1	-	-	3	5	2	-	-	-	-	28	-	42	74	12	15	14	157	22	71%	39:1	207
,, 1889-93	4	4	-	-	-	-	-	7	2	8	18	4	4	22	2	58	76	20	7	1	162	51	70%	23:1	237
	65	30	54	5	30	13	30	39	7	9	18	4	4	74	2	158	354	52	37	29	630	308	62	16:1	1014

1) 10 cases of Eichorist and 5 cases of Friedreich
2) and 3) Many cases in Duchenne's book: 2 nd and 3d editions
4) 8 cases of Meryon
5) 10 cases in Duchenne's monography
6) 24 cases of Gowers

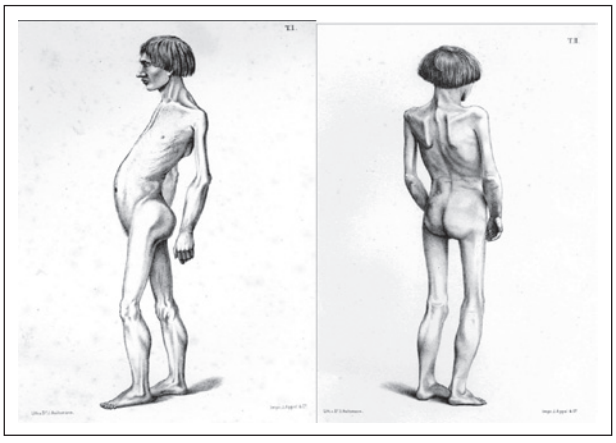


Figure 3. Observation I. (1874 year). Patient A.K., aged 22. Basic muscular tabes, typical form.

cow in 1895 under the name "Muscular Tabes. General part: Progressive muscular atrophy" (Fig. 2) at which he worked during 20 years. For this monograph Dr. Roth was awarded the degree in Medicine and the title of Extraordinary Professor of Neurology. This book includes 478 printed pages plus 19 isolated pages with photographs and drawings of Roth's own patients and histological preparations with their description (6).

This book included:

1) historical review with short clinical analysis of published cases and results of autopsies, histological and elecrtrodiagnostic studies in different neuromuscular diseases;
2) review and grouping of casuistic material;
3) review and grouping author's own observations and description of author's own observations (cases with muscular tabes i.e.myopathy, mainly as well as amyotrophic lateral sclerosis and primary and secondary spinal muscular atrophy) (pp.115-394);

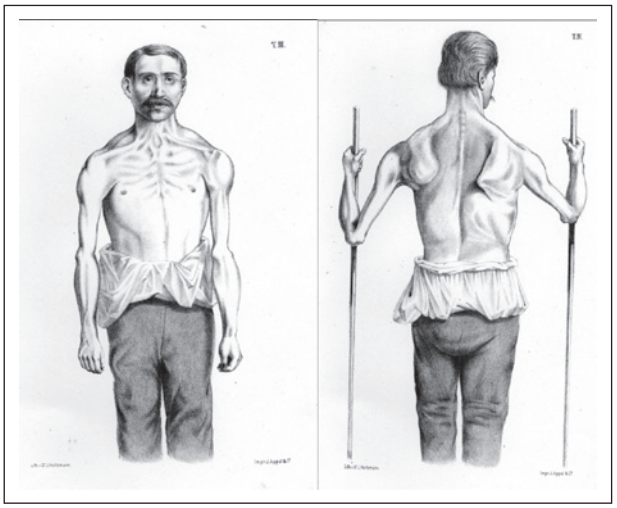


Figure 4. Observation XXIV (1879 year). Patient A.U., aged 31. Basic muscular tabes, descending form.

4) references and alphabetic list of cited authors.

We give a very brief characteristic and only some from these parts.

1) Historical review included the following items:
   - I. Article of Aran on progressive muscular atrophy. Opinion of the physicians on muscle atrophy before XVIII century. Gerardi L.B. van Swieten. Pathology of muscle system and cases of progressive muscular atrophy before the Aran period. Hasse, Graves, Parry, Ch. Bell, Costa &. Gioja, H. Maoy, Froriep, Dubois, Partridge, Duchenne, Romberg, etc.
   - II. Period of Aran, Duchenne and Cruveilhier.
   - III. Further development of progressive muscular atrophy studies. First attempts of clinical differentiation between different forms. Prevalence of myopathic theory in Germany and England. Meryon, Oppenheimer, Wachsmuth, Roberts, Friedberg, etc.
   - IV. Neuropathic theory of the disease. Different anatomic facts. Sympathetic theory of the disease. Jaccound, Valerius, Dumenil, ets. Development of spinal (poliomyelitic) theory. Predecessors of Charcot. Work of Bergmann. Successes in studies of the anatomy nervous system. Autopsy of Luys, Clarke, Dumenil. Spinal cord affection in children's paralysis. Charcot theory of trophic role of large cells of the spinal cord anterior horns.
   - V. Confirmation of Charcot theory by new facts in pathologic anatomy. Autopsy of Hayem, Charcot-Joffroy. Development of teaching of paralysis bulbaris progressiva and it connection with pathogenesis of progressive muscular atrophy. Duchenne, Leyden, Kussmaul. Amyotrophic lateral sclerosis and deutoropathic amyotrophy. Studies of systemic myelitis. Triumph of Charcot's poliomyelitic theory (Vulpian, Charcot).
   - VI. Studies on Friedreich.
   - VII. Muscle pseudohypertrophy. Casuistic material. Monograph of Seidel and Duchenne. Autopsy of Cohnheim and Charcot. Friedreich's opinion in the attitude of pseudohypertrophy to progressive muscular atrophy.
   - VIII. Revival of myopathic theory in Germany. Leyden's opinion on the devision of progressive muscular atrophy into two different forms. Autopsy of Lichtheim. Möbius's opinions. Second monograph of Seidel. Autopsy of Erb–Schultze. Autopsy of Roth. Clinical grouping of the cases progressive muscular atrophy: Leyden, Damaschino, Gowers and Erb.

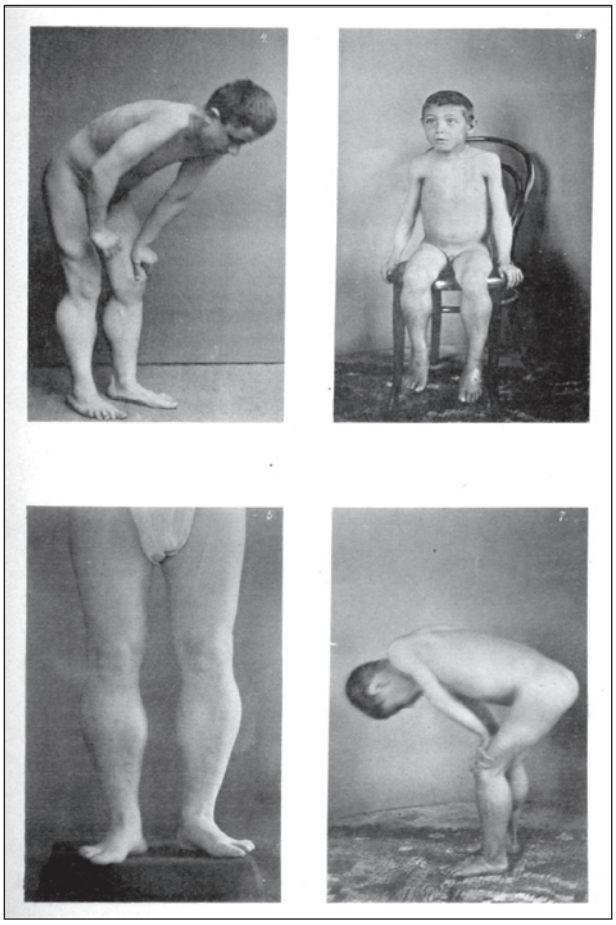


Figure 5. Two photos at the left side - Observation IV. (1891 year).Patient M.S., aged 8. Basic muscular tabes, ascending form (in childhood onset). Upper right - Observation V. (1888 year). Patient I.L., aged 16. Basic muscular tabes, ascending form (in childhood onset but with slow course); Lower right - The boy with pseudohypertrophy of muscles after influence is presented.

IX. Work of Erb. Juvenile form. Dystrophia muscularis progressive. Question of progressive muscle atrophy at the VIII International Medical Congress. Moebius's opinion. Autopsy by Landouzy and Dejerine. Facio-scapulo-humeral type. New opinion of Charcot-Marie-Tooth.

X. Peripheral type of muscular tabes. "Special form" of Charcot-Marie: peroneal type Tooth; Hoffman's neural muscular atrophy. Autopsy of Dubreuilh and Hanel. Hypertrophic neuritis Dejerine. New spinal forms: radial type and hereditary form of Bernhardt. Autopsy of Strumpell. Children's spinal form of Werdnig and Hoffmann. Polioencephalomyelitic form.

XI. Casuistry of the basic muscular tabes. Theory of the vascular origin of this disease. Neuritic theory of Holland authors. Success of tropho-neuropathic theory. Pathogenic theory the disease of Gradenigo, Babinsky and Onanoff and Roth. Opinions of Schultze, Vulpian, Erb and Strumpell.

2) Review and grouping of cases from literature (Table 1): this table shows a review of 1014 cases of progressive muscle atrophy and basic muscular tabes collected by Dr. Roth in the World literature between 1830-1893.

3) Review and grouping of Roth's own observations (Table 2): this table shows

Table 2. Review and grouping of author's own observations (1874-1894 author's note)(A total of 125 observations).

	Glyomatosis of spinal cord	Lateral amyotrophic sclerosis	Don't confirm new cases of aran-Duchenne type	Pure cases of Aran-Duchenne type	Humero-scapular type spinal muscular atrophy	Humero-scapular type did not differentiated	Atrophia which beginning with posterior cervical mescles	Radial type	Atypical cases with affection of forth limbs	Accidental amyotrophies in neuritis, migrenis, poliomyelitis, procasa et others	Unknow origin	Peripheral muscular tabes	Basic muscular tabes, descending variant	Basic muscular tabes, ascending variant in children	Basic muscular tabes, ascending variant in adults	Typical basic muscular tabes	Atypical cases of basic muscular tabes	Total cases of basic muscular tabes	Total cases of progressive muscular atrophy
Author's observation	27	8	5	1	1	3	1	4	1	3	1	4	5	9	4	8	3	29	88
Ambulant patients observed at the Clinic of Neurology for 4 years (1890-1894)	12	2	1	-	-	1	1	2	-	3	-	1	2	1	-	2	-	5	28
Patients not admitted at the Clinic of Neurology in the same period	4	2	-	-		-	-	-	-	-	1	-	-	-	-	-	-	-	7
Patients treated in the Clinic of Neurology during 10 years (1884-1894)	13	12	-	1	-	2	1	1	3	1	-	1	4	2	-	3	-	9	44
All clinical patients excluding repeated cases	26	14	1	1	-	2	1	3	3	4	-	2	5	3	-	5	-	13	70
All author's cases and clinical cases excluding repeated cases	41	20	5	2	1	4	1	4	4	4	2	4	7	9	4	10	3	33	125

In the group of the patients with gliomatosis and lateral amyotrophic sclerosis the patients without muscular atrophies were not included.

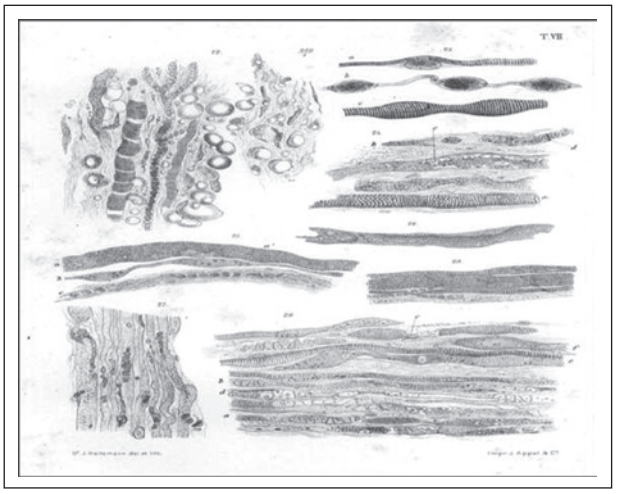


Figure 6. Upper right (Slide n. 22, m. sacro-lumbo-dorsalis). Patient A.K. Muscular tabes. Normal fiber in middle, atrophic fiber on the right, fiber with granular degeneration, capillaries, connective tissue growth and formation of fat cells on the left . Upper left (Slide n. 23, m. interosseius). -Observation XLII – syringomyelia. Down Left side (slides 24-26, 28-29, m. biceps brachii and slide 27, m. gastrocnemius). Observations XXXIV: amyotrophic lateral sclerosis. The histological study of XXXIV case was made in laboratories of Virchov and Klein. (The slides 23-29 were not described by authors of this article-author's note).

a review of 125 observations of progressive muscle atrophy and muscular tabes collected by Dr. Roth between 1874-1894. Dr. Roth subdivided muscular tabes into descending basic cases, ascending basic cases with onset in children and adults as well as the typical and atypical cases of basic muscular tabes and peripheral (distal) cases of muscular tabes.

4) Bibliography. Dr. Roth cites 1175 papers that he collected from the world literature from 1830 to 1893 and read in the original language, German, French and English. Of the most cited papers a very short summary of their content. Table 3, for example, presented, only two pages of references from 1830 to 1853 years.

The book "Muscular tabes" is very well illustrated with photographs and pictures of patients (performed by the painting-physician Dr. Heitzmann from Vienna) demonstrating various distinct phenotypes. Beside, detailed histological finding of muscle and central and peripheral nervous system are beautifully illustrated by color pictures. For example, we present from this monograph some photographs and drawings of patients with different types of muscular tabes (i.e. a myopathy) (Figs. 3-5, 8) and some histological slides (Figs. 6, 7).

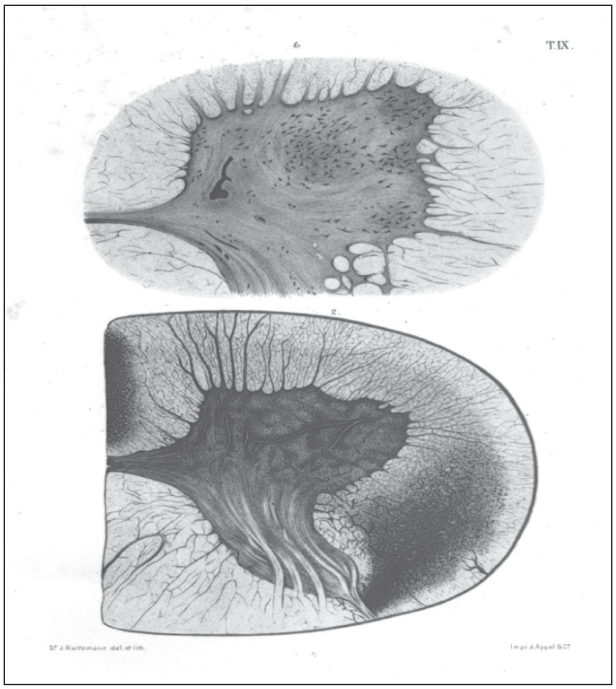
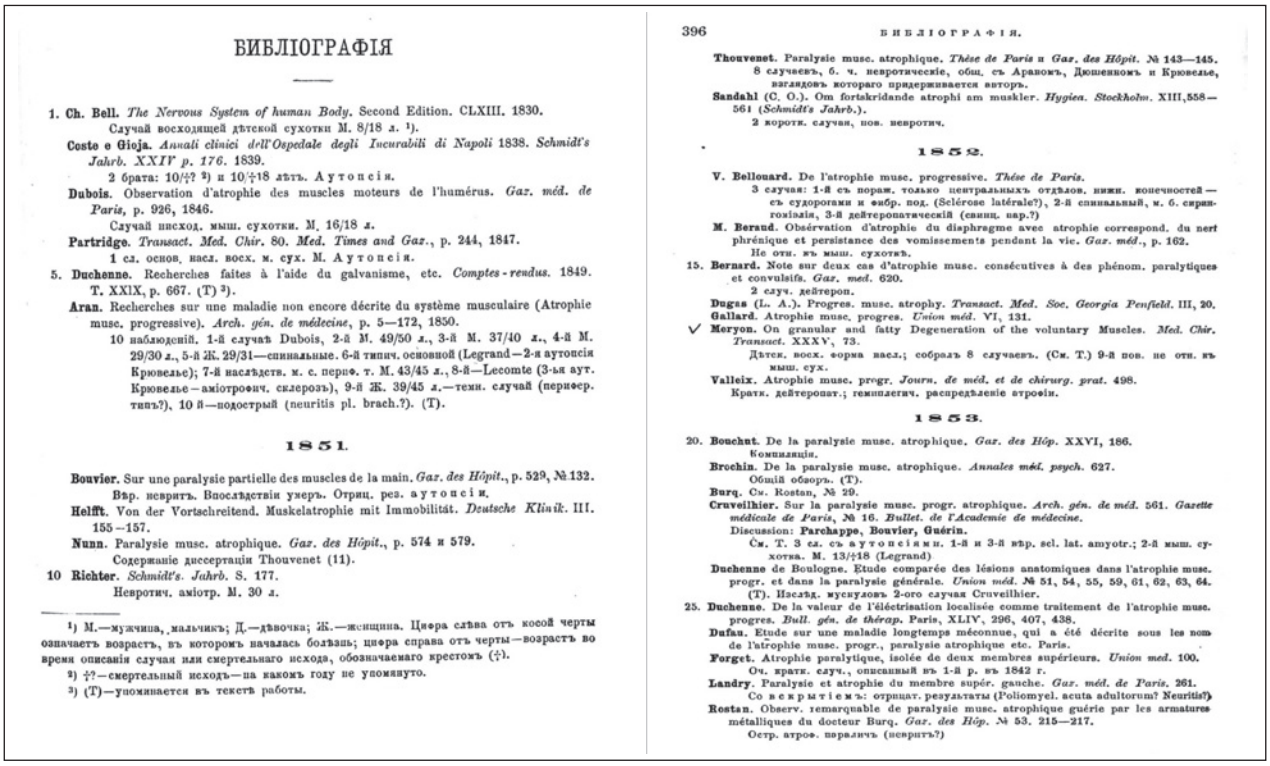


Figure 7. Up. Observation I. Basic muscular tabes. Abundance normal cells are shown in the anterior horn at the border between of C5-C6 nerve roots; **Down.** Observation XXXIV. Amyotrophic lateral sclerosis. Section of spinal cord at the C6 vertebra level. Sclerosis of the pyramidal tract and white substance around anterior horn. Absence of nervous cells in anterior horn and his hyaline infiltration. Atrophy of anterior and preservation of posterior nerve roots.

Distal myopathies in Roth's publications

There are four hereditary observations of peripheral muscular tabes (the same as a distal myopathy) in Dr. Roth's material: 2 observations with typical form (Observation XXXI, 1885, hereditary with severe weakness and atrophy of hand and feet, forearm and lower leg muscles (pp. 291-295); observation XXXIII, 1893**,** hereditary with severe weakness and atrophy of feet and lower leg muscles, only (pp. 298-301) and 2 atypical hereditary forms that Dr. Roth called as a "transitional forms of muscular tabes" (Observation XXVIII, 1886 (pp. 275-283); see description of this observation below) and observation XXIX, 1886, the sister of previous patient (pp. 283-286) (6). In all Dr. Roth's observations the disease began with affection of the lower leg muscles, extensors of feet and fingers. As an example, we present a very short description of two hereditary observations (a. with typical peripheral muscular tabes and b. with atypical, transitional form of muscular tabes).



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Вѣр. невритъ. Впослѣдствіи умеръ. Отриц. рез. аутопсіи.
Helfft. Von der Vortschreitend. Muskelatrophie mit Immobilität. *Deutsche Klinik.* III. 155—157.
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Содержаніе диссертаціи Thouvenet (11).
10 **Richter.** *Schmidt's. Jahrb.* S. 177.
Невротич. аміотр. М. 30 л.

[1]) М.—мужчина, мальчикъ; Д.—дѣвочка; Ж.—женщина. Цифра слѣва отъ косой черты означаетъ возрастъ, въ которомъ началась болѣзнь; цифра справа отъ черты—возрастъ во время описанія случая или смертельнаго исхода, обозначаемаго крестомъ (†).
[2]) †?—смертельный исходъ—на какомъ году не упомянуто.
[3]) (Т)—упоминается въ текстѣ работы.

396 БИБЛІОГРАФІЯ.

Thouvenet. Paralysie musc. atrophique. *Thèse de Paris* и *Gaz. des Hôpit.* № 143—145. 8 случаевъ, б. ч. невротическіе, общ. съ Аранонъ, Дюшенномъ и Крювелье, взглядовъ котораго придерживается авторъ.
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3 случая: 1-й съ поражм. только центральныхъ отдѣлов. нижн. конечностей—съ судорогами и фибр. под. (Sclérose latérale?), 2-й спинальный, м. б. сирингомиэлія, 3-й дегенеративный (спинц. нар.?)
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1853.

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Burq. См. Rostan, № 29.
Cruveilhier. Sur la paralysie musc. progr. atrophique. *Arch. gén. de méd.* 561. *Gazette médicale de Paris,* № 16. *Bullet. de l'Academie de médecine.*
Discussion: **Parchappe, Bouvier, Guérin.**
См. Т. 3 сл. съ аутопсіями. 1-й и 3-й вѣр. scl. lat. amyotr.; 2-й мыш. сухотка. М. 13/18 (Legrand)
Duchenne de Boulogne. Etude comparée des lésions anatomiques dans l'atrophie musc. progr. et dans la paralysie générale. *Union méd.* № 51, 54, 55, 59, 61, 62, 63, 64. (Т). Изслѣд. вскрытій 2-ого случая Cruveilhier.
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Остр. атроф. параличъ (невритъ?)

