

Top of Form

Neuromuscular Disorders

Bottom of Form
Top of Form

Official Journal of the World Muscle Society

Register or Login: Password: Auto-Login
[Reminder]

Search for
Advanced Search - MEDLINE - My Recent Searches - My Saved Searches - Search Tips

Bottom of Form

Top of Form

JOURNAL HOME

CURRENT ISSUE

BROWSE ALL ISSUES

SEARCH THIS JOURNAL

Bottom of Form
Top of Form

JOURNAL INFORMATION

• Aims and Scope

• Editorial Board

• Author Information

Volume 14, Issue 6, Pages 387-396 (June 2004)

◀ previous 10 of 12 next ▶

Top of Form

FULL TEXT
PDF (131 KB)

Bottom of Form

118th ENMC International Workshop on Advances in Myotubular Myopathy. 26–28 September 2003, Naarden, The Netherlands. (5th Workshop of the International Consortium on Myotubular Myopathy)

CITATION ALERT
CITED BY
RELATED ARTICLES
EXPORT CITATION
EMAIL TO A COLLEAGUE
VIEW DRUG INFO

Top of Form

E. Bertini a*, V. Biancalana b, A. Bolino c, A. Buj Bello d, M. Clague e, P. Guicheney f, H. Jungbluth g, W. Kress h, A. Musaro' i, H. Nandurkar j, L. Pirola k, N. Romero f, J. Senderek l, U. Suter m, C. Sewry n, H. Tronchere o, C. Wallgren-Pettersson p, M.J. Wishart q and J. Laporte d
Received 25 March 2004;
Bottom of Form

Article outline

- Introduction
- Genotype–phenotype correlation

- Abstracting/Indexing
- Contact Information
- Society Information
- Pricing Information

Bottom of Form

More periodicals:
Top of Form

FIND A PERIODICAL

FIND A PORTAL

GO TO PRODUCT
CATALOG

Bottom of Form

- Myotubularin function and myotubularin related proteins
- Charcot-marie-tooth and MTMR related proteins
- Centronuclear myopathy
- X-linked myotubular myopathy and trophic factors
- Conclusions
- Acknowledgements
- Appendix
- References
- Copyright

Introduction

 [return to article outline](#)

XLMTM (X-linked myotubular myopathy) is a rare recessive disorder. The MTM1 gene is localized on Xq28 and it encompasses 100 kb at the genomic level, with 15 exons coding for a 603 aa protein named myotubularin. Mutations in myotubularin result in the human disease XLMTM, which is characterized by the persistence of muscle fibres that retain an immature phenotype [1].

MTM1 gene is the founder member of the myotubularin family (14 genes in human) which constitutes a large group within the tyrosine/dual specificity phosphatases super family (PTP/DSP). Members are present in almost all Eucaryotic organisms, including yeasts and plants. Two other members were more recently found mutated in 2 forms of demyelinating Charcot-Marie-Tooth (CMT) neuropathy type 4B1 and 4B2[2]. In the last 4 years, after the past ENMC Consortium on Myotubular myopathy, which was held in 1999, a great number of findings have emerged concerning the function of myotubularin and myotubularin related proteins. Accordingly, the Consortium was extended to many new participants with expertise in biochemistry and cell signalling. The ENMC Consortium on advances in Myotubular Myopathy (MTM) held its 5th Workshop in Naarden, the Netherlands, the weekend from 26–28th September 2003. It was attended by 19 active participants from Australia, Finland, France, Germany, Italy, Switzerland, the United Kingdom and the USA.

Molecular diagnosis

The meeting opened with an overview by Wolfram Kress and Valérie Biancalana on the genotype–phenotype correlation of XLMTM

A mutational review by Valérie Biancalana, including 7 not reported cases found in the Strasbourg group, counted a total of 196 different mutations in 340 