Table 3. Fragments of the references (1830-1853) including a total of 1175 articles with short summaries from Roth's monograph "Muscular tabes." Moscow 1895

a. Observation XXXIII, peripheral muscular tabes

Patient, of German origin, merchant, 39 years old, presented with symptoms of anxiety. The examination revealed a motor disorder in the lower limbs existing for a long time, but which the patients did not complain. The patient had 4 brothers and 4 sisters. The father and 1 of 4 sisters had a similar gait. The brother was normal. Clinical examination. Able to walk unaided without a stick. Main impairment was lack of dorsal flexion of the feet. Dr. Roth noted that the muscle wasting and weakness predominantly affected the anterior compartment of lower legs (posterior compartment muscles were minimal affected). Dr. Roth gave a very accurate and detailed analysis of the mechanisms of course steppage gait in this patient and noted that the leg muscle weakness was symmetrical. Small muscles of the feet were not affected. There was the contracture of the Achilles tendon. No fibrillations. The knee and Achilles tendon reflexes were absent. No sensory changes. All other muscles have not motor disturbances. Electric diagnosys: Roth himself made electric diagnostics of 22 muscles including feet and lower leg muscles and hands as well as mm. vastus lateralis and medialis and also peroneal nerves bilaterally of this patient. Results: no reaction of degeneration, no electric excitability in paralyzed muscles and its decrease in a weakened muscle. There were no electric signs of neuropathy. Dr. Roth concluded that this was a hereditary case of peripheral muscular tabes (i.e. primary distal myopathy, author's note).

In two other hereditary observations (brother and sister) in which the disease began with the atrophy and weakness of the distal part of legs and arms and later of the proximal muscles of the limbs, pelvic and shoulder girdle muscles involved in a less degree. Dr. Roth called this disease as a transitional form of muscular tabes.

b. This was an observation XXVIII (Fig. 8), which we present with very short Roth's remarks:

"A distinctive feature of this case is that… muscles of the pelvic girdle are affected less than thigh muscles, whereas muscles of the lower leg are completely gone, while muscles of the feet are minimally affected". "…On the upper extremities muscles of the scapular and shoulder girdle and upper arm muscles are much less severely affected than muscles of the forearm and hand. The lesions are symmetrical and muscles of the body are preserved". … "All tendon reflexes are absent"… "No fibrillation" … "Here we also see terminal atrophy, hypertrophy and lipomatosis of some muscles, characteristic of this disease, however, distribution of atrophy is quite distinctive". …

"No reaction of degeneration, no electric excitability in paralyzed muscles and its reduction in weakened muscles". There were not electric signs of neuropathy (Roth himself made electric diagnostics of 22 muscles in arms and legs and peripheral nerves (radialis, ulnaris, cruralis, peroneus, tibialis) on both sides of this patient-author's note)…"In our opinion this case represents transitional form of muscular tabes between the basic (central) type and peripheral type".

Figure 8. Observation XXVIII. (1886 year). Patient L.G., aged 32. Transitional form of muscular tabes (the same as atypical form of distal myopathy- author's remark).

In 1884 (1, 2) Dr. Roth at the International medical congress in Copenhagen presented a report "Issue of amyotrophic lateral sclerosis and its relation to progressive muscular atrophy" where he presented a classification of progressive muscular atrophies (without affection of the anterior horns of the spinal cord and nerves) which he called "muscular tabes" (the same as a myopathy) and described two main forms: 1. The most frequent – basic (central) form of muscular tabes – affecting muscles of the body and proximal segments of the extremities, pelvic and shoulder girdle. Often the disease has a tendency to generalize; 2. the second – peripheral form of muscular tabes – with muscular atrophy and weakness of the lower legs and in a lesser degree in feet, hands and forearms. In case of the peripheral form generalization of atrophy is less frequent.

In the review of progressive muscular atrophies in 1887 (1, 3) Dr. Roth makes the following conclusions based on his own data and literature:

1. Some cases of progressive muscular atrophies depend on primary affection of the spinal cord gray matter cells - amyotrophia spinalis progressive (protopathia Charcot). The cases described can be divided into two types according to the distribution of atrophy: a) Hand type – should not be called the Aran-Duchenne type and cannot be used as a prototype of "progressive muscular atrophy", because it is primarily (and may be exclusively) found in deuteropathic spinal atrophies; b) Shoulder-scapular type of Vulpian, the existence of which has not been proven yet.
2. The most frequent cases include progressive protopathic essential form of muscle atrophy (muscular tabes - the same as a myopathy) subdivided into two main forms according to the distribution of the disease: basic (central) or peripheral. The basic (central) form can be ascending or descending in children and adults. The peripheral form of muscular tabes deserves to be distinguished by the basic (central) form due to the presence of its peculiar clinical manifestations (7).

In the period 1884-1895, Dr. Roth published 4 hereditary cases of distal myopathy which he called "peripheral form of muscular tabes" (2 typical cases) and "transitional form of muscular tabes" (2 atypical cases) and presented (in 1884) this special type of muscular tabes i. e. distal myopathy as a nosological entity.

However, in Welander's survey of the literature (1884-1947) on distal myopathy, the name of Dr. Roth was not mentioned (8), although Davidenkov – a great expert on neuromuscular diseases in Russia – in his textbooks on hereditary diseases on nervous system (9, 10) wrote: "A special type of myopathy with a peripheral onset, recently called the "Naville type", deserves a separate description…Probably the G. family described by Roth (cases XXVIII, XXIX from his monograph), where brother and sister, born by healthy, probably related, parents, were affected, belong to the same group of cases" (9) and " Distal form, distinguished from all other myopathic forms by the onset in distal parts of upper and lower extremities with the following spread in the proximal direction to limb girdle and body muscles, is probably an independent form. Similar cases were described by Roth, Naville, Rimbaud-Giraud, Kolmaus und Sweerts, Kristen, Frommel and Van Bogaert" (10).

Conclusion

The development of studies on neuromuscular diseases, in particular myopathies, began in Russia in the second half of the XIX century and is tightly connected with the name of Vladimir Roth, professor of Neurology at the Moscow University. However his contribution to the study of the clinical pictures, etiology, pathogenesis and classification of hereditary myopathies and spinal muscular atrophies has remained underestimated in the world literature on neuromuscular diseases.

By a historical point of view, the name of Vladimir Roth should be remembered among the Authors who first described new clinical forms of myopathies, in particular those with a distal distribution of atrophy and weakness.

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Acta Myologica • 2014 XXXIII: p. 43-81

PROCEEDINGS OF THE XIV MEETING OF THE ITALIAN ASSOCIATION OF MYOLOGY

Sirmione (BS), Italy

May 8-10, 2014

Associazione Italiana di Miologia

14° CONGRESSO NAZIONALE AIM

PalaCreberg Sirmione
8/10 maggio 2014

Azienda Ospedaliera "Spedali Civili" Brescia

Sin
SOCIETÀ ITALIANA DI NEUROLOGIA

Università degli Studi di Brescia

GIOVEDÌ, 8 MAGGIO

8.30 SALUTI E INTRODUZIONE
A. Padovani
Università degli Studi di Brescia
E. Agabiti Rosei
Università degli Studi di Brescia
M. Magoni
A.O. "Spedali Civili" Brescia

Apertura del Congresso
M. Moggio *(Milano)*, M. Filosto *(Brescia)*

8.45-9.45 **COMUNICAZIONI ORALI**

9.45-10.30 **Lettura magistrale**
Guidelines for diagnosis and treatment of inflammatory myopathies
M. De Visser *(Amsterdam)*

10.30-11.00 *Coffee break*

11.00-13.00 **Workshop congiunto AIM-SIR**
LE MIOPATIE INFIAMMATORIE: NEUROLOGI E REUMATOLOGI A CONFRONTO

11.00 Inquadramento clinico e diagnosi bioptica
M. Moggio *(Milano)*

11.30 Anticorpi miosite-specifici: significato clinico e patogenetico
A. Doria *(Padova)*

12.00 Approccio alla diagnosi ed alla terapia: l'esperienza del neurologo
R. Mantegazza *(Milano)*

12.30 Approccio alla diagnosi ed alla terapia: l'esperienza del reumatologo
R. Neri *(Pisa)*

13.00-14.00 *Pranzo*

14.00-15.30 **Visione e discussione poster**

15.30-16.30 **COMUNICAZIONI ORALI**

16.30 *Coffee break*

17.00-18.00 **Incontro con le Associazioni dei Pazienti**

18.00 **WORKSHOP**
NUOVI SVILUPPI NELLA TERAPIA DELLE MALATTIE NEUROMUSCOLARI

18.00 Distrofinopatie
G. Comi *(Milano)*

18.20 SMA
E. Mercuri *(Roma)*

18.40 Miastenia
A. Evoli *(Roma)*

VENERDÌ, 9 MAGGIO

8.30-9.30 **MUSCLE CLUB**

9.30-10.15 **Lettura magistrale**
Moderni aspetti nella diagnostica morfologica delle distrofie dei cingoli
R. Barresi *(Newcastle)*

10.15 *Coffee break*

10.45-12.45 **WORKSHOP**
IMPATTO DELLE METODICHE DI LABORATORIO NELL'ITER DIAGNOSTICO DELLE MALATTIE MUSCOLARI

10.45 Le indagini di laboratorio possono indirizzare verso una diagnosi piu' precoce?
A. Toscano *(Messina)*

11.15 L'imaging nella diagnostica differenziale delle miopatie.
G. Tasca *(Roma)*

11.45 Biomarkers nelle malattie neuromuscolari.
F. Gualandi *(Ferrara)*

12.15 Next Generation Sequencing e Whole Exome Sequencing: utilità e limiti.
V. Nigro *(Napoli)*

12.45-13.45 *Pranzo*

13.45-15.15 **VISIONE E DISCUSSIONE POSTER**

15.15-16.45 Aggiornamenti e proposte dei Gruppi di studio AIM

16.45-17.15 *Coffee break*

17.15-18.30 **COMUNICAZIONI ORALI**

18.30-20.00 Assemblea dei soci

21.00 Cena sociale

SABATO, 10 MAGGIO

8.30-10.30 **WORKSHOP**
NUOVI SVILUPPI NELLA TERAPIA DELLE MIOPATIE METABOLICHE

8.30 MNGIE ed altre malattie da deplezione del mtDNA.

9.00 La terapia genica nelle malattie mitocondriali.
M. Zeviani *(Cambridge)*

9.30 Glicogenosi V: dal modello animale allo sviluppo di nuove terapie.
A. Andreu *(Barcelona)*

10.00 Malattia di Pompe ad esordio tardivo: lo stato dell'arte.
M. Filosto *(Brescia)*

10.30-11.00 *Coffee break*

11.00-13.30 **COMUNICAZIONI ORALI**

13.30-14.00 Compilazione del questionario di valutazione ECM e verifica dell'apprendimento

Chiusura del Congresso

ABSTRACTS

(in alphabetical order of the 1st Author)

Functional characterization of three new recessive ClC-1 mutations causing myotonia congenita in Southern Italy

C. Altamura [1], S. Portaro [2], N. Licata [2], C. Rodolico [2], O. Musumeci [2], M.M. Dinardo [1], P. Imbrici [1], A. Toscano [2], D. Conte Camerino [1], J.F. Desaphy [1]

[1] Department of Pharmacy & Drug Sciences, University of Bari - Aldo Moro, Bari; [2] Department of Neurosciences, Psychiatry and Anestesiology, University of Messina

Myotonia congenita is an inherited disease characterized by impaired muscle relaxation after contraction, resulting in muscle stiffness. It is caused by loss-of-function mutations of the muscle ClC-1 chloride channel. We report the functional analysis of three new missense mutations found in patients with recessive myotonia congenita. The T82A and R453W mutations were found in compound heterozygosis with the known G190S mutation in two unrelated families, and the G270V mutation was found in an homozygous patient. Recombinant hClC-1 channel mutants were expressed in a mammalian cell line for patch-clamp studies of chloride current properties. The G270V and G190S mutations were found to induce a dramatic shift of activation voltage-dependence toward more positive potentials, resulting in nearly zero chloride current within physiological voltage range. Thus the effect of G270V can explain the myotonia in the homozygous patient. Conversely, the T82A and R453W chloride currents showed current amplitude, kinetics, and voltage-dependence similar to WT currents. Studies of mutant cotransfection reproducing the patients heterozygosis are being performed in order to elucidate the pathomechanism of T82A and R453W mutations. Supported by Telethon-Italy (GGP10101).

Predictors for cardiac conduction abnormalities in DM1. A 33 Yrs. prospective study in 102 DM1 patients with normal ECG at baseline

G. Antonini [1], E. Bucci [1], E. Gabriele [2], A. Frattari [2], L. Licchelli [1], N. Vanacore [3], M. Testa [2]

[1] Dept. NESMOS and [2] Cardiologic Institute. Faculty of Medicine and Psychology. University of Rome; [3] National Institute of Health, Rome

Since 1980, 151 continuous DM1 patients have entered a prospective research project on genotype/phenotype correlations of the disease. Aimed to find genotypic and phenotypic predictors of cardiac conduction abnormalities (CCA), we have recorded yearly ECGs in 102 patients (M/F 57/45, aged 5-79 yrs. Median 37 yrs., CTG range 50-1700), who had normal ECG at baseline. Patients were followed from 1 to 33 yrs. (median 8 yrs.) During the F-U 43 patients developed CCA (AVB-I: 32%, AVB-II: 9%, BBB: 23%); 12 patients underwent PMK/ICD implantation; 13 patients died for cardiac cause; 59 patients showed an increase of MIRS.
Follow-up was similar in patients who developed CCA and in those who did not. CCA occurred more frequently in males ($p = 0.008$) and in patients who showed MIRS progression ($p = 0.015$). CTG expansion showed a significant inverse correlation with age at onset of CCA ($R2 = 0.16$; $p < 0.0001$). Logistic regression analysis showed that, after correction for CTG expansion and age, both male sex and MIRS progression were independent predictors for CCA development (OR = 3.15; 95%CI = 1.28-7.72; $p = 0.012$ and OR = 3.86; 95%CI = 1.47-10.15; $p = 0.006$, respectively).

Novel *SEPN1* Mutation in 3 Patients: diagnostic clues of neck weakness and MRI pattern

A. Ardissone [1], C. Bragato [2], F. Blasevich [2], M. Mora [2], I. Moroni [1]

[1] Child Neurology Unit, [2] Neuromuscular Diseases and Neuroimmunology Unit, Foundation IRCCS C. Besta Neurological Institute, Milan, Italy

Mutations in *SEPN1* cause Selenoprotein N-related myopathy (*SEPN*-RM), characterized by early onset axial and neck weakness, spinal rigidity, respiratory failure and four distinct histopathological entities. A typical MRI pattern has been described in lower limbs, represented by a selective involvement of the sartorius muscle. We report on the clinical, histopatological and genetic findings in 3 patients from two families, presenting with an heterogenous phenotype and carrying novel *SEPN1* mutations. Two siblings arrived to the observation at the age of 10 and 14 years respectively, presenting with mild myopathic signs, neck weakness and spinal rigidity; the female showed also a severe diffuse muscle wasting. After two years follow-up the myopatic signs were stable, while a marked respiratory involvement was detected. The third patient, a three years old boy, presented with severe axial weakness, leading to a "dropped head" appearance, and lower limb girdle muscle weakness. The typical MRI pattern was present in both cases. *SEPN1* gene analysis disclosed the presence of the c.1176delA mutation in the siblings and of the c.726_727InsTCC mutation in the other patient. We underline the clinical diagnostic clues of early neck and axial weakness and of muscle MRI in addressing the diagnosis of *SEPN*-RM, and confirm the importance of investigating the progressive respiratory impairment in spite of mild myopathic course.

Beneficial effects of salbutamol in congenital myasthenic syndrome associated with new mutations in *CHRND*

G. Astrea [1], L. Maggi [2], R. Trovato [1], D. Kapetis [2], D. Cassandrini [1], S. Frosini [1], R. Brugnoni [2], P. Bernasconi [2], R. Mantegazza [2], R. Battini [1], F.M. Santorelli [1]

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Congenital myasthenic syndromes (CMS) are disabling, but potentially treatable disorder characterized by a neuromuscular transmission defect. Cholinesterase inhibitors are effective in most cases, but harmful in specific CMS. Salbutamol, a beta-adrenergic agonist, has demonstrated a partial improvement of clinical symptoms in some CMS.
A 15-year-old girl received a clinical diagnosis of CMS in her first year of life. The patient was put on acetylcholinesterase inhibitors since age 2.5 years and on 3,4 DAP since age 9 with limited improvement. At the age of 14 years, salbutamol was gradually added on (4 mg /day and, after six months, 6mg/day) because of lost of independent ambulation. Response to therapy was evaluated by using the MFM scale. We identified a novel missense mutation (c.215 A>C/p.Thr72Asn) heteroallelic to the c.521_524dupATAC/p.Ala176tyrfs* in CHRND. Molecular modeling demonstrated that replacement of Asn72 modifies local H-bond interaction environment between β1, β6 sheet domains and β1/ β2 loop.
Comparison of the pre- and post-treatment examinations showed a beneficial response to salbutamol with no side effects. Our findings highlight the importance of a molecular diagnosis in CMS and proposes salbutamol's use when conventional therapies fail to achieved a stable response.

Next Generation Sequencing in facioscapulohumeral muscular dystrophy patients supports the idea that FSHD is a complex genetic disease

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Facioscapulohumeral muscular dystrophy has been associated with reduction of the number of D4Z4 repetitive elements at 4q35. FSHD is characterized by great clinical variability within families in which D4Z4 reduced allele (DRA) segregates. An increasing number of cases are sporadic with no other affected relatives and several findings suggest that additional factors (genetic modifiers) might modulate FSHD expression. Thus molecular diagnosis, prognosis and genetic counseling have become more challenging.
To gain additional information on the complexity of FSHD, we tested 40 FSHD patients belonging to families with reduced penetrance by testing, a broad core panel of 93 genes involved in myopathies (Motorplex). This Next Generation Sequencing-based workflow permit the analysis of 2,544 exons.
We studied 40 samples from FSHD patients belonging to families in which other DRA carries are healthy. In all subjects we found putative pathogenic variations in genes causing different myopathies. In addition to DRA, all patients carried at least one damaging variations in other disease genes. These variants, if they had been detected alone in the context of a single gene testing, would have been considered as causative. The high number of damaging mutations identified in each sample support the hypothesis of "multiple factors" leading to the FSHD phenotype.
In conclusion, the use of a reliable, sensitive and specific method has been able to identify putative pathogenic mutations that can explain the variable penetrance of DRA. Importantly these large set of mutations observed in FSHD patients highlight the genetic complexity that might contribute to the disease expression.

Lack of association between CNS and muscle involvement in Steinert's Disease (DM1). What about a link between CNS and behaviour?

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Introduction. Myotonic dystrophy is a genetic, multisystemic disorder due to polynucleotide-expansions being only partially reliable to predict phenotypic expression. Beyond muscular involvement DM1 can be characterized by functional/morphological brain abnormalities to different extents. From a neuropsychological point of view executive, visuo-spatial dysfunctions, mood and personality impairments are reported.
Methods. Forty subjects with established clinical-genetic diagnosis, underwent complete neurological assessment, including psychological interview and neuropsychological evaluation. Main caregiver underwent patient's Quality-of-life interview. A subgroup of 15patients underwent brain-MRI investigation.
Results. We found reduced scores in neuropsychological tests for frontal (61%) and visuo-spatial abilities (66%); interestingly verbal abilities were rather preserved (80%). Behaviour was characterized by mixed-mood conditions (anxiety, depression, apathy) and by variable sets of pathological personality traits, though without fulfilling diagnostic criteria for major psychiatric disorder according to DSM-IV. Patient's and main caregiver's reports showed internal discrepancies (63%), with patients

tending to denial some aspects of their condition. Brain imaging revealed white-matter involvement in frontal (53%), parietal (27%) and temporal (73%) lobes. Statistical analysis showed significant relationships between reduced spatial memory performances and temporal lobe white-matter changes (Fisher-Exact-Test, $p < 0.05$).

Conclusions. Muscle and brain appear independently involved in DM1; white-matter lesions are common in DM1 patients independently from muscle involvement (MIRS). In our study CNS involvement in DM1 is characterized by cognitive/psychopathological dysfunctions, heterogeneously distributed; this could be a prominent feature in DM1, leading to an increased burden in management in health-institutions and at home. Cognitive/behavioural disorders could have significant relationships with white-matter lesions and should be investigated since the early phases of illness, in order to plan proper management.

Early limb-girdle and congenital muscular dystrophy co-occurrence within the same family carrying a new homozygous *ISPD* mutation

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Dystroglycanopathies represent an important subgroup of recessively inherited disorders within the group of muscular dystrophies. They are associated with a reduction in the functional glycosylation of dystroglycan, and their severity may vary from the mild forms of adult-onset limb-girdle muscular dystrophy (LGMD), to the severe congenital muscular dystrophies with cerebral and ocular involvement. Although mutations in at least 17 genes have been identified so far, about 50% of the cases with dystroglycanopathy still remain unsolved. Mutations in the isoprenoid synthase domain containing (ISPD) gene has been associated with loss of dystroglycan glycosylation. Similarly to other genes associated with dystroglycanopathies, ISPD gene mutations have been at first reported as a frequent cause of Walker–Warburg syndrome. More recently, ISPD mutations have been reported in seven families, with phenotypes ranging from congenital muscular dystrophy to LGMD. We report clinical, histopathological, immunochemical, genetic and muscular MRI findings in 2 consanguineous children of Pakistani origin, carrying a new homozygous missense mutation (Gly123Arg) in the ISPD gene. Case 1 is a 8 year-old female with an early limb-girdle phenotype, who lost ambulation at the age of 7.5 years. Case 2 is a 2.5 year-old male and second degree cousin of case 1, showing a congenital muscular dystrophy phenotype. Cognitive development, brain MRI, eye examination, electrocardiogram and echocardiogram were normal in both the patients. To our knowledge, this is the first report on the co-occurrence of both early limb-girdle and congenital muscular dystrophy within the same family carrying a new homozygous *ISPD* mutation.

Pilot study of flavocoxid in ambulant DMD patients

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Muscle degeneration in Duchenne muscular dystrophy (DMD) is exacerbated by the endogenous inflammatory response and increased oxidative stress. A key role is played by nuclear factor(NF)- κB. We previously showed that flavocoxid, a flavonoid with antioxidant and anti-inflammatory properties, ameliorates muscle pathology and function in *mdx* mice. This effect seemed to be mediated by the inhibition of NF-κB, tumor necrosis factor-α, cyclooxygenase-2/5-lipoxygenase and MAPKs expression in muscle. Moreover, flavocoxid has been shown to decrement serum levels of IL-1β and TNF-α in *in vivo* studies.

Primary end-point of this pilot study was to evaluate safety and tolerability of flavocoxid administered daily *per os* for one year in ambulant DMD patients. We also evaluated function, muscle strength and quality of life. The effects of flavocoxid on selected biomarkers was also assessed. We enrolled 20 patients. We did not report any treatment-related adverse event and clinically meaningful change in laboratory findings. Serum expression analysis of inflammatory cytokines showed a significant reduction of TNF-α and IL-1β and oxidative stress markers ($p < 0.05$). The results of the multidimensional clinical evaluation showed an overall stabilization of clinical course, even in patients older than 7 years and which showed deterioration in the year before baseline. Moreover, a significant worsening of North Star Ambulatory Assessment was shown at 6 months after end of treatment compared to all trial time points ($p < 0.05$). We demonstrated that flavocoxid at this dosage is safe, also in pediatric age and in association with corticosteroids, and able to exert its biological effects on inflammatory pathways relevant to DMD pathogenesis.

Mutations in *GMPPB* cause α-DG: report of an additional highlighting

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Congenital muscular dystrophies with hypoglycosylation of alpha-dystroglycan (α-DG) are a heterogeneous group of disorders in which the spectrum of severity observed ranges from classical congenital muscle dystrophy (CMD) presentation to children showing mild limb-girdle weakness variably associated with mild intellectual disability and/or structural brain anomalies. Recently, mutations in the guanosine diphosphate mannose (GDP-mannose) pyrophosphorylase B (*GMPPB*) gene have been associated with muscular dystrophy with hypoglycosylated α-DG.

Through an international collaborative effort, we identified recessive *GMPPB* mutations in an additional patient. LS is a 5-year-old boy who presented axial muscle weakness in the first months of life followed by ataxia and nystagmus, hyperCKmia, brain MRI with enlargement posterior fossa, moderate intellectual disability and autistic-like behaviour.
In light of no evidence of perturbed transferrin glycoforms and a reduced muscular immunostaining for glycosylated α-DG, we performed exome sequencing and detected a reported p. R287Q (Carss et al., 2013) and a novel p.I219T variant in *GMPPB*. The latter mutation is not present in Exome Variant Server or dbSNP but a variant in the same place is seen 3 of 9464 cases in the UK10K project Considering that there were no other suggestive variants in the exome of LS, most probably the 2 mutations in *GMPPB* are causative of CMD with low α-DG and autistic-like features.

Acute ataxia and psychomotor regression due to SDHAF1 mutation responsive to riboflavine

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Deficiency of complex II (succinate dehydrogenase, SDH) represents a rare cause of mitochondrial disease and is associated with a wide range of clinical symptoms. Recently, mutations of SDHAF1, the gene encoding for the SDH assembly factor 1, were reported in SDH-defective infantile leukoencephalopathy with typical brain MRI and spectroscopy features. No satisfactory treatment is currently available, and affected patients undergo a relentlessly progressive motor and mental deterioration. Riboflavin has been reported as possibly effective in reducing the progression of disease or even preventing from developing signs of neurological involvement when administered before the onset of the disease, but so far only few cases have been described.
We describe here the case of child with SDHAF1 mutation and typical features in brain MRI and spectroscopy with onset at 2ys 4mths, treated with riboflavin and CoQ and with a positive clinical and neuroradiological outcome after 3 years

Cognitive impairment in mitochondrial encephalomyopathies

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Objective. Mitochondrial encephalomyopathies are a genetically heterogeneous group of diseases characterized by multisystem involvement. We studied the neuropsycological profile, magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT) to look for common or specific cognitive defects and a possible correlation with neuroimaging data.
Patients and methods. 53 patients (thirthy three females, twenty males) aged 17-80 years: fourty-three PEO, five MELAS, four MERRF and one MNGIE. All the patients underwent neuropsychological evaluations and MRI neuroimaging; eighteen patients had also SPECT. Four main cognitive areas were explored: visuospatial perception, memory, executive, speech and praxic functions.
Results. Mental control, short term memory and visual selective attention were selectively impaired in 75% of patients., but we did not found any correlation between neuropsychological profile, and age, clinical phenotypes and genetics. SPECT was abnormal in nine patient with parietal and temporal hypoperfusion in six of them. A 60–year old woman with PEO showed selective hypoperfusion in right temporal lobe with temporomesial atrophy and developed an Alzheimer's like syndrome. MRI neuroimaging revealed signs of subcortical white matter impairment in thirty-four patients. Finally we observed four patients with short term memory or mental control impairment without SPECT abnormalities.
Conclusions. Cognitive impairment is frequent in mitochondrial diseases revealing a consistent pattern independently from phenotype; moreover no correlation were observed with MRI and SPECT imaging.

Body composition and energy expenditure in Duchenne muscolar distrophy: longitudinal study

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Background. Duchenne muscolar distrophy (DMD) is characterised by decreased fat-free mass (FFM) and increased fat mass (FM). Skeletal muscle metabolism is the major determinant of the resting energy expenditure (REE). A reduction of REE, according to the severe muscle loss, is hypothetical in DMD subjects but in the literature there are few and conflicting data regarding this relationship.
Objective. To provide longitudinal data about the natural evolution of body composition and REE in DMD and to investigate their relationship.
Methods. At baseline we studied 11 subjects with DMD median age 11 years (IQR: 9-13). They were divided in normal-weight and obese according to Italian BMI growth norms table. Only five patients were assessed at follow-up after 12 months. Body composition (FFM, FM, FFMI) was measured using DEXA; REE was assessed by indirect calorimetry; dietary energy intake was also investigated.
Results. At baseline in obese subjects mean FM% was significantly greater than in normal-weight (51.2; IQR:50.2-58.1vs39.1; IQR:29.7-46.4, $p = 0.014$). Also the FFM was greater in obeses. The REE values were smaller in normal weight

subjects (1325.5;IQR:1006-1467.5vs1633;IQR:1402-1683 kcal/day) but similar when adjusted for kg/FFM (50.5; IQR:48.1-57vs51.1; IQR:48.8-53.2). The primary longitudinal outcomes show a mean weight gain of 3 kg and a mean FM% increase; even the mean FFM significantly increase (26.2; IQR:20.4-31.6, kg p = 0.043). REE and REE/FFM mean values decreased. The caloric intake was stable respect to basal observation.

Discussion. In the obese patients FM was greater but also FFM values. This higher value of FFM in obese may be due to the difference in mean age between groups beside possible genetic determinants of body size. The REE was significantly lower than the value obtained from the literature in healthy children of the same age and it was significantly lower in the normal-weight children than in the obese subjects. The REE/FFM, nevertheless, was similar between the two groups, due to the higher values of FFM in the obese subjects. At the follow-up the significant increase of FFM is probably due to the influence of growth and of sexual hormones. Moreover we can suppose that the absence of significant changes in REE was secondary to the too short follow-up.

Conclusion. DMD patients suffer from progressive weight gain and increase fat mass but in young boys the hormonal pattern probably influence FFM and its decrease may be detected later.

MLASA syndrome: a new pathogenic mutation in the *PUS1* gene

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Myopathy-Lactic-Acidosis-Sideroblastic-Anemia (MLASA) syndrome is a rare autosomal recessive disease that involves skeletal muscle and erythroid cells. Mutations on *PUS1* and *YARS2* genes have been reported. Both genes are involved in post-transcriptional modifications of mitochondrial and cytoplasmic tRNAs. We studied a 33 years old female presenting since childhood a mild cognitive retardation and a severe sideroblastic anemia that required several blood transfusions. She developed hepatopathy, cardiomyopathy and insulin dependent diabetes. Slowly progressive and generalized muscle weakness appeared in adolescence and, at the age of 33, patient was unable to walk and showed a diffuse muscle hypotrophy with severe weakness that involves both proximal and distal muscles. Patient had normal CK, but elevated lactic acid. Brain MR showed cerebral atrophy with hyperintensity of the cortical-spinal tract and muscle MR a severe muscle atrophy with fibro-fatty infiltration. Muscle biopsy showed myopathic changes with ragged red fibers and a decrease of COX activity. *YARS2* gene study was normal, but two different mutations in the *PUS1* gene were identified: c.487delA and a novel c.884 G > A resulting in p.R295Q. This patient showed all MLASA stigmata but a classic phenotype is hard to define. Severity of cognitive, muscular and systemic involvement differ in all reported cases. Clinical and molecular findings of this patient widen genotype-phenotype spectrum in MLASA syndrome.

Toll-like receptors and innate immunity: new key players in the pathophysiology of oculopharyngeal muscular dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a late-onset muscle disease caused by short (GCN)11–17/polyalanine expansions within the polyadenylate-binding protein nuclear 1 gene (*PABPN1*). *PABPN1* plays a key role in the regulation of RNA metabolism, by modulating post-transcriptional processes including transcript stability, nuclear export and translation. Here we hypothesized that accumulation of the expanded mutant PABPN1 protein and the consequent impairment of protein homeostasis might represent an endogenous danger signal able to activate Toll-like receptors (TLRs) and innate immunity, promoting degenerative downstream processes in skeletal muscle. The analysis of mRNA transcript levels of TLR3, TLR4, TLR7 and TLR9, molecules involved in the recognition of endogenous and exogenous nucleic acids, and of the TLR-inducible cytokine interferon beta, showed their up-regulation in OPMD muscle samples compared to controls. By immunofluorescence we observed a highly positive staining for all the TLRs investigated, particularly for TLR4. TLRs were expressed on the sarcolemma or diffusely in the cytoplasm of some muscle fibers.

Overall, our findings suggest that TLRs might play a pathogenetic role in OPMD. These results might have important implications for new therapeutic approaches.

Cardiac imaging in emerinopathies: a cohort study

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The X-linked Emery Dreifuss muscular dystrophy is a clinical entity characterized by cardiac compromise, muscle weakness on a humero-peroneal distribution and contractures. We performed cardiac magnetic imaging studies on a small cohort of subjects carrying the same STA gene alteration. The cohort includes three affected males, whose cardiac compromise varies from mild to severe, and three females carriers. The pattern of heart alterations detected by heart magnetic imaging techniques is peculiar and sheds light on the likely pathophysiology mechanisms responsible for heart manifestations.

Progression of muscle histopathology but not of spliceopathy in myotonic dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is caused by a CCTG repeat within the first intron of the *CNBP* gene. Mutant transcripts are retained in cell nuclei and alter the functions of MBNL1 and CUGBP1 splicing factors leading to missplicing of several genes. To understand the molecular mechanisms that play a role in DM2 progression, the evolution of skeletal muscle histopathology and biomolecular findings have been studied in 5 DM2 patients who underwent two successive biopsies at different years of age. All DM2 patients examined show a worsening of muscle histopathology and an increase of MBNL1 sequestration and of CUGBP1 protein expression. The progressive worsening of myotonia in DM2 patients may be due to the decrease of CLCN1 mRNA observed in all patients examined. However, a worsening of alternative splicing alterations has not been evidenced overtime. These data indicate that DM2 is a slow progression disease since histological and biomolecular alterations observed in skeletal muscle are minimal even after 10-year interval. Muscle histopathological alterations evolve more rapidly than the molecular changes indicating that muscle biopsy is a more sensitive tool than biomolecular markers to asses disease progression at muscle level.

Heterogeneity of an Italian family affected with Adult Polyglucosan Body Disease

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Adult Polyglucosan Body Disease is a rare autosomal recessive leukodystrophy, affecting mainly Askenazi Jewish, due to mutations of glycogen branching enzyme gene (*GBE1*), leading to accumulation of polyglucosan bodies (PB) in central/peripheral nervous system.
We describe three affected siblings from a non-Jewish family. The proband presented distal paresthesia at the age of 55 years, followed by gait ataxia and urinary urgency. His nerve conduction study (NCS) demonstrated a sensory-motor demyelinating neuropathy. His sister developed paraparesis at the age of 52 years, complicated by neurogenic bladder. The youngest sister presented a transitory episode of orthostatic vomit and mild ataxia at the age of 53 years. In all cases, MRI showed diffuse hyperintense infra/supra-tentorial white matter abnormalities, with bulbar/spinal cord atrophy. In both sisters NCS was normal, whereas muscle biopsy showed non-specific alterations. In the proband, muscle/nerve biopsies showed PB, which prompted genetic investigation for *GBE1*: all siblings were compound heterozygous for c.1604A > G mutation, previously described, and the novel c.1064G > A.
In conclusion, common clinical signs occurred together with "atypical" ones (demyelinating neuropathy/transient symptoms), featuring a peculiar intrafamilial variability. Indeed, PB detection at muscle/nerve biopsy correlates with NCS alteration.

Prevalence of sub-sarcolemmal mitochondrial aggregates (SSMA) and age at biopsy in paediatric mitochondrial disease

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The diagnosis of mitochondrial disease (MtD) in children is challenging and muscle biopsies often lack ragged red and COX negative fibres. Instead, SSMA are thought to be more prominent and various %SSMA cut-offs (> 2%, ≤ 4%) have been proposed as markers of MtD.
By both by conventional light microscopy and a novel image analysis (IA) tool for precise quantitation, we aimed to assess the prevalence of %SSMA in muscle biopsies of patients with MtD (N = 31) and age-matched controls (CTRL) (N = 39) from 0-16 years and examine their relationship to the age at biopsy and mtDNA copy number.
We found that %SSMA was significantly lower in mtD (4%) versus CTRL (13%), it increased by 4%/year in the first 3 years of life and showed a positive correlation with mtDNA copy number. At multivariate analysis, disease group, age at biopsy and mtDNA copy number were significantly associated with %SSMA.
The lower prevalence of %SSMA in MtD and their correlation mtDNA copy number suggest that SSMA may be a good pathological marker of MtD in pediatric muscle biopsies. However, age-dependent prevalence of SSMA may limit using absolute SSMA cut-off for the diagnosis of MtD in children.

iPSC-derived neural stem cells act via kinase inhibition to exert neuroprotective effects in Spinal Muscular Atrophy with Respiratory Distress Type 1

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The aim of the study was to demonstrate that neural stem cells (NSCs) from human induced pluripotent stem cells (iPSCs) have therapeutic potential in the context of SMARD1 disease. Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an hereditary motor neuron disease caused by mutations in the IGHMBP2 gene, without a cure. We generated iPS cell lines derived from human skin fibroblasts with a

non-viral non integrating method based on the expression of reprogramming factors with episomal vectors. We differentiated iPSCs using a protocol to promote neuronal stem cells fate. The phenotype of these cells was analyzed by morphological, gene expression, and protein analysis. Finally, iPSC-purified NSCs were transplanted by intraspinal cord injection into nmd mice, an animal model of SMARD1. NSCs from iPSCs are self-renewing and multipotent and can differentiate in vitro into the three neuroectodermal lineage as well as in motor neurons. We show that upon transplantation NSCs can appropriately engraft and differentiate into the spinal cord of SMARD1 animals, ameliorating their phenotype, by protecting their endogenous motor neurons. To further evaluate the effect of NSCs in the context of human disease, we generated human SMARD1-iPSCs motor neurons that had a significantly reduced survival and axon length. Notably, the co-culture with NSCs ameliorate these disease features, an effect attributable to the production of neurotrophic factors and their dual inhibition of GSK-3 and HGK kinases. Our data support the role of iPSC as SMARD1 disease model and the translational potential of pluripotent cells for cell-mediated therapies in motor neuron disorders.

Critical illness myoneuropathy complicating akinetic crisis in parkinsonism

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Introduction. Akinetic Crisis (AC) is a life-threatening complication of parkinsonism characterized by an acute severe akinetic-hypertonic state, alterations of mental status, dysphagia, dysphonia, and dysautonomia with transient unresponsiveness to current treatment or to an increment of dopaminergic drug doses. Hyperthermia and muscle enzymes elevation frequently occur. It is frequently complicated by infections, pulmonary embolism, disseminated intravascular coagulation, and cardiac arrhythmias. Critical illness myopathy and/or neuropathy (CIMN), often occurring in intensive care settings, is primarily associated with sepsis, systemic inflammatory response syndrome, multi-organ failure, inactivity and steroid treatment.
Methods. To describe the occurrence of CIMN during the course of AC.
Results. Of 25 patients referred to our Clinic for AC in the last 3 years, three (12%) developed acute disappearance of hypertonia substituted by flaccid quadriparesis. Electrophysiological studies evidenced primary involvement of both muscle and nerve. In one patient myopathy was bioptically demonstrated.
Discussion. Although AC encompasses most of the putative risk factors for CIMN, its occurrence is difficult to recognize and was never reported. In AC patients CIMN should be suspected when hypertonia-rigidity subsides despite persistent akinesia and a bimodal pattern of CK and myoglobin increments occurs.

Pharmacogenetics of hNav1.4 sodium channel mutations causing myotonia

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Gain-of-function mutations of the Nav1.4 sodium channel are responsible for paramyotonia congenita or sodium channel myotonia. The sodium channel blocker mexiletine has received orphan drug designation in myotonia. Yet some patients show limited benefits from mexiletine due to side effects or lack of effects. We previously showed that the G1306E hNav1.4 mutant causing myotonia permanens is less sensitive to the drug in vitro, and that patients carrying G1306E can gain benefits by shifting treatment to flecainide, another sodium channel blocker, thereby opening the way toward a bench-to-bedside pharmacogenetics strategy (Desaphy et al. Neurology 2001; J. Physiol. 2004; Eur. J. Clin. Pharmacol. 2012). Here we report the case of a girl with severe myotonia associated to the new P1158L hNav1.4 mutation. The patient obtained unsatisfactory response to mexiletine and shifted treatment to flecainide with some success. Using patch-clamp, we found alteration of P1158L channel fast inactivation that can explain the myotonic phenotype. The P1158L currents were less sensitive to mexiletine compared to wild-type, while sensitivity to flecainide was not altered. This study supports our hypothesis of pharmacogenetics strategy, which we propose to extend to a larger number of sodium channel myotonias. Supported by Telethon-Italy (GGP10101).

Pilot study of serial casting of ankles in muscular dystrophy patients

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Contractures of Achilles tendons (TAs) deteriorate the performance in daily living activities of patients with neuromuscular diseases. Nocturnal use of ankle-foot orthoses (AFOs) helps to prevent the progression of deformities and to obtain optimal position of the joint. In clinical practice ankle serial casting is used to reduce TA contractures and to allow an improving in AFOs fitting, however only scanty reports focused on these aspects. The aim of this work was to assess the effect of TAs' serial casting on: 1) patient's perspective (using a self reported questionnaire), 2) joint physical examination (range of Motion (ROM)) and 3) functional performances (six minute walking test (6MWT)) in ambulant patients affected by Duchenne muscular dystrophy (DMD) and limb gird muscular dystrophy (LGMD) 2A. The protocol included three casting five days apart and was proposed to ambulant patients with contractures < 35°. After the cast removal on day 5 and 10, the new cast was reapplied with the TA on a stretch. We included 12 patients (10 DMD, age range: 4-12 yrs, 2 LGMD 2A). Our results showed in

all patients a significant improvement of ROM of ankles, in ten out of twelve patients a reported improvement of mobility and autonomy (questionnaire). Only the youngest patients had an improvement at 6MWT. The procedure has been well tolerated by all patients, no adverse events have been reported during the procedures. All patients received indication of daily stretching of TA and use of AFOs after treatment. Although further studies will be required to evaluate the effect of this procedure in a larger cohort, our results suggest that serial casting may be a valid alternative to surgery, avoiding therefore the needed immobilization.

Symptomatic heterozygosity due to definite GAA mutations, in late onset Pompe disease

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Genotype and phenotype are reported of 14 members of the third generation of a late-onset Pompe disease family which counts 36 individuals. Clinic, laboratories and GAA enzymatic and genetic studies disclosed widespread myalgias and low back pain as well as mild weakness of pelvic girdle muscles in 5 individuals (3F, 2M; aged 24 to 30 years) 3 of whom had slight increase of CPK. Symptoms onset was during the second decade of life. GAA enzyme activity ranged 2-4µmol/h/L in all patients. Direct sequencing of the GAA gene carrying the mutations already identified in their parents, disclosed the R40X mutation in the 5 symptomatic individuals whereas the splicing mutation c.2647-7G > A was found in the remaining 9 who did not show any symptom of neuromuscular disease.
Although the Pompe phenotype is by definition due to two mutations in the GAA gene, rare symptomatic heterozygous have been reported. The most relevant findings of our study are the identification of several symptomatic heterozygous in the same family who all share the identical GAA mutation. Thus suggesting that specific deleterious mutations even in heterozygous may address the prognosis. These symptomatic carriers, represent a unique model to identify factors modifying the phenotype.

Lipodistrophy and progeroid features associated with mutation of DNA polymerase δ (POLD1)

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Mandibular hypoplasia, Deafness and Progeroid features with concomitant lipodystrophy and insuline resistance, define a multisystem disorder named MDP syndrome. MDP has been recently associated to heterozygous microdeletion in POLD1 gene affecting the active site of DNA polymerase δ.
Here we report a case of a 13-yr-old girl, second-born of non consanguineous parents, who showed retarded growth, anemia and xerotic skin at age 1. Other early clinical features included sensorineural deafness at age 8, and a notable subcutaneous fat loss from her extremities with accumulation at abdomen level and insuline-resistance. At last neurological examination she presented generalized hypotonia and severe muscular wasting with joint contractures. She also has facial dismorfisms including, micrognathia, low-set and small ears. A muscle biopsy performed to exlude a congenital myopathy was normal. Molecular analysis of genes responsible for laminopathies and lipodystrophy such as LMNA and BSCL2 excluded pathogenetic mutations.
We analyzed the POLD1 gene and identified the heterozygous in frame deletion (c.1812_1814delCTC, p.S605del), previously detected in the three patients with MDP syndrome.
The case underlines the genetic heterogeneity of patients with progeroid features and lipodistrohy and the overallapping clinical presentation with congenital myopathy.

Exploring mitochondrial dysfunction in *CAPN3* related myopathy

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Limb-girdle muscular dystrophy type 2A (LGMD2A) is the most frequent form of recessive LGMD worldwide and it is caused by a defect of calpain-3 gene. Calpain-3 is a muscle specific, calcium dependent, multi-substrate cysteine protease. The exact pathomechanism underlying muscle damage due to calpain-3 deficiency, remains largely unclear. Animal model (CAPN3 KO mice) exhibits morphological and biochemical evidence of mitochondrial abnormalities in muscle, including irregular distribution of mitochondria and reduced in vivo mitochondrial ATP production. These findings have never been reproduced nor quantified in patients' tissue.
In this work we investigated pathological effects of calpain-3 mutations in myoblasts from LGMD2A patients, in terms of a putative mitochondrial dysfunction. In particular we examined the activity and amount of respiratory chain (RC) enzymes, cellular ATP level and ROS production.
Routine histochemical stains for oxidative metabolism in muscle biopsies revealed the typical aspect of subsarcolemmal accumulation of mitochondria. Measurement of RC enzymes revealed reduction of complex I and IV in one case and of complex III in another case, whereas the immunodetection pattern of the RC complexes was within normal values.
Luminometric measurement of ATP in patient's cultured myoblasts showed a specific reduction of ATP content compared to control cells in the presence of 2-deoxyglucose and pyruvate, a condition that supports only mitochondrial ATP synthesis.
We also observed a statistically significant increase of ROS production in patients fibroblasts after a short term H2O2 treatment.

Taken together these data support evidence for a secondary mitochondrial damage with energy production defect and increased oxidative stress also in human calpainopathy.

Novel mutations in CHKB gene in the first Italian case presenting with mental retardation, congenital deafness, seizures and muscular dystrophy

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CHKB gene encodes the choline kinases B, an enzyme that catalyze phosphorylation of choline by ATP, the first step of the enzymatic pathway for biosynthesis of phosphatidylcholine.
Homozygous or compound heterozygous mutations in the CHKB gene have been recently associated with a form of muscular dystrophy characterized by early-onset muscle weakness and mental retardation. Interesting muscle biopsy shows peculiar enlarged mitochondria that are prevalent toward the periphery of the fibres.
We describe a case from Italy presenting at birth with hypotonia and congenital deafness in which we have identified two novel mutations in the CHKB gene (c.140_146del p.Arg47Pro fs*21 in exon 1 and c.1066_1067delTG p.Trp356Val fs*72 in exon 11). The patient now age 9 is mentally retarded with particular impairment of speech. She also suffers of epileptic seizures since age 6. Brain imaging showed no structural change. At last neurological examination she presented proximal weakness and wasting of limb muscles, waddling gait and hyperlordosis. Hypertricosis was also present. Muscle biopsy was consistent with previous CHKB cases, showing necrotic and regenerating fibers, endomysial fibrosis, and abnormal COX staining with reduction at the centre of the fibres and abnormally large mitocondria at the periphery of the fibres.
Our case confirms the effect of CHKB defect in human pathology and extends the clinical phenotype.

Myotonic Dystrophies in a National Italian Registry: IRCCS Policlinico San Donato - start up

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Patients' enrolment in the National Registry for DM began on November 30th, 2013 at the Coordinator Center, IRCCS Policlinico San Donato.
Patients' compliance was adequate, they were highly willing to take part in the project and to undergo clinical evaluations. Website accessibility was good and easy and data entry was simple and clear. To date, we have enrolled a total of 80 patients, 70 of whom completed the data collection sheets with data entry required; all the data were validated. Thirty-one medical sheets were completed and validated too. The data collected so far have been analyzed and processed, highlighting results in line with the literature. The oncoming involvement of several other italian Neuromuscular Centers in data collection will permit to join a greater number of patients.

A *DOK-7* myasthenic syndrome: a treatable disorder?

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Mutations in *DOK-7* gene are responsible for postsynaptic Congenital Myasthenic Syndromes (CMS). In *DOK-7* related CMS cholinesterase inhibitors treatment is usually not effective But salbutamol, a selective β2 adrenergic agonist, has been associated with improvement. We studied a 32 years old male presenting since childhood with diffuse muscle hypotrophy and weakness, ligamentous hyperlaxity, kyphoscoliosis, ptosis, faccial weakness and arched palat. He showed hypotonia and cianosis at birth, and laryngospasm and asphyxia episodes during the first year of life.
Muscle weakness did not progress, but few day-long symptoms worsening was reported, occasionally related to infections. Laboratory findings were normal, including thyroid function test, CK, anti-AchR and anti-Musk antibodies. Brain MR, ECG and echocardiogram were negative. Respiratory function tests showed severe restriction. Electromyography revealed myopathic changes and a decreasing action potential amplitude at repetitive nerve stimulation test. Patient underwent two muscle biopsies: at age13 and 32. Both, showed polydimensional fibres and central hyporeactive cores at oxidative reactions.
Diagnosis of CMS was confirmed by detection of heterozygous *DOK-7* gene mutations: 1124_1127 dupTGCC and c.480C > A (p.Y160X). After diagnosis salbutamol treatment was initiated with significant clinical improvement: patient was able to climb stairs without support and walk unassisted. The exact mode of action of salbutamol in DOK7 CMS is unknown; however skeletal muscle β2-receptor stimulation may results in numerous effects on muscle function including muscle hypertrophy and transition of slow to fast fiber type.

Heart arrythmia in genetically confirmed facioscapulohumeral muscular dystrophy

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Background. Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy with prevalence

for 1:20000. Clinically significant cardiac disease is uncommon in FSHD but the heterogeneous clinical observations reported to date have shown that the heart alterations are possible in FSHD.
Patients and methods. 85 FSHD patients, aged between 14-80 years, attend our Institute. The diagnosis of FSHD was made on a clinical basis and was confirmed by genetic tests. 58 of these undergo annual clinical heart examination, 12-lead electrocardiography, 24 hour Holter monitoring and echocardiography.
Results. 9 patients (15%) had conduction defects or arrhythmia, in the absence of cardiovascular risk factor and with normal left ventricular function (3 cases with juvenile onset disease).
Supraventricular arrhythmias were detected in 5 cases. The AV node conduction was abnormal in 3 patient and infranodal conduction was abnormal in one. These heart abnormalities were symptomatic in 3 patients (palpitations associated with supraventricular arrhythmia). Two cases needed antiarrhythmic drug therapy. In addition, 6 patients (10%) manifested minor ECG abnormalities.
Conclusions. Data confirm the evidence of cardiac involvement in FSHD (in agreement with literature data of prevalence around 5-27%) and suggest regular cardiac follow-up in FSHD. Therefore, results support the hypothesis of possible conduction degeneration in FSHD and suggest a cardiac longitudinal study.

'Dropped head' in a case of recessive Oculopharyngeal Muscular Dystrophy: description of an unusual clinical phenotype

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Recessive cases of OPMD are rarely reported. We report a case of a 69-years-old woman admitted to our Department for progressive head ptosis and dysphagia begun two years before. She and her two sisters received the diagnosis of schizophrenia during youth. No other symptoms had been reported in the other members of her family. Neurological examination, other than severe weakness of neck extension with dropped-head and dysphagia, showed mild weakness of orbicularis oculi and proximal limb muscles. Electrophysiological assessment showed myopathic changes in weak muscles and neuromuscular transmission disorders were ruled out. CK levels were mildly elevated (350 UI/L). Anti-AChR and anti-MuSK antibodies were negative. Neck MRI showed cervical paraspinous muscles myopathic changes. Deltoid muscle biopsy showed increased variability in fibers size with some small angular dark stained fibers on NADH, with predominance of type I fibers and the presence of rimmed vacuoles in many fibers. No inflammatory infiltrates and MHC-I overexpression was found. Molecular study for Oculopharyngeal Muscular Dystrophy (OPMD) was performed and an homozygous expansion of GCN11 repeats in the PAPBN1 gene was found.
The elective involvement of the extensor muscles of the neck with "dropped head" is an unusual phenotype for OPMD. Probably, clinical phenotypes of recessive cases of OPMD are more heterogeneous than dominant ones and muscle biopsy findings. Muscle biopsy can be helpful to identify patients without familiar history who should be genetically tested for OPMD.

'Cap myopathy' in a family with unusual clinical phenotype

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The "Cap Myopathies" are rare congenital myopathies characterized by the presence of structures peripherally located in the muscle fibers, mainly composed of material containing thin and thick filaments, fragments of Z lines and cellular debris. We report the clinical and morphological features of the muscle biopsies from two patients (mother and daughter, P1 and P2 respectively) with an unusual clinical phenotype. Both patients showed a complex clinical syndrome concerning 1) signs of congenital myopathy, characterized by the delay in the motor milestones, exercise intolerance and mild weakness in the lower limbs, 2) dysmorphic features with retractions (short neck, micrognathia, retraction of the fingers) and 3) the pseudo-paralytic episodes, most evident in P2. In addition, the mother (P1) showed cardiac rhythm disturbances requiring a pacemaker implantation at the age of 56.
Muscle biopsies performed at the age of 26 years (P1) and 59 years (P2) showed the presence of areas sousarcolemmali evocative of "Caps" structures, variability of the of the fiber size and a mild predominance of fibers type1. Ultrastructural analysis confirmed the presence of peripheral, well demarked, subsarcolemmal areas, corresponding to the type of "Caps".
None mutation of known genes associated with the "Cap Myopathies" (TPM2, TPM3, ACTA1) and Anderser - Tawil Syndrome (KCNJ2) has been found. For these patients an exome sequencing study is now in progress.

P2X antagonist oxidase-ATP (oATP) treatment in alpha-sarcoglycan null mice

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Limb-girdle muscular dystrophy 2D (LGMD2D), caused by mutations in the gene encoding alpha-sarcoglycan (a-SG), is a rare disorder characterized by progressive weakness and degeneration of skeletal muscle. Pathological features of muscle biopsies from these patients show myofiber degeneration and necrosis, endomysial fibrosis, and reactive inflammatory response. In this scenario, extracellular ATP (eATP) molecules released from the cytosol of dying cells, contribute to the initial phase of the immune response

and later to the amplification of the inflammasome reaction. Intriguingly, a-SG binds eATP and displays an ecto-ATPase activity, thus controlling eATP concentration at the surface of cells expressing P2 receptors, attenuating the magnitude and/or the duration of eATP-induced signals. alpha-SG deficiency leads to membrane instability and decreased ecto-ATPase activity causing increased eATP concentration. Excessive eATP causes protracted P2X7 activation with alteration in muscle intracellular calcium homeostasis as well as recruitment of inflammatory cells.
We performed a systemic treatment with P2X antagonist oxidase-ATP (oATP) in mice deficient in α-sarcoglycan (Sgca-null mice), a model for human LGMD2D, and we analyzed the clinical and histological parameters of muscle disease progression. We observed an improvement in the forelimb muscular strength and in the diaphragm muscle morphology with a reduction of the number and areas of the reactive inflammatory infiltrates.

Pharmacological purinergic antagonism improves muscle dystrophy in *mdx* mice

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Extracellular Adenosine-5'-Triphosphate (eATP) plays a crucial role in the priming of immune response and directly regulates calcium homeostasis in muscle cells.
Primary muscle cells express various eATP-purinergic (P2X) receptor subtypes; significant up-regulation of P2X7 occurs in skeletal muscle from *mdx* mice as well as in Duchenne Muscular Dystrophy patients.
We analyzed the consequences of P2X7 pharmacological inhibition in *mdx* mice through a systemic treatment with the irreversible P2X antagonist periodate oxidase-ATP (oATP).
Blockade of P2X receptors improved muscular function and morphology and enhanced myofiber regeneration in *mdx* mice. The beneficial effect exerted by purinergic blockade was associated with a reduction of the number and area of the inflammatory infiltrate, a decrease of muscle levels of Il1 and Il6, a decrease of muscle infiltrating CD3+ T cells with a parallel 2-fold increase of FOXP3, a marker of regulatory T (Treg) cells.
oATP inhibitory effect on innate and adaptive immunity translated into a decrease of the expression of the pro-fibrotic factors TGFβ and Connective Tissue Growth Factor (CTGF).
In conclusion, purinergic antagonism led to a functional and histological improvement of the dystrophic process bound to dystrophin deficiency. This effect was mediated by a double action on the inflammatory response: down-regulation of the innate inflammasome pathway and induction of Treg cell population.

MRI of scapular muscle involvement in facioscapulohumeral muscular dystrophy (FSHD)

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FSHD is one of most common muscular dystrophies, characterized by extreme variable degree of facial and scapular muscle involvement. Clinical and genetic determination may be sometimes difficult, as genetic analysis is not always definitive and other similar muscle disorders, as scapulo-peroneal syndromes, are still lacking of molecular characterization. Here we evaluated the MRI of scapular muscles in 30 patients with clinical and molecular diagnosis of FSHD, in order to determine any specific pattern of muscle involvement. A Philips 1.5T-scanner was used and two examiners blindly evaluated fourteen muscles per patient. Muscle fatty replacement and atrophy were measured by using a four-point semi-quantitative visual scale. We observed that the most frequently and fatty replaced muscles were trapezius and serratus anterior, followed by teres major and pectoralis major. Instead, sopraspinatus, infraspinatus and scalenus muscle were relatively spared. Asymmetric scapular muscle involvement was observed. Interestingly enlargement of brachial plexus nerve trunks was observed in 33% of patients. Scapular muscle MRI was very sensitive to detect the selective muscle involvement of FSHD patients and useful to assess the involvement of non-clinical testable muscles. MRI imaging may be considered a potential tool to differentiate FSHD from other muscular dystrophies to drive the molecular analysis.

Reference values of fat infiltration and residual muscle volume for morpho-functional predictive behaviour in Duchenne Muscular Dystrophy: a longitudinal MRI study

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Purposes. i) quantitative assessment of fat infiltration (FI) and residual muscle volume (volume of muscle index, VMI) in lower limbs of children with Duchenne Muscular Dystrophy (DMD) by Magnetic Resonance Imaging (MRI); ii) establishment of a morpho-functional relationship between MRI values and functional outcomes.
Materials and Methods. 26 children with DMD were longitudinally assessed by lower limb 3T MRI and functional tests (Gowers, 10-meter time, North Star, 6-minute walking test). 5 age-matched healthy controls were also examined. A total of 85 MRI studies were performed. T1 Signal Intensity Ratio (SIR) of muscle and nearby fat was used to quantify FI. Muscle volume was measured by applying thresholds on T1-weighted images, and results were normalized for the whole muscle volume to obtain a VMI.
Results. SIRs and VMIs were significantly different from control values, except for sartorius and gracilis. Age-related curves with percentile values were calculated for SIRs and VMIs. SIRs and VMIs significantly correlated with all clinical measures, and could reliably predict functional outcomes.
Conclusion. SIRs and VMIs are objective measures that can improve DMD staging. MRI-based curves display the multistep

muscle involvement and provide reference values of FI and residual muscle for both clinical and research settings.

Magnetic Resonance of the lower limbs in Duchenne Muscular Dystrophy: longitudinal semi-quantitative study of muscular degeneration

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Purpose. Semi-quantitative assessment of Duchenne Muscular Dystrophy (DMD) progression in lower limb muscles by Magnetic Resonance Imaging (MRI).
Materials and methods. 26 DMD patients were longitudinally studied with 3T MRI of the lower limbs (72 MRI studies with T1, T2, STIR, 3DTHRIVE sequences). Two neuroradiologists evaluated 44 muscles per study, visually scoring the presence/absence of fat infiltration (FI), atrophy, hypertrophy and oedema. The severity of FI was also scored (0-3).
Results. In 6/7-year-old patients, FI was mainly present on gluteus maximus/medius(100%), adductor magnus(86%), rectus femoris(42%) and biceps(42%). At the age of 8/9, FI worsened and involved vasti(68%), gastrocnemii(72%), soleus(50%), and peronei(54%). In 10-12 year-old patients FI was also present on gluteus minumus (62%), obturators(62%), semitendinosus(62%), sartorius(62%) and gracilis(25%). The most frequently affected muscles were also the most severely scored in terms of FI.
Atrophy was noted since the age of 8/9 on gluteus maximus(18%), quadriceps and biceps(18%), with sparing of gracilis(0%), sartorius(0%) and calf muscles, which were hypertrophic since the age of 6/7(42%). FI, atrophy and hypertrophy were always present and progressing, whereas oedema was inconstant over time and mostly appeared in hypertrophic muscles.
Conclusion. MRI displays a typical proximal-to-distal pattern of muscular involvement with sparing of gracilis and sartorius. This finding can be useful in DMD diagnosis and staging.

Neuromuscular diseases in pregnancy: multidisciplinary management of elective c-section in 7 women

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Background. Neuromuscular diseases can have a tremendous impact on pregnant women ad affect offspring. Patients and Healthcare providers need to be fully aware of the potential complications they and their babies may encounter.
Objective. We describe the multidisciplinary management of 7 women affected by different neuromuscular disorders (NMD) and the outcome through a multi-step and multi-professional organization.
Methods. 7 women (LGMD, n = 3, SMA type II, n = 1, ALS, n = 1, axonal polyneuropathy, n = 1) with variable degrees of disability and skeletal deformities were subjected to complete neurological, respiratory and cardiological assessments. Grandround discussion with gynaecologists and anaesthesiologist occurred in each case. Before the procedure all patients were subjected to respiratory training (air stacking, cough-assist machine and preventive NIV)
Results. Fetal assessment was normal in all but 1 patient, in whom a congenital cardiac problem was found. Elective Caesarean delivery and spinal anesthesia was possible in all but 1. Clinical outcome was positive for all mothers.
Conclusions. Appropriate multidisciplinary management of neuromuscular patients in childbearing age including family counselling, gestation and delivery in an adequate setting is mandatory. A Centre fully dedicated to NMD, such as NEMO, permits to provide individualized care plan, tailored to meet the specific needs of this new growing population.

Isolated camptocormia – a two cases report

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Camptocormia may be a manifestation of disorders of the CNS, such as in Parkinson disease (PD); more rarely it is due to myopathies or motor neuron disease. The morphological changes associated with camptocormia are still under debate and the pathophysiology is unknown also in PD in which myopathic changes have been observed. A muscular pathogenesis of isolated axial myopathy was found in rare cases of DM1, IBM, and nemaline myopathy.
Here, we describe two patients with muscular disease manifesting with isolated camptocormia.
The first patient, a 65 years old man, initially presented weakness of the truncal muscles reaching fully developed camptocormia in about 18 months. CTscans and MR imaging showed progressive para-spinal dorsal-lumbar muscles atrophy and adipose tissue substitution. Paraspinal muscle biopsy revealed neurogenic aspects with central cores like recently observed in bent spine syndrome described in families from North Europe with a defective Ryr gene.
The second patient, a 70 years old man suffering from neoplasm, presented weakness and mialgia of the axial muscles. Paraspinal muscle biopsy revealed a myositis. To date, only few cases of camptocormia secondary to idiopathic inflammatory myopathies have been described.
Our data emphasize the role of primary muscle disorder in the etiology of camptocormia and the need to consider these common myopathies as a cause of the paraspinal muscle weakness.

Stepping gait: when should ankle foot orthosis be prescribed? preliminary data in a cohort of patients with DM1

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Background. Patients with Myotonic Dystrophy type 1 (DM1) often complain of gait instability. This is often related to ankle dorsiflexor weakness and steppage. Ankle foot orthosis are thus prescribed.
Aims. To determine: (i) the frequency and severity of proximal and ankle dorsiflexor weakness in a cohort of DM1 patients complaining of gait instability; (ii) if foot-leg orthosis can improve gait pattern and reduce falls.
Methods. 26 patients with classical DM1 phenotype (mean age: 46.26 ± 12 were subjected to: Manual Muscle Strength Testing (modified MRC scale) and performed the 6 minute walking test (6MWT). Ankle foot orthosis (AFO) were applied if ankle dorsiflexor weakness was ≤ 4 MRC. The 6MWT was repeated and subjective assessment of efficacy was accounted for with self-reported measures. To obtain additional information on gait parameters we performed a quantitative spatio-temporal analysis using BTS Bioengineering (Milan) G-Walk.
Results. Ankle foot orthosis improve walking distance (0-20 meters) in a minority (1/3) of our cohort of patients. G-walk analysis confirms 6MWT results. Despite this, patients who accepted AFO have positive perspective of efficacy.
Conclusions. Detailed analysis of gait including assessment of vestibular and proprioception causes, central and cardiovascular causes is mandatory to target management and tailor physiotherapist treatment and care in patients with DM1.

FOR-DMD: Double-blind randomized trial to optimize steroid regime in Duchenne Muscular Dystrophy (DMD)

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The FOR DMD study ("Find the optimum corticosteroid regime for DMD") is open to recruitment. The study compares benefits and side effect profile of three commonly prescribed corticosteroid regimes: daily prednisolone, daily deflazacort and intermittent prednisolone (10 days on and 10 days off). The study aims to enrol 300 steroid-naïve DMD boys, between the age of 4 and 8 years to be followed according to the current care recommendations over three to five years.
The FOR DMD study will provide information to allow families and clinicians to make informed decision about dosage and regimens of corticosteroids with regard to efficacy and tolerability. By standardizing corticosteroid treatment and prevention of side effects, the FOR DMD study will provide a more stable and uniform baseline which will impact the assessment and success of future studies with new investigational therapies. Additionally, the FOR DMD study is a unique opportunity to further investigate phenotype-genotype correlations, bone health, behavioural issues and the correlation of functional change with quality of life.
The study set up has been a long process with extended delay in study site activation and recruitment. This has been largely due to issues related to funding, approval and contract agreements between different countries and sites. This has underlined the lack of harmonisation in the regulations for international academic studies between US and Europe and among different European countries which need to be urgently addressed to ensure timely development of translational research in this field.
Regardless, the FOR DMD study is currently open with 39 sites actively recruiting in 5 different countries: Italy (6 sites), UK (8 sites), Germany (5 sites), US (15 sites) and Canada (5 sites). 112 boys have been screened for the study and 80 have been already enrolled and are taking study medication. We aim to actively continue recruitment with the aim to reach the enrolment target within the first half on 2015.

Functional characterization of novel CLC-1 mutations associated to myotonia congenita from Italian families

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Myotonia congenita (MC) is caused by loss-of-function mutations in the skeletal muscle CLC-1 chloride channel leading to impaired muscle relaxation after forceful contraction (Desaphy et al. Exp Neurol, 2013). We chose to functionally characterize five novel mutations, selected among the largest cohort of Italian families reported to date, on the basis of the associated clinical phenotype and position in the protein 3D-structure (Brugnoni et al. J Hum Gen, 2013). The dominant mutations (F484L, L198P and L520P) are located outside the common hot-spot for dominant genetic variants, instead residing within the channel pore, whereas the recessive mutations (V640G and L628P) occur in the C-terminal domain close to the proposed ATP binding sites. Whole cell patch-clamp recordings from wild-type and mutant chloride channels expressed in HEK293 cells revealed a huge reduction of current amplitude for F484L, L198P and V640G. In addition the open probability for F484L and L198P is dramatically right shifted compared to wild-type, which likely contributes to impaired muscle repolarization. Further experiments are required to clarify the dominant inheritance for mutations located outside the dimer interface, to address the mechanism of ATP modulation, and to clarify genotype-phenotype correlations. Pharmacogenetics studies are in progress. This work was supported by the Italian Ministry of Health (Grant No. 1580433).

Modulation of neuronal nitric oxide synthase by the isoflavone genistein promotes muscle regeneration in mdx mice

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Genistein has been reported to have pro-proliferative effects, promoting G1/S cell phase transition through the induction of cyclin D1, anti-apoptotic properties and increases nNOS expression. Apoptosis and the exhaustion of muscle regenerative capacity are implicated in Duchenne muscular dystrophy (DMD) pathogenesis and therefore are relevant therapeutic targets. Dystrophin lack in DMD, causes neuronal nitric oxide synthase (nNOS) membrane delocalization which in turn promotes functional muscle ischemia, and exacerbates muscle injury during exercise. We tested whether genistein could have beneficial effects on morphological, biochemical and functional pattern in *mdx* mice. Five-week old *mdx* and wild type mice received for five weeks genistein (2 mg/kg i.p. daily) or vehicle. Genistein treatment: 1) reduced muscle necrosis and enhanced regeneration with an augmented number of myogenin-positive satellite cells and myonuclei; 2) increased cyclin D1 and nNOS expression; 3) showed an antiapoptotic effect, modulating the expression of BAX and Bcl-2; 4) increased forelimb strength (+ 31%, $p < 0.01$) and strength normalized to weight (+ 28%, $p < 0.01$). Our results suggest that this isoflavone might restore the altered nNOS expression and increase regeneration modulating cell-cycle and apoptosis. Further studies with longer time treatment or using different experimental approaches are needed to investigate the underlying mechanisms of such encouraging results.

New mutation in CHKB gene in two Italian patients with congenital muscular dystrophy and enlarged mitochondria

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Congenital Muscular Dystrophies (CMD) are a heterogeneous group of autosomal recessive disorders characterized by infancy onset of muscle weakness, CNS and variable heart involvement with dystrophic changes at the muscle biopsy. Recently CHKB (choline kinase B) gene, which participates to the biosynthesis of the major membrane phospholipid phosphatidylcholine, has been associated to congenital muscular dystrophy and cardiomyopathy in 15 patients. We describe two sisters from unrelated healthy parents presenting since the first month of life with psychomotor delay, cognitive impairment, ichthyosis, muscle weakness and hypotonia. The older sister died at 10 years because of a severe dilatative cardiomyopathy, while the 8 years old sister still do not present any heart involvement. Electron microscopy performed in patients' muscle biopsy and in the cardiac tissue of the died girl showed enlarged, peripheric mitochondria, associated with some dystrophic features; respiratory chain activity was normal. We identified a homozygous deletion delTTTG 565_568, p.L188frx7 in CHKB gene in both patients. We may hypotize that the anomalous biosynthesis of phosphatidylcholine could alterate the mitochondrial membrane potential, producing the peculiar morphological aspect of mitochondria in muscle and heart. In conclusion we describe the first Italian patients carrying a new mutation in CHKB gene.

Genetic analysis of non-dystrophic myotonias in Italian patients

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Non-dystrophic myotonias (NDMs) are a heterogeneous group of skeletal muscle diseases presenting myotonia as a main feature. NMDs are caused by mutations in *CLCN1* and *SCN4A* genes, coding for the chloride (ClC-1) and sodium ($Na_v1.4$) muscle channels respectively. NMDs comprise autosomal dominant (Thomsen) and recessive (Becker) myotonia congenita, autosomal dominant paramyotonia congenita and sodium channel myotonia.
We describe the genetic screening of 194 subjects screened at our laboratory in the last 7 years (2007-2014). We achieved a genetic diagnosis in 106 subjects (54.6%): 73 patients (68.6%) presented with *CLCN1* mutations, whereas 33 patients showed mutations in the *SCN4A* gene (31.4%, of which 33.3% paramyotonia congenita and 66.6% sodium channel myotonia). 34.2% of *CLCN1* patients had Thomsen myotonia, 54.8% Becker, and in 10.2% only one allele was determined. This screening revealed 24 new mutations increasing the knowledge of genetics of NDMs and confirming the need for tight collaboration between clinicians and geneticians in this expanding field.

20% subcutaneous immunoglobulin HIZENTRA® in inflammatory myopathies: the experience of Ancona

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Introduction. Polymyositis and Dermatomyositis are chronic immune-mediated systemic diseases which mainly affect skel-

etal muscle. The best know diagnostic criteria are the Bohan and Peter's criteria. Among immunomodulatory treatment options, the role of intravenous Ig (IVIg) is that of an add-on therapy. However, the use of IVIg is associated with high care cost (need of venous access, hospitalization) and poor quality of live for patients (adverse systemic effects, absence from work). The beneficial effect of SCIg administration (as add on treatment) in these inflammatory myopathies, due to a different way in modulating pathogenic immune response from IVIg, has been previously documented.

Material and Methods. We describe seven patients with a new onset or recently relapsed PM or DM in whom 20%SCIg (Hizentra®) has been add to glucocorticoid therapy using a standardized protocol of infusion consisting of the administration of a weekly dose of 0.2 g/kg/week of SCIg. Mean follow-up, after the treatment start, was 18 ± 3 months.

Results. In all patients an improvement of muscle strength, assessed by MRC score, and of Rankin modified scale, have been documented; a decrease of serum CK levels was also recorded. It had been also possible to decrease the daily glucocorticoid dose. Adverse systemic events or local reactions in the infusion sites have been not observed.

Conclusion. Hizentra ® is an effective and well tolerated add on treatment option in active inflammatory myopathies. It reduces the risk of adverse effects and improve patient's quality of life.

Muscle channelopathies in a large cohort of Italian patients

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Skeletal muscle channelopathies are rare diseases, including non-dystrophic myotonia and periodic paralysis, which are associated with a great inter- and intrafamilial phenotypic variability, making challenging genotype-phenotype correlations. Hence studies on large populations of patients are needed.

We included patients referred to Carlo Besta Neurological Institute molecular laboratory with a clinical diagnosis of periodic paralysis or nondystrophic myotonia and mutated in CLCN1, SCN4A, KCNJ2 or CACNA1S.

We investigated 301 patients, among which 195 (64.3%) patients mutated in CLCN1 gene, 74 (24.6%) in SCN4A, 28 (9.3%) in CACNA1S and 4 (1.3%) in KCNJ2. We found 22 novel mutations: 8 in CLCN1 gene, 13 in SCN4A and 1 in CACNA1S. All the mutations detected in SCN4A, CACNA1S and KCNJ2 genes were missense, except for an unreported 9-nucleotide deletion in SCN4A. On the contrary CLCN1mutations were missense in 131/195 (67.2%) patients and remaining cases showed nonsense, splice site or deletion mutations.

Our study confirms genetic heterogeneity of muscle channelopathies, although a relatively small number of mutations is responsible for most of the cases.

Sport therapy and nutritional supplementation in a case of Facioscapulohumeral dystrophy

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Introduction. We describe the effects of 6mo exercise therapy and nutritional supplementation in a 43-year-old woman severely affected by Facioscapulohumeral dystrophy (FSHD).

Methods. Resting energy expenditure and the VO2peak were calculated by indirect calorimetry and submaximal handbike incremental test. Maximum voluntary contraction (MVC) of elbow flexors, elbow extensors, shoulder abductors groups was calculated with a dynamometer. Body composition was analyzed with Bioelectrical Impedance Analysis (BIA).

Endurance training consisted in 10min up to 20min at 90% VO2peak with assisted handcycling equipment 2 times/wk. Strength training consisted in 2 series of 8 repetition for each district with elastic band (2times/week, green Thera-band®). Respiratory training consisted in isocapnic hyperpnea (2min, 4 times/wk, 50% vital capacity, 20/min). Supplements were: Branched Chain Amino Acid Mixture (BCAAem, BigOne Professional Dietetics, 0.1gr/kg/day) and creatine monohydrate (CreaATP Syform, 0.1gr/Kg/day) plus conjugated linoleic acid (CLA Syform, 2.4gr/day).

Results. An improvement was observed in terms of cardiorespiratory fitness and body composition. Additionally we observed steadiness of respiratory volumes.

Conclusion. A mixed exercise program combined with nutritional supplementation can be safely used with beneficial effects in selected patients with FSHD.

The National Registry of Limb Girdle Muscular Dystrophy: clinical and molecular characterization of a sample of 466 Italian patients

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Limb girdle muscular dystrophies (LGMD) are highly heterogeneous disorders characterized by predominant limb girdle weakness. Molecular analysis and clinical-genetic correlations are fundamental for genetic counselling, definition of natural history and insight into pathogenesis.
We collected detailed clinical, biochemical, histological and molecular data of 466 Italian LGMD patients, belonging to 8 neuromuscular Italian centres.
Among them 309 patients are molecularly defined, 111 (24%) are still un-diagnosed and 46 (10%) carry heterozygous mutations in genes determining autosomal recessive forms. Relative frequency was as follows: 5.5% LGMD1B, 11% LGMD1C, 25.2% LGMD2A, 27% LGMD2B, 9.2% LGMD2I, 9.1% LGMD2D, 6% LGMD2E, 4% LGMD2C, 2.1% LGMD2L, 0.3% LGMD2F, LGMD2R (0,3%) and LGMD2S (0,3%). Onset spans from the first decade to adulthood; LGMD2E being the most precocious (6.2 ± 5.3 years) and LGMD2L the latest (36.6 ± 7.1 years). Creatine-kinase values were generally increased, especially in sarcoglycanopathies, LGMD2B, LGMD1C. Cardiomyopathy was more frequent in LGMD1B (100%), LGMD2E (47%) and LGMD2I (50%) and restrictive pulmonary involvement in LGMD2I (53%) and LGMD2E (47%). 30% of patients was wheelchair-bound.
Overall this study defined the relative frequency of Italian LGMD and improved the knowledge about clinical, morphological and molecular spectrum as far as their natural history. Furthermore the study of undiagnosed patients will potentially lead to identification of new LGMD causative genes.

ISPD mutations account for a small proportion of Italian Limb Girdle Muscular Dystrophy cases

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Isoprenoid synthase domain containing (ISPD) gene mutations are a recently described cause of Limb Girdle Muscular Dystrophy (LGMD) associated with defects in α-dystroglycan (α-DG) glycosylation. Since now few cases have been described and the frequency in Italian population is unknown.
We studied α-DG expression and ISPD gene mutations in 44 undiagnosed patients selected from a cohort of 180 Italian LGMD patients, which included 9 LGMD2I subjects showing α-DG reduction.
At immunohistochemical (IHC) analysis, performed in 32/44 undiagnosed probands, 29 subjects had normal α -DG expression, 2 partial deficiency, 1 complete absence. ISPD direct sequencing, performed in patients showing α -DG reduction, revealed in the last subject two novel heterozygous mutations: c.836-5T > G (leading to Exon6 deletion with production of an out-of-frame transcript) and c.676T > C (p.Tyr226His). This 43 years-old man presented sural hypertrophy and tiptoes walking at the age of 6 years and developed proximal progressive weakness at the age of 30 years, associated to severe respiratory insufficiency.
Overall ISPD mutations are a rare cause of LGMD in our population accounting for 0.7% of the entire cohort (FKRP mutations represent 5%). However, considering the increasing number of genes involved in α-DG glycosylation, α-DG IHC should be always performed in un-diagnosed LGMD, in order to detect reduction to be further investigated.

Mitochondrial DNA single deletion and related phenotypes: data of the Italian Network of Mitochondrial Diseases

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The Italian Network of Mitochondrial Diseases

Mitochondrial DNA (mtDNA) single deletion is one of the major causes of mitochondrial disease. It is commonly associated with progressive external ophthalmoplegia (PEO), Kearns-Sayre syndrome (PEO associated to pigmentary retinopathy and cardiac conduction block), and Pearson syndrome (pediatric refractory sideroblastic anemia associated with pancreatic insufficiency), but its variability is incompletely understood. Aim of this study is to revise the phenotypic spectrum associated with mtDNA single deletion in more than 150 Italian patients, by a retrospective, database-based study ("Nation-wide Italian collaborative network of mitochondrial diseases"). The great majority of our patients had a PEO phenotype (≈ 70%), whereas KSS (≈ 15%) and Pearson syndrome (≈ 5%) were rarer. The remaining patients had non-specific (encephalo)myopathic clinical pictures. Furthermore, our results showed higher clinical heterogeneity than commonly thought, with the presence, in several cases, of cardiomyopathy, neuropathy, migraine, tremor, dementia, psychiatric disorders and/or other clinical features.

Prevalence of congenital muscular dystrophy in Italy: a population study

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Congenital muscular dystrophies (CMD) are rare diseases and small patient number represents the major impediment to progress in research and care. Classically the term CMD includes a group of genetically, clinically and biochemically distinct entities sharing clinical and pathological features such as early presentation of weakness and hypotonia and dystrophic features on muscle biopsy. In the last years the identification of several new genes responsible for different forms of CMD has not only expanded the spectrum of the known forms of CMD, but has produced exciting progresses in the understanding of the mechanisms underlying this group of disorders.
The really incidence and prevalence of CMD in populations is not sufficiently known.
The aim of this study is to establish the prevalence of CMD in Italy.

Our inclusion criteria were all registered patients with a diagnosis of CMD currently seen in the participating Centers.
The diagnosis of CMD was based on the following criteria: dystrophic or severe myopathic features on muscle biopsy and early presentation of weakness and hypotonia.
Preliminary results show 346 cases of CMD in Italy: 42.77% dystroglycanopathies, 22.83% laminin α2 deficient, 19.36% collagen VI deficient, 5.7% laminopathies, others 6%.
As the study includes all the Italian tertiary care centers for pediatric neuromuscular disorders, we can presume that our findings will provide an estimate of at least all the cases of CMD in Italy.

Neuropsychiatric comorbidities in Duchenne Muscular Dystrophy

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Amongst boys with Duchenne muscular dystrophy (DMD), intelligence quotient (IQ) is on average 1 SD below the general population mean, with working memory impairments being especially prominent. We previously described in smaller cohorts that 23% of DMD boys present with clinically significant traits of Autistic Spectrum Disorder (ASD) and 35% of DMD boys meet the criteria for Attention-Deficit/Hyperactivity Disorder (ADHD). Other comorbid psychopathologies included internalising (27%) and externalising (15%) behavioural problems. The objectives of our study were to describe to what extent neuropsychiatric comorbidities coexist in the same individuals, and to describe the bearing that the genotype has on the neuropsychiatric phenotype in DMD. We recruited 135 DMD boys who underwent standardised neuropsychological assessments including: WISC-IV, 3Di, Conners-3 Questionnaires, and CBCL.
We demonstrated that intellectual disability (ID) and psychopathologies are highly prevalent in DMD, with overall 22% of boys scoring in the ASD range and 29% scoring in the mood disorder range. Different psychiatric symptoms coexist in the same individual: 50% of the boys with ADHD score in the ASD rage, and 75% of boys with ASD manifest ADHD. The presence of internalising and externalising behavioural problems significantly correlated with the diagnosis of ASD and ADHD, but not with ID. We observed a genotype effect: mutations downstream of exon 31 (disrupting Dp260, 140,Dp116 and Dp71) are at a higher risk of psychiatric comorbidities suffering from ID ($p = 0.001$) ASD ($p = 0.03$) and clustering of neuropsychiatric symptoms. These striking findings suggest the need for a systematic assessment of the neurobehavioural problems and targeted support in routine clinical care.

Homozygous IVS1-13T>G mutation in two patients with Late-Onset Pompe Disease (LOPD): a rare genotype

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Pompe disease is an autosomal recessive metabolic myopathy due to mutations in the acid alpha-glucosidase (GAA) gene causing deficiency of lysosomal alpha-glucosidase activity. To date, more than 400 pathogenic mutations havebeen reported in the literature. In the late-onset form (LOPD), the IVS1-13T > G is the most frequently mutation found in the Caucasian population, accounting for approximately 70% of cases. Thisis classified as a "potentially mild mutation", because it results in low levels of normal transcript. In large LOPD population studies, the majority of patients are compound heterozygous for IVS1-13 T > G with a second mutation on the other allele whereas patients homozygous for IVS1-13 T > G have been very rarely reported.
We describe herein two brothers affected by LOPD, carrying a homozygous IVS1-13T > G mutation.
The proband was a 51 years-old man, complaining of exercise-induced muscle cramps since at least 20 years. He showed macroglossia, postural hands and head tremor and a mild proximal muscle weakness. He showed hyperckemia (max 837 UI/L) and a moderate vacuolar myopathy with glycogen accumulation on muscle biopsy. Muscle GAA activity was severely reduced (13% of residual activity); diagnosis of LOPD was made and confirmed by GAA genetic analysis that documented the IVS1-13T > G mutation on both alleles. His brother, 42 years-old, carried an asymptomatic hyperckemia (630 UI/L), a mild postural tremor at the hands. DBS for GAA activity showed a clear reduction (0.179 umol/h/L) and genetic analysis resulted in the same genotype as his brother.
Although GAA low levels, our data showed that homozygous IVS1-13T > G was associated with a mild LOPD phenotype in our patients. Those findings are somewhat in contrast with few other cases described in the literature where this genotype is associated with a more severe phenotype.

Early diagnosis and early treatment in LOPD: when asymptomatic patients should be treated

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Pompe disease is a lysosomal disorder caused by GAA deficiency. LOPD is characterized by progressive muscle weakness and/or respiratory failure but, sometimes, only by an asymptomatic hyperCKemia. Being a muscle degenerative disorder, it has been suggested that an early diagnosis could be more useful for a timely ERT start. According to the current treatment guidelines, ERT is recommended for patients who have symptoms or signs of Pompe diseas and in presymptomatic patients who have detectable proximal weakness or reduction in respiratory parameters. In a recent high risk population study, involving several Neuromuscular Italian Centers, we were able to diagnose 17 new LOPD patients.
Among those patients, 35% showed an asymptomatic hyperCKemia, 59% hyperCKemia and limb girdle muscle weakness (LGMW) whereas 6% manifested only LGMW. In these patients, the median time from the onset of symptoms/signs to the diagnosis was 7.7 years. ERT has been initiated in 11 patients. 8 out of the 11 showed LGMW with hyperckemia and two of them also had severe respiratory involvement. The last 3 only had hyperCKemia without any symptoms. Despite the presymptomatic condition, muscle morphology showed severe muscle damage and the muscle MRI revealed an adipose substitution in proximal muscles at lower limbs.
Conclusions: Of the 17 newly diagnoses Pompe patients, remarkably 35% of patients with only asymptomatic hyperCKemia were early identified but a combination of clinical and morphological data prompted us to start ERT early. To initiate ERT we suggest to consider, apart from the clinical symptoms, different parameters such as muscle MRI or muscle morphology to optimize the treatment efficacy.

RNA therapy for Spinal Muscular Atrophy by SMN increase or modulation of secondary cell death events

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The aim of our research is the development of RNA-based therapeutics to treat Spinal Muscular Atrophy (SMA) using a stem cell in vitro platform. SMA is a genetic motor neuron disorder caused by mutations of the survival motor neuron gene (SMN1). No effective treatment is available so far, but antisense therapy to increase the SMN level is a promising strategy. We obtained induced pluripotent stem cell (iPSC) lines reprogramming SMA/wild-type human fibroblasts with lentiviral constructs and with a non-viral non integrating method based on the expression of reprogramming factors with episomal vectors. We differentiated iPSCs using a protocol to promote motor neuronal commitment. The phenotype of these cells was analyzed by morphological, functional, gene expression, and protein analysis. RNA strategy based on antisense morpholino and shRNA aiming at increasing SMN level or inhibiting Fas activation were investigated. We demonstrate that SMA iPSC-motor neurons recapitulate the disease phenotype with a significantly fewer and smaller motor neurons at later time periods in culture compared to wild-type subject iPSC lines. These features were rescued in SMA motor neurons treated with antisense morpholino or U1 shRNA that increase SMN level. During motor neuron development, SMA lines showed an up-regulation of Fas ligand-mediated apoptosis and increased caspase-8 activation. Importantly, this could be mitigated by Fas silencing. Our data support the utility of SMA iPSCs as in vitro disease model, suggesting that RNA therapy can be a possible therapeutic strategy for SMA trough SMN up-regulation and modulation of disease pathways that can be achieved with different therapeutic tools.

Infantile Bilateral Striatal Necrosis: if not a mitochondrial disorder, what else?

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Infantile bilateral striatal necrosis (IBSN) is a unique and very distinctive neuroradiological phenotype characterized by a specific involvement of the striata, with initial swelling of the putamina and caudates followed by degeneration, leading ultimately to cellular necrosis.
IBSN has been associated with different genetic conditions, including mitochondrial disorders.
We report a child with neuroradiological findings compatible with IBSN referred to us with a suspect of mitochondrial disorder. Reduction in the activity of the respiratory chain complexes (in particular complex III) was documented on muscle biopsy.
A diagnosis of Aicardi–Goutières syndrome was made in this patient, subsequently confirmed with identification of two mutations in the ADAR1 (AGS6) gene.
We speculate that in our case mitochondrial dysfunction could be secondary to interferonopathy, the pathogenetic process of AGS and that the mitochondrial dysfunction could contribute to selective involvement of basal ganglia in AGS.
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36 month longitudinal data in ambulant boys with Duchenne muscular dystrophy

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The 6 minute walk test (6MWT) has been recently chosen as the primary outcome functional mobility, endurance, and ability to walk. Its use has been reported in international multicenter clinical trials in DMD ambulant patients.
The aim of the study was: to assess the spectrum of changes over 36 months in ambulant boys affected by Duchenne muscular dystrophy, to establish the difference between the first the second and the third year results, and to identify possible early markers of loss of ambulation.
Three children (3%) lost the ability to perform the test within 1 year, another 13 between year 1 and year 2 (14%) and other 11 between year 2 and year 3 (12%). The 6MWD showed an average overall decline of −15.8 meters in the first, and of −58.9 in the second year and -104.22 in the third year.
The changes were significantly different in the two baseline age groups: children below 7 remained on the average stable. Children above 7 had a decrease during the first, the second and the third year.
The changes were also significantly different according to steroid treatment.
These results can be of help at the time of using inclusion criteria for a study in ambulant patients in order to minimize the risk of patients who may lose ambulation within the time of the trial.

Assessment of Upper Limb function in DMD patients:12 month changes

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As a result of an international effort, a new tool, the Performance of Upper Limb (PUL) has recently designed to assess upper limb function in DMD boys. The purpose of the PUL is to assess changes that occurs in motor performance of the upper limb over time from when a boy is still ambulant to the time he loses all arm function when non-ambulant.
The test has proved to be reliable, suitable for multicentric studies with excellent inter and intra-observer reliability.
Cross-sectional results in 322 DMD patients (mean age 12.7; range 4.1-35.1), showed a progressive deterioration of scores with age, with early involvement of the proximal muscles that was more obvious after the age of 10 years. Even the oldest and weakest DMD patients were still able to perform some of the distal items, suggesting that the scale is capable of measuring small distal movements (lifting small weights, tracing a diagram) that are important as they relate to activities of daily living such as using a mobile or using a computer mouse.
Results. The results showed some variability I the 12 month changes.
Conclusion. The PUL Scale demonstrated to be a useful tool for upper limb motor disease assessment in DMD ambulant and non-ambulant patients, both for evaluation in clinical trials and for therapy follow-up.

Juvenile dermatomyositis: two case report

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The idiopathic inflammatory myopathies, including juvenile dermatomyositis (JDM) are rare systemic autoimmune diseases characterized by proximal muscle inflammation and weakness. JDM affects children younger than 18 years with skin and muscle features similar to adult DM and is characterized by histopathologic findings as perifascicular and perivascular inflammatory infiltrates. Diagnostic criteria include characteristic skin findings, muscle weakness, elevated muscle enzyme levels, and evidence of an endomisial, mononuclear inflammatory infiltrate on muscle biopsy. Those criteria are not ever obvious. Actually, we propose two children with increased CPK, muscle weakness, cramps, faticability, no serum inflammatory signs and skin involvement. Histopathology shows minimal perifascicular atrophy and scant endomisial mononuclear infiltrate. Electron Microscopy played a key role in the differential diagnosis showing Tubular Reticular Structures (TRI) in endothelial cells of muscular small vessels. TRI are considered an ultrastructural pathognomonic sign of DM and its presence could even anticipate skin involvement in these myopathies.

Serum sclerostin in myasthenia gravis: an index of bone fractures risk in glucocorticoids-induced osteoporosis. A protective role of pyridostigmine on bone metabolism

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Myasthenia gravis (MG) is a neuromuscular junction disease which has been associated to an increased risk of glucocorticoid-induced osteoporosis. Recently it has been reported that, in MG, the use of oral glucocorticoids is not associated with an increased risk of fractures, suggesting that pyridostigmine may have an anabolic effect on bone, mediated by its interaction with osteoblasts receptors. Sclerostin is a glycoprotein which inhibits osteoblasts and bone formation, binding to the co-receptor LRP5/6. We have compared DEXA scan, serum sclerostin expression and other bone remodeling markers (CTX, osteocalcin, alkaline phosphatase, calcium, phosphorus, vitamin D, parathyroid hormone, creatinine) in 24 AChR-related MG patients, exposed to a prolonged steroids and pyridostigmine treatment, versus 16 MuSK-reletaed MG patients exposed only to steroids. We have found that markers of osteoporosis are more evident in patients affected by MuSK-related MG treated with the same dosage of glucocorticoids, compared to those affected by AChR-related MG, who don't disclose the same severe pattern of osteoporosis. Furthermore, we found lower sclerostin levels in patients on pyridostigmine treatment, suggesting its protective role on bone metabolism. Further investigations must be performed to confirm, on

a large scale, the possible protective role of pyridostigmine against glucocorticoids-induced osteoporosis.

Bone mineral density and body composition in 39 duchenne muscular dystrophy patients: a two-years follow-up

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Individuals affected with Duchenne muscular dystrophy (DMD) show significantly altered whole-body composition compared with normal control population. We assessed for at least two years 39 DMD-patients to evaluate how their bone mass and body composition change during time.
All patients underwent clinical evaluation and dual-energy-X-ray-absorptiometry (DXA) assessment every six months. By DXA we obtained subtotal total body (without head) and spine bone mineral density (BMD), lean tissue mass (LTM) and body fat percentage (PFAT). We compared data using Wilcoxon tests. At baseline visit the average age was 7 ± 1.62 (range 4-11) years, 11 patients were already treated with steroids since a mean of 3 years, 17 started therapy after that visit and 4 about 1 year later. At baseline we found significant Spearman correlations between all four DXA parameters each other and with weight, height and BMI. We observed an increasing trend (with statistically significant differences) in all DXA parameters during the two-years follow-up, especially in PFAT. Comparing boys over 10 years old with others we found significant differences in all parameters. These results confirm usefulness of DXA as tool both for DMD follow up and for treatment adjustment. Correlation with clinical outcome measures will be presented.

Phenotypic characterization of DM1 individuals carrying premutation alleles

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Myotonic dystrophy type 1 (DM1) is caused by the expansion of an unstable CTG repeat. Larger alleles are associated with a more severe phenotype and an increase in length from one generation to the next, accounting for the clinical anticipation. DM1 premutation alleles – giving rise to new DM1 families – can be identified in distant relatives of DM1 probands, often asymptomatic.
To investigate the phenotype of DM1 individuals carrying premutation alleles, the clinical records of 218 individuals from 50 DM1 families identified at our Service, during the period 2002-2013 were re-evaluated and the following parameters analysed: CTG repeat length, presence of myotonic phenomenon, heart conduction defects, cataract and serum CK values.
A total of 27 subjects (16M;11F, current age range 23-73 years) were identified as premutated patients, sharing a CGT expansion ≤ 100 (mean 76 ± 12,7; range 45-100).
Cataract before 45 years was the most frequent observed symptom (59.2%), occurring more frequently in premutated DM1 males (62.5%) than in females (25%). Atrio-ventricular blocks were the second most frequent (37%) feature observed in these patients, followed by myotonic phenomenon (18.5%).
Ophthalmologists and cardiologists should explore the aetiology of cataract or the presence of AV block in their patients, as an early identification of DM1 premutation alleles, could avoid the transmission of larger alleles and more severe phenotypes in subsequent generations.

Oculopharyngeal muscular dystrophy genotypic and phenotypic features of 13 patients

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Oculopharyngeal muscular dystrophy (OPMD) is a late onset muscular disease, caused by a short GCG expansion in the PABP2 gene, usually dominantly inherited. The mean symptoms onset is after the fourty years, with progressive eyelids ptosis, dysphagia and proximal limbs weakness. We report the clinical and genetic characteristics of 13 patients (3 families), followed in our neuromuscular center since 1990. 6/13 patients showed ptosis as first symptom, mean age 47.5 years; 4/13 complained dysphagia, mean age 45.7; only one patient reported silmutaneous onset of ptosis and dysphagia at the age of 55 years and two patients, aged 49 and 42 years, were asymptomatic. Six patients showed mild proximal limbs weakness, four patients had progressive external ophthalmoparesis, one patient suffered from dysphonia. Four patients underwent to blepharoplasty, with stable improved vision in three of them. Undernourishment was reported in six patients, at a marked degree in two. In one of these patients upper esophageal sphincter myotomy resulted in increase of body weight. Muscle biopsy, performed in four patients, showed myopathic findings with rimmed vacuoles. This is the second larger serie of Italian OPMD patients (Mirabella M. et al, 2001), documenting: GCG6/GCG9 repeat mutation is the most frequent in the Italian population, it shows a meiotic stability within the same family, with a moderate phenotypic associated to this genotype.

sEMG descriptors of central and peripheral fatigue in a case of Facioscapulohumeral dystrophy

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Introduction. In Facioscapulohumeral muscular dystrophy (FSHD) fatigue is a precocious symptom but it is unknown the relative contribution of central and peripheral mechanisms to perceived fatigue and whether it may temporally precede the

appearance of detectable alterations of skeletal muscle by magnetic resonance imaging (MRI).
Aim. To examine the myoelectric manifestations of central (FD, fractal dimension) and peripheral (CV, conduction velocity) fatigue of a FSHD patient (Score 1, Lamperti 2010 *Muscle Nerve* 42, 213); to assess whether fatigue precedes or follows detectable muscle alterations by MRI.
Methods. sEMG signals were recorded in biceps brachii (BB) during isometric contractions at 20% and 60% of the maximum voluntary contraction (MVC) for 1 min and 20s respectively at 90 degrees knee joint angle. Initial values and rate of change (slope) of Mean Power Frequency (MNF), CV and FD were calculated. Total body muscle MRI was also obtained.
Results. MRI revealed no abnormalities in the BB bilaterally. In the right biceps brachii a change in CV slope was observed at 20% MVC (0.0014 vs 0.0021). This change was associated with higher initial value and positive MNF slope and equal initial value and unchanged FD slope. All estimated parameters displayed a significant change in the slope at 60% MVC.
Conclusion. Peripheral fatigue was observed during contraction at 20% MVC whereas peripheral and central fatigue and a progressive reduction of fibers recruitment were observed at higher output only in the right BB. Importantly these changes were observed in absence of phonotypical alterations detectable with MRI.

Muscle magnetic resonance imaging findings in two sisters carrying two new CLCN1 gene mutations

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Few data are available on muscle MRI findings in patients with non-dystrophyc myotonia (NDM). A recent study (Morrow JM et al, 2013) has shown MRI abnormalities in all out of 21 NDM patients, almost half of them showing at least mild extensive T1w changes and a "central stripe" of STIR hyperintensity within the medial gastrocnemius muscle, more frequently in patients (10/11) with CLCN1 myotonia (myotonia congenita).
We report two sisters aged 53 and 56 respectively, who developed myotonic symptoms in early infancy. Both presented with widespread muscle myotonia with limb muscle hypertrophy, the older one with slight proximal muscle weakness. Molecular genetic analysis of CLCN1 gene revealed two new mutations (p.Gly233Ser; p.Thr328Ala). Segregation studies confirmed that these mutations were in trans.
Muscle MRI study showed selective T1w changes at the level of the inferior limb, different at the level of the thigh from those described in literature so far, being the sartorius muscle the most selectively involved. The "central stripe" of STIR hyperintensity within the medial gastrocnemius muscle was also evident in one of them.
This report widens the spectrum of MRI appearances in myotonia congenita, so helping differentiating it form other myotonic dysorders.

New RYR1 mutations discovered by Next Generation Sequencing in Congenital Myopathy patients with different phenotypes

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Congenital Myopathies (CM) are clinical and genetic heterogeneous disorders of skeletal muscle. The clinical spectrum ranges from severe forms with congenital hypotonia, severe muscle weakness and early mortality, to milder forms characterized by slowly progressive weakness. There are different subtypes based on the pathological features: nemaline myopathies, myopathies with cores, myopathies with central nuclei, myopathies with fiber type disproportion. More than half of CM can be attributed to mutations in RYR1 gene that encodes the skeletal muscle ryanodine receptor. The use of Next Generation Sequencing (NGS) allowed us to diagnose in a faster way mutations in RYR1 gene in three CM families, presenting with a bioptical pattern suggestive for Core Myopathy and Fiber Type Disproportion. We identified 6 variants, 4 of them are new. In addition the analysis of a family with four affected members revealed a different clinical course associated to the same haplotype, characterized by the presence of two mutations, one linked to central core and the other associated to malignant hyperthermia. Overall NGS appears promising for the analysis of complex and heterogeneous pathologies like CMs.

Late onset ophtalmoplegia in a typical neonatal congenital myopathy

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Introduction. Congenital myopathies (CM) are a group of muscle disorders clinically characterized by hypotonia and weakness, usually from birth, a static or slowly progressive clinical course, with genetic and phenotype heterogeneity.
Case report. We report a case of a 31 years old female, suffering from a typical congenital myopathy. The father and the younger sister suffer respectively from an adult onset CM and a benign / asymptomatic CM. She presented at birth: generalized hypotonia, facial weakness, cleft palate, breathing difficulties requiring intubation and feeding difficulties requiring nasogastric tube. Only at 5 months of age, she could breath and feed autonomously. Ambulation was acquired at 2 yrs; scoliosis and pectus excavatum were observed early in childhood. Normal CK levels, myogenic signs at EMG, fiber type variability with type I fibers predominance and no irregularity of the oxidative enzymes stain on muscle biopsy completed the clinical feature. At 31 yrs she presents long face, mild facial and proximal lower limb weakness, hypotonia,

and external ophtalmoparesis. This last sign was firstly observed when she was around 22 yrs, and mild progression has been recorded during the years, with no other bulbar signs.
Conclusion. Ophtalmoplegia is frequently associated to severe form of CM and mostly observed since birth, even in cases with a mild skeletal myopathy; it is very rarely reported in adult onset CM. To our knowledges, our case is one of the few in which a mildly progressive ophtalmoparesis appears in young adulthood in a typical benign congenital myopathy.

Psychological benefits of caregiving in relatives of young people with muscular dystrophy

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This paper focuses on the psychological benefits of caregiving in key-relatives of patients with Duchenne, Becker, or Limb-Girdle Muscular Dystrophies (MD). The sample included 502 key-relatives of patients 4-25 years-old in charge for at least six months to one of the 8 participating centers, living with at least one relative 18-80 years-old. 88% of key-relatives stated that they had gotten something positive out of the situation, 96% considered their patients to be sensitive, and 94% viewed their patients as talented. Positive aspects of caregiving were more recognized by key-relatives more convinced that the patient was sensitive and who perceived they received higher level of professional help and psychological social support. These results suggest that most key-relatives consider their caregiving experience as a positive impact on their lives, despite the practical difficulties. Professionals should help relatives to identify the benefits of the caregiving without denying its difficulties.

Sodium channel related mytotonia: different phenotypes and revision of the literature

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Non-dystrophic myotonias are rare diseases caused by mutations in skeletal muscle chloride and sodium ion channels. Mutations of SCN4A encoding the skeletal muscle sodium channel Nav1.4 cause several types of disease, the sodium channel myotonias, including paramyotonia congenita, potassium aggravated myotonia, myotonia fluctuans. Common symptoms are muscle stiffness, transitory weakness, fatigue and pain. Herein, we describe clinical features, neurophysiological and molecular data of five families affected by sodium channel myotonias followed by our Centre: three patients, belonging to two different families, are affected by paramyotonia congenita (c.4367G > A; c.2095G > A); two patients, belonging to the same family, are affected by potassium aggravated myotonia (p. Ala699Thr); two patients, belonging to the same family, are affected by myotonia fluctuans (p. Gly1306Ala); one patient is affected by paramyotonia congenita, with the very rare neonatal onset form, which include the severe neonatal episodic laryngospasm (SNEL) (p. Gly1306Glu). We confirm the clinical variability of these patients and the importance of a correct neurophysiological approach, according to Fournier's guidelines, to guide the molecular analysis. Furthermore, we found a novel mutation responsible for paramyotonia congenita, which need to be biophysically studied, considering that it is responsible for very different clinical phenotypes within the same family. Recognizing patients with channelopathies and confirming this diagnosis is important, as treatment and management strategies differ based on mutation and clinical phenotype.

Familial disto-proximal mitochondrial myopathy with inflammation: a new phenotype in search for a gene

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Patients. Three brothers (two females and one male) were affected by adult-onset myopathy with disto-proximal progression characterized by severe involvement of distal muscles of lower and upper limbs and less pronounced weakness of limb girdle muscles; they had also cardiac arrhythmia and long QT syndrome (LGTS) that required Implantable Cardioverter Defibrillator in one patient. Congenital strabismus was present in all three brothers.
Methods and results. Muscle biopsies were performed in two patients showing a chronic myopathy with evident mitochondrial proliferation and presence of necrosis, myophagia, regenerating fibers, small lympho-mononuclerar perivascular infiltrates and strong expression of MHC1 in 80% of the fibers. Immunocytochemistry, beside a diffuse immunopositive desmin reaction in regenerating fibers, didn't revealed other proteins aggregates. Interestingly, heart biopsy also showed both mitochondria and inflammation. A therapy with IVIg and steroids induced a partial, but clear improvement of muscle weakness. Mitochondrial enzymes activities and analysis for mitochondrial DNA and LQTS genes were normal. Whole exome sequencing is underway, but all nuclear-encoding genes for mitochondrial proteins were excluded.
Conclusions. We report a family with a distinctive autosomal recessive myopathy and LQTS; the association of mitochondrial abnormalities and inflammation is puzzlingly but consistent, bolstering the emerging debate on the role of mitochondria as a player in the innate immune response in specific disorders.

Novel SPG11 mutation in a case of HSP/ALS overlap phenotype

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Autosomal recessive hereditary spastic paraplegias with thin corpus callosum (AR-HSP-TCC) represent the most common form of complicated hereditary spastic paraplegias (HSP). Autosomal recessive juvenile ALS is a rare form of genetic ALS occurring before age of 25. Although such diseases are genetically heterogeneous, mutations in SPG11 have been reported in both of them. We report the case of a female patient, which presented at the age of 18 years with progressive weakness and spasticity affecting the lower limbs. The neurological examination revealed paraparetic gait associated with bilateral steppage and pyramidal signs.
EMG showed active and chronic partial denervation in all limb muscles and a axonal/demyelinating sensorimotor peripheral neuropathy. Muscle biopsy confirmed a pattern of chronic neurogenic denervation. Motor evoked potentials pointed to an increased central conduction time. Brain MRI showed diffuse white matter abnormalities and thinning of the corpus callosum. Direct sequencing of SPG11 gene identified a novel frameshift mutation, a c.7081insT variant in compound heterozygosis with an already known c.733_734delAT mutation.
Even though a diagnosis of AR-HSP-TCC was initially made, some clinical findings could suggest a juvenile ALS. It thus appears that the lines between ALS and HSP phenotypes are blurred. Given the need for a correct nosologic categorization, strong criteria to differentiate ALS from HSP phenotype linked to SPG11 are warranted.

Reliability and validity of the nine hole peg test in myotonic dystrophy type 1

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Background. Myotonic dystrophy type 1 (DM1) is a multisystemic inherited disorder. Distal weakness is one of the main features of the disease, leading to a manual skill impairment. The Nine Hole Peg Test (NHPT) is a timed test of fine manual dexterity validated in other neurological diseases. The objective of this study is to evaluate the reliability and validity of NPHT in DM1 patients.
Methods. NPHT was administered to 42 DM1 patients and 28 age-matched healthy controls. Both DM1 and controls were retested within one week from the first test. Handgrip and pinch strength were measured in DM1 using a Handheld Dynamometer. Differences in NHPT values between DM1 and controls were evaluated by unpaired t-test. Pearson's correlation coefficient was used to demonstrate NHPT test-retest reliability, and to analyze the relationship between NHPT performances and manual strength.
Results. In both DM1 patients and controls, NHPT in dominant and non-dominant hand showed test-retest reliability ($r > 0.7$, p value < 0.05). Time of NHPT execution was significantly increased in DM1 group compared to controls ($p < 0.001$). Handgrip and pinch strength values inversely correlated with NHPT ($p < 0.05$) in both dominant and non-dominant hand.
Conclusions. NHPT is a simple, rapid, inexpensive and repeatable test to assess manual dexterity in DM1 patients. NHPT could represent a valid instrument to monitor the natural history of the disease or the therapeutic effect in clinical trials.

Exercise Test in skeletal muscle channelopathies: potential applications as learned from 26 patients

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Skeletal muscle channelopathies are rare diseases with overlapping phenotypes. Distinguishing clinical features have been searched for, with unconclusive results. By showing the electrical correlates of myotonia and paralysis, and evaluating the effect of stressors on membrane excitability, the exercise test (ET) may help drive the molecular diagnosis.
We adopted a standarized EMG protocol (Fournier et al, 2004-2006) evaluating 18 controls and 26 mutated patients (44.2 ± 12.2 years, 10 females), among which 19 in SCN4A, 6 in CLCN1, and 1 in CACNA1S. Among SCN4A-mutated patients we include 10 Paramyotonia Congenita-PC, 6 Sodium Channel Myotonia-SCM, and 3 Hyperkaliemic Periodic Paralysis-HPP. The Short-ET was normal in 100% SCM and HPP, abnormal in 100% PC and most CLCN1-Recessive patients, with distinct pattern on exercise repetition and decreased cMAP persistence. Cold sensitivity, though relevant in PCs only, allowed the detection of other, but still not all, CLCN1 patients. On Long-ET, abnormal in all PC and in 2/3 HPP, we found one false negative (HPP with mild phenotype) and one false positive.
In conclusion, the ET narrows the diagnosis, but is not always precise in predicting the abnormal ion channel, being normal ET found in SCM and in some patient with dominant myotonia congenita. The ET is a particularly attractive tool to understand the pathological consequences of new mutations, and for quantitative evaluation of pharmacological interventions.

An updated comprehensive clinical evaluation form for detailed characterization of genetic and phenotypic features associated with D4Z4 reduced allele

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Wide phenotypic variability has been observed in D4Z4 reduced allele (DRA) carriers, sometimes with unexpected mode of inheritance. In some cases the high frequency of FSHD molecular signature might have generated a biased evaluation of families in which a myopathy and a DRA were detected influencing diagnosis and interpretation of clinical and genetic data. Starting from the Italian National Registry for FSHD, in order to capitalize on the rich repository of material from FSHD subjects, we have designed a Comprehensive Clinical Evaluation Form (CCEF) that defines phenotypic subgroups by combination of different clinical features. We believe that the precise phenotypic and genetic classification of patients and families will be central to define the natural history of disease, to propose suitable measure of outcome and to identify new susceptibility/causative factors contributing to FSHD.

Clinical characterization of carriers of borderline D4Z4 alleles from the Italian National Registry of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) has been associated with reduced numbers (≤ 8) of D4Z4 repeats at 4q35. Since 3% healthy individuals carry D4Z4 reduced alleles (DRA) with 4-8 repeats molecular diagnostic in FSHD can be troublesome especially in presence of atypical clinical presentations. Particular attention has to be paid to cases carrying alleles with 9-10 D4Z4 repeats, considered borderline. To define the clinical significance of these alleles, 228 subjects carrying 9-10 DRA (123 index cases and 105 relatives) have been selected from the INRF. We discuss the ongoing evaluation protocol that include: -detailed clinical characterization of probands and relatives; -analysis of disease expression severity; -study of mode of inheritance (familial/sporadic cases); -study of disease penetrance among relatives. Results are discussed, including alternative diagnosis for the atypical/overlapping forms.

Clinical and pathological features of a case with vacuolar myopathy

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A 44-years-old man came to our attention because of a ten months history of exercise-induced muscle pain and early fatigue in daily activities. His family history was inconsistent for neuromuscular diseases. The neurological examination revealed a moderate proximal muscle weakness at lower limb. Serum creatine kinase resulted repeatedly increased (1000-1500 U/L). Needle electromyography recorded a myopathic pattern. Cardiac evaluation was normal. The morphological study of muscle biopsy showed several fibers with clear vacuoles in sections stained with haematoxylin and eosin and Gomori-modified trichrome. Vacuoles had an oval shape or lobulated border. Some vacuoles contained fine amorphous material. Vacuoles did not stain with acid phosphatase, but some of them showed a rim of PAS positive material. Ultrastructural analysis revealed the presence of highly electrondense inclusions, mostly polygonal with rectangular or quadrangular shape, and, sometimes, irregular contour. They were located among myofibrils and were often associated with glycogen particles. The pathological features were suggestive of a surplus protein myopathy. Further details will be added on in site discussion.

ASAH1-related spinal muscular atrophy with no myoclonic epilepsy: expanding phenotypic and mutational features

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ASAH1 gene encodes for the N-acylsphingosine amidohydrolase that is involved in the degradation of ceramide into sphingosine and free fatty acids within lysosomes. Homozygous mutations in ASAH1 are responsible for rare cases of spinal muscular atrophy (SMA) with progressive myoclonic epilepsy (SMAPME) which is phenotypically characterized by childhood onset of proximal muscle weakness and atrophy due to spinal motor neuron degeneration followed by occurrence of myoclonic seizures. The disorder is progressive and patients usually died in the teenage years.

We report the case of a 29-year-old pregnant woman affected with very slowly progressive proximal muscle weakness and atrophy since age of 3 yrs. EMG and muscle biopsy analysis suggested a chronic neurogenic process as usually seen in SMA

but the search for deletions or point mutations in the SMN gene resulted negative. No history of seizures or myoclonus has been reported and EEG was unremarkable.
The molecular study of ASAH1 gene showed the presence of the homozygous nucleotide variation c.124A > G that causes the amino acid substitution p.T42A. This variation has not yet been described and affects the same base involved in the disease-causing mutation described in association with the classical *ASAH1* phenotype (p. T42M). This change is likely to have a pathogenic effect by inserting an alternative splice acceptor site. A similarly affected sister harbored the same mutation.
Our case describes for the first time the association between *ASAH1* mutations and an adult SMA phenotype with no myoclonic epilepsy, thus expanding phenotypic spectrum of *ASAH1*-related SMA.

Muscle mitochondrial dysfunction due to defective mitochondrial biogenesis in SMA patients

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Spinal muscular atrophy (SMA) is characterized by degeneration of motor neurons with skeletal muscle weakness. Depletion of mitochondrial DNA (mtDNA) had been reported in SMA patients as a consequence of the fiber atrophy. We studied muscle samples from 24 genetically SMA patients In all subjects, skeletal muscle biopsy showed a chronic neurogenic pattern. We observed a severe COX deficiency in most samples, in particular in SMA I and SMA II subjects. The enzyme defect was evident in both atrophic and normal/hypertrophic fibers. All respiratory chain complexes were severely impaired. Using custom array gene expression studies, we linked these alterations to the down-regulation of PGC1-alpha and of its downstream targets, including transcription factors NRF1, NRF2 and TFAM. In conclusion, PGC-1α expression and mitochondrial content are significantly reduced in SMA muscles. Therapeutic strategies aiming at counteracting these changes may reveal beneficial for SMA patients.

Myasthenia gravis: diagnostic pitfalls for the atypical phenotypes

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Myasthenia gravis (MG) is an autoimmune disorder characterized by weakness and fatigability, associated to different profiles of antibodies directed against several neuromuscular junction components (AChR, MuSK, LRP4). Although MG has long been considered a well-established autoimmune disease associated with autoantibodies, which are convincingly pathogenic, accumulating data indicate that clinical heterogeneity is very wide. Clinical presentation is distinct from patient to patient, the gravity of symptoms does not correlate to antibodies title and sometimes the clinical phenotype is uncommon and this lead to time consume and misdiagnoses. Herein, we describe various atypical MG phenotypes: three patients with distal upper limbs muscle weakness; a case of cervico-inflammatory myositis complicating by MG involving the arms; a case with acute facial dyplegia; three cases with post-exertional axial muscles weakness; one case with isolated bilateral weakness of the triceps brachii; one case of isolated peroneal muscle weakness. MG should be suspected in patients with "fatigable muscle weakness", even if it is isolated and restricted to "unusual" muscle groups.

A family with epilepsy, movement disorders, mental retardation and exercise-induced myoglobinuria: a complex phenotype caused by two different rare disorders

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We report a 44-years-old man, who presented, since the age of 20, recurrent episodes of rhabdomyolysis after exercise or prolonged fasting; he also showed a mild mental retardation and sporadic choreo-athetoid movements. His 14-years-old son had a psychomotor developmental delay with episodes of drowsiness and head drops, occurring mainly at fasting, and exercise-induced choreo-athetoid movements but no history of pigmenturia. Neurological examination revealed microcephaly, mild spastic ataxia and mental retardation.
EEG was normal in the proband but in the son showed, during fasting, diffuse spike-wave discharges disappearing after food intake. Brain MRI was normal in both. CSF analysis in the son revealed hypoglycorrhachia (40 mg/dl). Clinical and laboratory findings suggested a search for mutations in SCL2A1 (GLUT-1) gene that revealed in both subjects, an already reported pathogenic heterozygous mutation (R333W). GLUT-1 Deficiency Syndrome (DS) is a rare encephalopathy, caused by impaired glucose transport into the brain, presenting with early-onset epilepsy, movement disorders, developmental delay and microcephaly but rhabdomyolysis has never been reported in similar cases. To better define the origin of recurrent exercise-induced rhabdomyolysis in the father, he underwent forearm ischemic test (normal), EMG (myopathic pattern) and muscle biopsy that evidenced unspecific changes. Muscle biochemical studies excluded the most common metabolic causes of recurrent rhabdomyolysis, but VLCAD gene analysis in the father showed two known heterozygous mutations (p.G185S and p.R385W) whereas his son carried only the p.G185S.

Nowadays, it is evident that cases of "double trouble" are increasing and, when a known phenotype is accompanied by some atypical features, we should think of an alternative explanation of unusual presentations.

Reversible acute dropped head syndrome as a presenting feature of mitochondrial myopathy

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Dropped head secondary to weakness of neck extensor muscles has been frequently reported in a wide range of neuromuscular disorders. However, it has been rarely described as a presenting feature of a mitochondrial myopathy.
We here report a 72-year-old man who complained of sudden onset of neck pain followed, after a 15 day period, by weakness of neck extensor muscles and dysphagia.
Since many years he presented a nuanced gait disturbance and diffuse muscle wasting.
Neurological examination showed the presence of dropped head and weakness/atrophy of the upper limb proximal muscles (MRC 4-/5). EMG analysis showed a mixed neurogenic and myogenic pattern at both the upper and lower limbs.
Deltoid muscle biopsy showed severe myopathic changes with prominent features of mitochondrial respiratory chain dysfunction (over 80 COX-negative fibers, many fibers with subsarcolemmal accumulation of mitochondria and ragged blue fibers). Rare small inflammatory infiltrates, scattered fiber atrophy and increase in lipid droplets were also observed. No MHC I expression in muscle fiber was present.
The sequence analysis of the 22 tRNAs in mtDNA did not show any variation. The remaining mtDNA sequencing is ongoing.
In the next two months, the patient experienced a gradual spontaneous improvement until he was able to hold his head upright again and no longer complained of dysphagia.
The role of mitochondrial abnormalities and the mechanisms underlying spontaneous recovery in this patient remain to be explained.

Sporadic PEO caused by a novel *POLG1* variation and a *Twinkle* mutation: digenic inheritance?

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Progressive external ophthalmoplegia (PEO) with multiple deletions of mitochondrial DNA (mtDNA) is associated with several mutations in nuclear genes. They include *POLG1*, *POLG2*, *ANT1* and *Twinkle*. However, digenic inheritance in mitochondrial disorders has been documented in a few cases over the years.
Here we describe an 80-year-old man with sporadic PEO, dysphagia and diffuse muscle weakness. Muscle biopsy showed mild variation in fiber size, about 5% of fibers negative for cytochrome c oxidase (COX) staining while modified trichrome staining did not reveal ragged red fibers. Long range polymerase chain reaction (PCR) analysis of muscle DNA showed multiple mtDNA deletions. Sequencing the *POLG1* revealed a novel heterozygous mutation (c.2831A > G; p.Glu944Gly), predicted as damaging, in the patient who also carried a heterozygous mutation in *Twinkle* (c.1142T > C; p.Leu381Pro).
This case provides a second report of a digenic PEO caused by different mutations in the *POLG1* and *Twinkle* genes. These data support the hypothesis that the PEO phenotype can be determined by the co-existence of two abnormalities in separate genes, both involved in the maintenance and stability of mtDNA. Finally, this study expands the spectrum of *POLG1* mutations and highlights the need to sequence the whole set of nuclear genes associated with PEO and multiple mtDNA deletions.

A new mutation in MYH7 gene occurs with complex fenotype

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Background. Mutations in the beta-myosin heavy chain gene (MYH7) cause different muscle disorders: Laing distal myopathy (LDM), myosin storage myopathy (MSM), hypertrophic familial cardiomyopathy (FHCM) and fiber type dispropotion (CFTD).
We describe three members of a family with a new mutation in the MYH7 gene (c.5401G > A) and a complex phenotype. A 65 year old man and his two sons, 37 and 35 years old, presented in the third decade a non-compact cardiomyopathy, followed in time by heart rhythm abnormalities; the proband and his eldest son underwent pacemakers implants. All our patients since the age of about 30 years showed a moderate weakness of both proximal and distal muscles of lower limbs, while at upper limbs there was a mild proximal involvement whit sparing of the wrist and finger extensor muscles. At last neurological examination all patients showed an anserine gait with bilateral steppage; the proband needs a cane and all of them need help to stand up from chair. The proband and his eldest son underwent to muscle biopsy, which showed a CFTD picture.
Conclusion. To the best our knowledge the association of non-compact cardiomyopathy, LDM like phenotype and CFTD picture at muscle biopsy have never been described in MYH7 gene mutations.

Facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy and platelet storage pool disease: "triple trouble" overlapping syndrome?

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Introduction. Facioscapulohumeral muscular dystrophy is a common hereditary myopathy characterized by an autosomal dominant inheritance, usually associated with a contraction of variable size of 3.3 kb tandem repeated units (D4Z4) on chromosome 4q35. Limb-girdle muscular dystrophies (LGMD) represent a genetically heterogeneous group of myopathies characterized by a variable phenotype. LGMD 2A is the most common type of recessive LGMD (14) and is caused by mutations in the calpain-3 gene.
Case report. We report the case of a patient with concomitant detection of a heterozygous mutation of the calpain3 gene and a contracted D4Z4 fragment presenting with limb-girdle and facioscapulohumeral muscular dystrophy-like phenotype and platelet storage pool disease.
Discussion. We suggest that our case could represent another example of "double" or even "triple" trouble overlapping syndrome. Therefore, we think that patients with atypical phenotypes should undergo more extensive genetic testing, which may provide further useful information for prognosis and genetic counseling.

Blood vessels depletion in MNGIE patients

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Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder characterized by ptosis and progressive external ophthalmoplegia, peripheral neuropathy, severe gastrointestinal dysmotility, cachexia, leukoencephalopathy and mitochondrial DNA depletion, multiple deletions, or both. This disorder is caused by loss-of-function mutations in the gene encoding thymidine phosphorylase (TP) a cytosolic enzyme that catalyzes phosphorolysis of thymidine to thymine and deoxyribose 1-phosphate. In MNGIE patients. TP activity is very low or absent resulting in dramatically elevated levels of plasma thymidine and deoxyuridine. TP is expressed in most human tissues but is not expressed in skeletal muscle usually affected in MNGIE suggesting that TP deficiency causes the disease through a toxic intermediate. In addition, TP is associated with angiogenesis and high concentrations of thymidine inhibit microvessels formation. In our preliminarly study vessels number between two MINGIE patients and eleven controls was compared. Histologic slides were stained with Alkaline Phosphatase and ratio between blood vessels and fibres number was calculated for each sample. Even if cases and controls numbers are low and they have to be increased, a significative differerence between MINGIE and control patients was revealed suggesting that angiogenesis inhibition could be involved in MINGIE pathogenesis.

Frequency of cerebrovascular abnormalities in patients with Late Onset Pompe disease (LOPD): our experience

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Although muscle skeletal dysfunction is generally the prominent manifestation of LOPD, the disease can present with a broad spectrum of clinical manifestations reflecting multisystem involvement with glycogen accumulation in several tissues including smooth muscle and blood vessels. In recent years cerebrovascular abnormalities have been also described. We report data from our population of 10 LOPD patients which underwent magnetic resonance or TC angiography. Three patients revealed to have significant brain vascular abnormalities (including basilar artery dolichoectasia, ectasia of intra and extra cranial arteries, vascular development abnormalities). Moreover, one patient presented three different aneurysms both on intra and extra cranial cerebral vessels, and has been proposed for surgical intervention. None of patients reported clinical symptoms related to the arteriopathy.
Our data confirm that LOPD patients have a predisposition to dilative arteriopathy, in particular of cerebral vessel, which often can be completely asymptomatic. According to this, rupture of a cerebral aneurysm has recently been described as presenting symptom in a LOPD patient.
We would like to highlight the importance of cerebrovascular investigation for the early recognition of such abnormalities in order to consider surgical intervention and prevent potentially fatal cerebrovascular complications.
Real incidence of vascular abnormalities in LOPD, their pathogenesis and the effect on them of enzyme replacement therapy are issues needing more investigations.

Dichlorphenamide in Hypokalemic Periodic Paralysis: effects on attack frequency and quality of life

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Background. Dichlorophenamide (DCP) reduces attack frequency in HypoPP but its effect on quality of life and interictal strength is not known.
Objective. To assess the efficacy of (DCP) in the prevention of attacks in hypokalemic periodic paralysis.
Trial design. Multicenter, randomized, double-blind, placebo-controlled trial of DCP in which participants were followed for 9 weeks. The primary outcome was the average attack rate over Weeks 2-9. Secondary outcomes included acute worsening, severity-weighted attack rate, and changes from baseline in muscle strength, muscle mass, and quality of life as measured by the SF-36.
Results. Participants on DCP (n = 24) had reduced attack frequency and severity relative to those on placebo (n = 20). Five

participants, all taking placebo, reached the endpoint of acute worsening. The most common adverse event were paresthesia (5% placebo vs 38% DCP) and confusion (10% placebo vs 21% on DCP). Despite these effects on cognition, participants on DCP showed significant improvements in physical and social functioning aspects of quality of life compared to placebo. There were no significant effects of DCP on muscle strength or muscle mass.

Conclusions. DCP is safe and effective in the prevention of episodic weakness and improves quality of life in hypokalemic periodic paralysis.

Myopathy with rimmed vacuoles in a girl with juvenile neuronal ceroid lipofuscinosis (CLN3)

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The neuronal ceroid lipofuscinosis (CLNs) are clinically and genetically heterogeneous neurodegenerative diseases characterized by intracellular accumulation of autofluorescent lipopigment and a clinical picture of progressive dementia, vision loss and epilepsy.

The CLNs were originally classified according the onset age, with CLN3 as the juvenile-onset form (JNCL), occurring between 4 and 10 years of age; now CLNs are classified according to the underlying gene defect. CLN3 refers to CLN caused by mutation in the CLN3 gene, whose hallmarks are the ultrastructural pattern of lipopigment with a 'fingerprint' profile and lysosomal vacuoles in blood lymphocytes.

We have carried out a study on a patient with the homozygote deletion (1.0 kb) of the CLN3 gene, who presented with dementia, epilepsy, retinal dystrophy and – in the late stages of the disease – creatine kinase increase. Muscle biopsy showed a myopathy with degenerative aspects and various fibers with rimmed vacuoles.

Even though there are numerous electron microscopy studies on CLNs, the literature lacks in the muscular histopathological aspects of the disease. The presence of a myopathy with rimmed vacuoles in our patient supports the pathogenetic hypothesis of an impairment of autophagy in CLN3. Moreover, if confirmed, the CLNs, mainly the CLN3, may be included in myopathies with rimmed vacuoles.

Clinical and molecular characteristics of dysferlinopathy

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Dysferlinopathy is a muscular dystrophy due to mutations in dysferlin gene. Muscular weakness can be prominent in lower limbs distal muscles (Miyoshi myopathy) or in proximal muscles (limb girdle muscular dystrophy 2B). Cardiac involvement is rare and respiratory involvement usually mild and occurs later in the disease.

The objective was to determine the clinical characteristics dysferlinopathies, and to investigate its genetic background. To identify the most relevant outcome measures to describe the progression of the disease.

The past medical history was collected, disease course was evaluated by specific questionnaires. Molecular analysis of *DYSF* gene was reviewed, as well as immunoblot analysis of the protein. A specific evaluation protocol was used, including clinical-instrumental quantitative evaluation of motor, respiratory and cardiac function.

Seventeen patients (12M, 5 F) from 15 families were included in the study, the age ranging from 19 to 55 years. Four patients were non-ambulant. All patients presented muscular weakness, variably expressed in severity and distribution. No patients referred signs and symptoms of respiratory insufficiency or cardiac involvement. Both mutations were identified in 16/17 patients, and eighteen different mutations were recorded. On western blot analysis dysferlin was absent in 9/14 patients and markedly reduced (< 10%) in 5. No clear correlations between genetic background and clinical features were observed.

The rarity of the disease and its clinical and molecular heterogeneity impose a multicenter longitudinal study. The choice of correct outcome measures is crucial to be prepared to the advent of possible new therapies for rare diseases.

The challenge of phenotypic heterogeneity in facioscapulohumeral muscular dystrophy

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Introduction. Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of hereditary myopathy. In the majority of cases FSHD is associated with a contraction of variable size of 3.3 kb tandem repeated units on chromosome 4q35.

Case report. We report the case of a family with a history of FSHD with the same findings at the after molecular diagnosis but with significant variability in clinical expression among the members affected.

Discussion. Our case corroborate that FSHD presents important inter- and intrafamilial clinical variability that makes it difficult to formulate clinical prognosis and to do genetic counseling. Other interesting evidence is the concurrence of FSHD and autoimmune disease in some of the members affected, which may be coincidental or maybe related with the pathogenesis of FSHD. Further work is needed to understand what the genetic, epigenetic and environmental factors that may influence phenotypic expression are to help genetic counseling and future therapeutic approaches.

Very late-onset ataxia with eyelid ptosis due to *POLG* mutation

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Genetic ataxias are especially suspected in early-onset cases. Here we report the case of an 82-year-old man who complained of progressively worsening dizziness since age 80 years. Neurological examination showed marked gait ataxia with central nystagmus and mild limb ataxia and eyelid ptosis. At last, mitochondrial polymerase γ gene (*POLG*) sequencing revealed the point mutation 1550G > T leading to the substitution p.517G > V. Most likely, this mutation is an incompletely penetrant dominant mutation with variable clinical features; other genetic variations (in *POLG* itself and/or in other loci) can elicit or modify the pathogenic effect, but further studies are needed. *POLG* is a very complex gene associated with incomplete penetrance and variable expressivity, which must be considered in ataxic patients, even in late-onset cases, especially when other signs of mitochondrial dysfunction (such as eyelid ptosis) are observed.

Myopathy in a carrier of *SDHD* (succinate dehydrogenase, subunit D) gene mutation. Report of a case

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The succinate dehydrogenase, subunit D (*SDHD*) gene encodes a subunit of the complex II of the respiratory chain, responsible for the oxidation of succinate. The encoded protein is one of the two integral membrane proteins anchoring the complex to the mitochondrial inner membrane. Mutations in this gene are associated with inherited tumors, namely hereditary paragangliomas. The transmission of hereditary paragangliomas occurs almost exclusively through the paternal allele, suggesting that this locus may be maternally imprinted.

We describe here the case of a 20-years-old man carrier of the c.242 C > T mutation in heterozygosity, in the *SDHD* gene. His mother is affected by multiple paragangliomatosis. He came to our attention because of exercise intolerance with muscle cramps in the upper limbs. Neurological examination was normal. Creatine kinase and lactate levels were normal. Electromyography revealed a myopathic pattern. Muscle biopsy showed mild changes suggestive of mitochondrial myopathy. The patient started therapy with reduced coenzyme Q10 (ubiquinol) with marked benefit.

Our case suggests the need of further, systematic studies to better elucidate the consequences of *SDHD* gene mutations on mitochondrial function in skeletal muscle.

Safe anaesthesia table and undiagnosed myopathy: a three year's experience

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Because of possible severe general anaesthesia complications, myopathic patients represent a challenge for anaesthesiologists. Review of medical literature indicates that currently the great majority of critical side-effects concerns surgery of subjects with clinically-unapparent and thus undiagnosed myopathy (C.U.Myopathy). To help anaesthesiologists in prevention of anaesthesia complications in such patients, we recently outlined [1] a "Safe Anaesthesia Table" (S.A.T.), listing anaesthetic drugs to be avoided and those considered harmless for myopathic patients. Our data concerning methods aimed to identify possible C.U.Myopathy before surgery and the ensuing S.A.T. appliance during their surgery, are presented. Throughout a three-year period, at pre-surgical anaesthesiological examination, 1500 subjects were searched for C.U.Myopathy applying one of the following: a Questionnaire self-administered by patients or, as alternative, a "Correlation-Table" [1] about signs/symptoms suggesting myopathy, derived from Questionnaire and managed by anaesthesiologist. Table and Questionnaire appeared equally useful to the purpose. C.U.Myopathy was recognized in 49 subjects (41: hyperCKemia; 3: clubfoot; 3: dystrophic patients' siblings; 1: ptosis; 1: myotonia). Consequently, the same patients (3.2% from 1,500) underwent surgery with S.A.T. Altogether, recognition of patients with possible C.U.Myopathy and related surgery with S.A.T. appliance enabled anaesthesiologists to avoid anaesthesia complications without delaying surgery.

Late-onset myopathy with undefined features: mitochondrial or lipid storage myopathy?

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We report the case of a 62 year old woman presenting with ptosis, dysphagia, respiratory distress and muscular fatigue after minimal physical exercise. She reported a history of dyslipidemia, high blood pressure, diabetes and hypothyroidism. Neurological examination showed unilateral ptosis, hypophonia, and normal muscle strength. Needle EMG evidenced myopathic changes and nerve conduction studies showed a sensory-motor polyneuropathy. Serum lactic acid level was elevated. Muscle biopsy showed small lipid droplets in type 1 fibers and the presence of COX-negative fibers. Molecular analysis helped in refining the diagnosis.

Late-onset dystrophinopathy due to a novel intronic mutation resulting in skipping of the exon 11: an exception to the "reading frame rule"

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The dystrophinopathies are characterized by a wide spectrum of disease ranging from asymptomatic hyperCKemia to more severe forms including Duchenne/Becker muscular dystrophy and cardiomyopathy. The currently most used rule to predict whether a mutation will result in a severe or mild phenotype is the "reading frame rule". We here report on a 29-year-old patient complaining of left thigh muscle weakness since his 23. Previously, he was a semi-professional football player. When he was 27, he started complaining of moderate proximal weakness at both lower limbs with difficulty in climbing stairs and running. No winging of the scapulae and hypertrophy of calf muscles were observed. Deltoid muscle biopsy showed few cell necrosis and degeneration, marked fiber size variability with atrophic and hypertrophic fibers, rare fiber splitting and only mild focal endomysial fibrosis. The immunohistochemical study by using anti dystrophin antibodies showed globally reduced staining with focal loss of staining in some muscle fibers.

MLPA (multiplex ligation-dependent probe amplification) analysis did not detect deletions, duplications or complex rearrangements in the dystrophin gene. Direct sequence analysis of the dystrophin gene exons and flanking intronic regions revealed a novel c.1150-3C > G substitution in intron 10. Reverse transcription analysis showed the absence of incorporation of exon 11 in the dystrophin RNA.

To date, isolated deletion of exon 11 was not reported in the databases. Mutation alters the reading frame of the gene and is predicted to result in a severe DMD phenotype.

Despite these considerations, our patient presents with a mild and late-onset clinical picture. Therefore, this seems to be a singular rare exception to the "reading frame rule".

Our report expands the clinical and allelic heterogeneity of dystrophinopathies.

Sodium channel gene as modyfing factor of DM2 phenotype

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Myotonic dystrophy type 2 (DM2) is an adult onset muscular dystrophy generally characterized by mild and inconsistent myotonia. New evidences have shown how co-occurrence mutation in chloride channel (CLCN1) may influence the phenotype. To date a possible effect of SCN4A mutation on DM2 phenotype has never been evaluated. In our study we investigated two DM2 patients with severe and early onset myotonia without mutation in CLCN1 gene. In one patient we identified a variant c.215C > T (p.Pro72Leu) in SCN4A gene suspected to be pathogenetic. Whole-cell voltage-clamp analysis showed a hyperpolarizing shift (-5mV) of the voltage dependence of activation that may increase cell excitability. In the other patient we found on SCN4A gene a S906T polymorphism that has been reported to influence channel biophysical properties (Kuzmenkin et al. 2003). In both cases SCN4A variants seem to determine the atypical phenotype of the patients. A SCN4A gene screening is suggested in DM2 patients with early and severe myotonia.

Paternal germline mosaicism in colvi related myopathies: a case report

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Ullrich congenital muscular dystrophy (UCMD) can be due to autosomal recessive mutations in one of the three genes of collagen VI with a related 25% recurrence risk. In the majority of UCMD cases nevertheless, the underlying mutation is thought to arise de novo and the recurrence risk is considered as low. Here we report a family with recurrence of UCMD in two sibs only by father's site. In both, the molecular analysis revealed heterozygosity for the c.896G > A mutation in COL6A1 exon 10 (Gly299Glu) and for the COL6A1 c.1823-8G > A variation within COL6A1 intron 29. The Gly299Glu mutation, despite not previously reported, is likely to be pathogenic, leading to the disruption of the Gly-Xaa-Yaa motif in the triple-helix domain of collagen VI alfa(1)-chain. The intronic variation was inherited from the father and RNA analysis in skin fibroblasts allowed to exclude its role in affecting COL6A1 transcript processing. The Gly299Glu mutation occurred apparently de novo in the two sibs. The described mutational segregation strongly suggests the occurrence of paternal germline mosaicism. The reported family represents the first observation of gonadal mosaicism in collagen-VI related myopathies and, similarly to other collagen-related diseases as osteogenesis imperfecta, this possibility deserve to be considered in genetic counseling and recurrence risk estimation.

Air stacking treatment improves cough efficiency in neuromuscular diseases

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In neuromuscular diseases (NMD), respiratory muscle weakness or glottis dysfunction impairs cough leading to ineffective clearance of airway secretions from lungs.

Air stacking (AS) technique with manual resuscitation bag provides lungs expansion optimizing lung recoil pressure and so increasing peak cough flow (PCF) as previously showed in Duchenne Muscular Dystrophy (DMD).

We have evaluated cough efficiency, by means of PCF and peak expiratory flow (PEF), in 30 NMD patients (16 DMD, 6 SMA type II, 3 Congenital Myopathies, 3 Congenital Muscular Dystrophies, 2 Myotonic Dystrophy) (mean age 17.1; 8.6-31.9 years) with restrictive lung disease (mean FVC 37.1% pred.) before and after six months of daily AS treatment.

FVC and PEF were collected by standard spirometry. PCF was measured by standard peak flow meter connected to an anesthesia mask. Data values were compared by a paired t-test.
In all patients, after a single AS treatment we observed a significant increase in PCF ($p < 0.0001$), in line with what already observed in DMD patients. In addition, at six months of regular daily AS treatment, a significant increase of PCF and PEF ($p < 0.005$ and $p < 0.03$, respectively) was observed in all cases.
Our data show that AS treatment improves the cough efficiency by increasing PCF and PEF, after a single treatment, and particularly after a six months of regular daily treatment not only in DMD, but also in other patients with different neuromuscular diseases.

Improving the diagnosis of Duchenne muscular dystrophy

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Despite the recent advances in the care and management of Duchenne muscular dystrophy (DMD) there has not been a significant improvement in the age of diagnosis over the last 30 years. Recent data suggest that early initiation of steroid treatment could be associated with better long-term outcomes. In addition there are potential promising therapeutic interventions on the horizon and these are likely to be of greater benefit in younger children with less damaged muscles. Early diagnosis of DMD is therefore crucial.
We reviewed the diagnostic process for DMD in boys without a family history at our tertiary centre for Neuromuscular Diseases in England over the last 10 years. The mean age of first reported symptoms of DMD was 2.7 years (32.5 months). First engagement of a health care professional occurred at a mean age of 42.9 months (presentational delay = 10.4 months). Diagnosis of DMD was confirmed at a mean age of 51.7 months (4.3 years). The diagnostic delay (8.8 months) was almost entirely due to delay in testing Creatine Kinase (CK) levels (7 months). The total delay from parental concern to genetic diagnosis was 19.2 months (1.6 years).
Our study showed an improvement in the age of diagnosis in DMD in the UK, however there continues to be a presentational delay, in addition to a delay in obtaining a CK test. To address this and further lower the age of diagnosis of DMD we need to raise awareness of DMD in primary care. We propose screening for DMD with a simple mnemonic as part of the Child Health Care programme in the UK. Any child who presents in primary care with hallmark features of DMD (including unexplained motor or speech development delay) should be offered a CK test as soon as possible. Comparing the diagnostic process for DMD in different countries would also help to identify where and why the delay in diagnosis is still occurring and to identify areas of interventions to improve the care for boys with DMD worldwide.

cPEO and Huntington Disease with reduced penetrance: a singular double trouble

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We report on a patient presenting with the unusual association of two different clinical pictures: a mild movement disorder consistent with HD and a mitochondrial myopathy suggestive for a Chronic Progressive External Ophthalmoplegia (cPEO).
A 70-year-old woman presented with progressive eyelid ptosis, bilateral ophthalmoparesis, dysphagia, dysphonia, mild proximal limb weakness, numbness and fatigue since age of 55. By the age of 64 she noticed some abnormal involuntary movements involving head and limbs, imbalance and gait instability. Mini Mental State Examination was rated 20/30. Family history was unremarkable.
Molecular analysis showed expansion of CAG repeats in the range of "reduced penetrance" (repeat count 38) on HTT gene. Muscle biopsy showed presence of ragged red fibers, fibers with subsarcolemmal accumulations of mitochondria and several cytochrome-C-oxidase negative fibers. No major rearrangements in mitochondrial DNA were detected by Southern Blot analysis. The 22 mitochondrial tRNA genes were directly sequenced and a novel m.5613T>C heteroplasmic mutation was identified in the tRNA Alanine gene, which disrupts a strongly conserved site and fulfills the accepted criteria of pathogenicity.
This is the first reported case of mitochondrial myopathy/HD "double trouble".
Mitochondrial involvement is an emerging key determinant in the pathogenesis of HD and mutant huntingtin influences mitochondrial complex II/III function also in non-neuronal tissue as skeletal muscle. Significant abnormalities on muscle histochemistry are usually not observed in HD patients but few cases displaying an excess of SDH positive and COX negative fibers have been reported.
The supposition of an additive effect of the HD mutation on muscle mitochondrial abnormalities and cPEO phenotype in this patient is intriguing and deserves further studies.

Natural history of muscle pathology in 40 DMD patients aged 1 to 10 years: morphologic and morphometric analysis

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We performed a morphologic/morphometric analysis of muscle biopsies in 40 DMD patients aged 1 to 10 years. We considered the following parameters: fibrotic tissue, necrosis, regeneration, hypercontracted fibers, internal nuclei, fiber size variability, inflammatory reaction.

We found that the increase in connective tissue, already present at birth and stable until the age of six years, rapidly increases from 18.5% to 29.7% at seven years of age. The number of necrotic fibers decreases from 2.12/ light microscopy grid area (0.24 sqmm) at five years of age to 0.81 at the age of six years. Regenerating fibers remain stable throughout the years.
These data allowed us to both clarify the natural evolution of muscle alteration and to define the turning point (around 6 years of age) at which fibrotic degeneration exponentially increases.
These data are extremely useful when deciding the starting age of a clinical trial: indeed, the efficacy of any treatment also depends on the initial degree of muscle alterations, thus, it is possible to speculate that when connective tissue affects a large percentage of muscle fibers, muscle tissue restoration will be difficult. Moreover, establishing the morphologic natural history of the disease evolution is useful to figure out if a particular treatment has any effect.

Features in muscle biopsies of late-onset Pompe Disease patients before and after ERT.

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Glycogenosis type II is an autosomal recessive disorder caused by a deficiency in the glucosidase alpha acid (GAA) enzyme leading to deposition of glycogen in heart, skeletal, and respiratory muscles.
The histopathological hallmarks are muscle fiber vacuolization and autophagy.
GSDII is clinically classified into infantile, juvenile, and late-onset. Recombinant human GAA is the only approved enzyme replacement therapy (ERT). It is effective in most infantile patients whereas the outcome is variable in adults.
We analyzed muscle biopsies from 14 late-onset patients.
All patients clinically improved or remained stable after ERT; morphologically, seven patients improved, two patients worsened and all other subject remained unchanged.
Immunohistochemical results show a variable binding of the autophagic antibodies: EEA1 (early endosome antigen 1), LC3 (microtubule-associated protein 1 light chain 3), and LAMP2 (lysosome associated membrane protein 2).
Five patients show a mild increase in GAA by both biochemical and WB analysis in skeletal muscle.

Validation of the Pediatric Quality of Life Inventory™ Neuromuscular Module and correlation with functional assessments over 12 month follow-up in the Italian DMD population

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The main aim of the study was to translate and validate the Pediatric Quality of Life Inventory™ 3.0 Neuromuscular Module (PedsQL NM) in combination with other selected outcome measures, PedsQL TM 4.0 Generic Core Scales (PedsQL GCS), 6–minute walk test (6MWT), North Star Ambulatory Assessment (NSAA) and timed items, in a large cohort of Italian patients with Duchenne muscular dystrophy (DMD). We also aimed to collect longitudinal data over 12 month interval of all the measurements and to verify possible correlations among them.
So far no systematic study has been planned to correlate QoL results with the outputs of the "gold standard" outcome measures for DMD. We enrolled 98 ambulant DMD patients (age range 5-12,8 yrs) with assessment at baseline and 12 months thereafter. A moderate direct correlation was obtained between PedsQL score (General and NM modules) and NSAA and between PedsQL score (General and NM modules) and 6MWT. The analysis of 12-month changes showed a significant correlation among QoL measures and NSAA and 6MWT results. Interestingly the correlations were stronger in patients' older group (> 7 yrs) and in parents' versions. These results suggest that these tools reflect functional changes and are valid to explore parents' and patients' perspectives in the cohort above the age of 7. Further studies are needed to validate new appropriate tools for younger children.

Acute statin-induced neuromyopathy: a rare condition with severe prognosis

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The clinical spectrum of statin-induced myopathy includes asymptomatic hyperCKemia, myalgia, myositis and rhabdomyolysis. Although statins may increase the risk of developing nerve damage, no clear-cut correlation between their use and peripheral neuropathy has been definitely demonstrated.
We here describe a 74-year-old woman who began to complain of muscle pain and rapidly progressive lower and upper limb weakness one week after starting hypolipidemic therapy with simvastatin 20 mg daily.
Neurological examination revealed proximal and distal limb weakness with diffuse loss of deep tendon reflexes and inability to walk. CK was more than 8000 U/L whereas anti-synthetase and anti-signal recognition particle (anti-SRP) antibodies were absent. Antibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) are still pending. EMG-ENG study showed a marked axonal sensorimotor polyneuropathy associated with muscular spontaneous activity and early recruitment, short duration, low amplitude and polyphasic shape of motor-unit potentials. The quadriceps muscle biopsy showed severe myopathic changes characterized by multiple necrotic and regenerating fibers with no inflammatory infiltrates.
Simvastatin was stopped but, because of the rhabdomyolysis, hemodyalisis was necessary. A three day cycle of therapy with IVIg at 0,4 g/kg daily was started and prednisone 1 mg/kg daily was added. Three weeks later, only a slight improvement in upper limb strength was observed.
Our report illustrates the rare concomitant acute involvement of peripheral nerves and skeletal muscles following treatment with statins. The mechanisms underlying this "full peripheral involvement", being presumably autoimmune, deserve further studies to be clarified.

AUTHOR INDEX

NEWS FROM AROUND THE WORLD

MSM

The 11th Congress of the Mediterranean Society of Myology was held in Athens, Greece on October 31st – November 2nd . The congress was chaired by Prof. Marinos dalakas, President of the Organizing Committee. President of the Scientific Committee was Prof. Luisa Politano, and honorary President Prof. Giovanni Nigro. There were nine invited lectures given by Haluk Topaloglu, Luisa Politano, Cyproula Christodoulou, Vincenzo Nigro, Valery Askanas, Marinos Dalakas, Reinhardt Rüdel, Corrado Angelini and Francesco Muntoni.

During the General Assembly of the Society, the current board has been confirmed till the next Congress, that will be in Naples, Italy in the spring of 2015. A meeting of the board will be held in autumn 2014, for organizational purposes.

GCA

During the Gala dinner of the 11th Congress of the Mediterranean Society of Myology held in Athens, Greece on 1st November 2013, in the splendid setting of the floodlit Parthenon, the 2013 Gaetano Conte Prizes have been awarded ex aequo to Karin Jurkat-Rott and Giuseppe Novelli for the basic research and to Zohar Argov and Giovanni Meola for the clinical research.

AIM

The 14th Congress of the Italian association of Myology will be held 8 to 10 May 2014 at the Pala Creberg, Sirmione (BS). The scientific Board is chaired by Maurizio Moggio, Milano cooperated by

Antonio Toscano, Messina; Claudio Bruno, Genova; Paola Tonin, Verona; Angela Berardinelli, Pavia; Massimiliano Filosto, Brescia; Giovanni Marrosu, Cagliari; Lucia Ovidia Morandi, Milano; Elena Pegoraro, Padova; Gabriele Siciliano, Pisa

The local Scientific Board is chaired by Massimiliano Filosto with the cooperation of Alessandro Padovani, Brescia, while the local Organizing Committee is composed by Massimiliano Filosto, Alice Todeschini, Fabrizio Rinaldi, and Silvia Rota from Brescia.

Further information will be available in the website of the society www.miologia.org.

The proceedings of the Congress are reported in the present issue.

WFN

The XIII International Congress on Neuromuscular Diseases (ICNMD XIII), will be held in Nice, France, July 5-10, 2014 in the modern facilities of the Acropolis Congress Centre. ICNMD is the regular meeting of the Research Group on Neuromuscular Diseases-World Federation of Neurology (RGNMD-WFN) hosted all around the world as a every 4-year event from now more than 50 years. It is firmly established by the global neuromuscular community as the most important forum and the unique opportunity to share scientific advances by those involved in the fields of improving care, understanding disease pathogenesis, and developing innovative treatments in muscle, neuromuscular junction, peripheral neuropathies and motor neuron diseases.

WMS

The 19th International WMS Congress, will be held in Berlin, Germany, 7-11 October 2014,

The symposium will be in the traditional WMS format with 3 selected topics:

1. Protein Aggregation, Autophagy and Proteomics
2. Limb-Girdle Muscular Dystrophies
3. Advances in Therapy for Neuromuscular Disorders

One day of the symposium will be dedicated to each of the selected areas. Invited keynote speakers will summarize the state of the art on the selected topics, covering clinical, molecular and other aspects. The sessions will comprise contributed oral papers and poster presentations with guided discussions.

FORTHCOMING MEETINGS

2014

March 8-14

RE(ACT)[2014] International Congress on research of Rare Diseases. Gehry building, Novartis Campus, Basel, Switzerland. *Information*: www.blackswanfoundation.ch

April 11

11th Italian Congress on Laminopathies. Bologna, Italy. *Information:* website: www.igm.cnr.it/laminopatie

May 8-10

14th Congress of the Italian Society of Myology. Sirmione (BS), Italy. *Information:* website: www.miologia.org

May 9-10

ECRD 2014 : The European Conference on Rare Diseases & Orphan Products. Berlin, Germany. *Information*: website: www.eurordis.com

May 31 - June 3

The European Human Genetics Conference, Milan, Italy. *Information*: website: www.esgh.org

July 4-6

Frontiers in CardioVascular Biology 2014. Barcelona, Spain. *Information:* website : www.esc.org

July 5-10

XIII International Congress on Neuromuscular Diseases. Nice, France. *Information*: website: www.icnmd2014.org

October 7-11

19thWorld Muscle Society Congress. Berlin, Germany. *Information*: website: www.world musclesociety.org

October 18-22

ASHG Annual Meeting. San Diego, CA,USA. *Information*: website: www.ashg.org

2015

May (date to be announced)

12th Mediterranean Society of Myology Congress. Ischia (NA), Italy. Information: giovanni.nigro@unina2.it; luisa.politano@unina2.it

June 6-9

The European Human Genetics Conference, Glasgow, United Kingdom. Information: website: www.esgh.org

October (date to be announced)

20thWorld Muscle Society Congress. London, UK. *Information*: website: www.world musclesociety.org

October 20-24

ASHG Annual Meeting. Baltimore, MD,USA. *Information*: website: www.ashg.org

2016

April 3-7

The European Human Genetics Conference. Kyoto, Japan. Information: website: www.esgh.org

September 4-9

International Congress of Human Genetics 2016. Yokohama, Japan. *Information*: website: www.esgh.org

October 20-24

ASHG Annual Meeting. Vancouver,Canada. *Information*: website: www.ashg.org

2017

October 17-21

ASHG Annual Meeting. Orlando, Florida, USA. *Information*: website: www.ashg.org

2018

October 16-20

ASHG Annual Meeting. San Diego, CA,USA *Information*: website: www.ashg.org

2019

October 22-26

ASHG Annual Meeting. Toronto, Canada. *Information*: website: www.ashg.org

2020

October 27-31

ASHG Annual Meeting. San Diego, CA,USA .*Information*: website: www.ashg.org

For application or renewal to MSM

MEDITERRANEAN SOCIETY OF MYOLOGY* (MSM)

G. Nigro, President
L.T. Middleton,G. Serratrice, Vice-Presidents
Y. Shapira, Secretary
L. Politano, Treasurer

APPLICATION/RENEWAL FORM

Application/Renewal for **1yr** ☐ **2 yrs** ☐

Prof. Luisa Politano, MSM Treasurer, Viale dei Pini 101, 80131, Napoli, Italy
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* The membership fee is inclusive of free subscription to Acta Myologica.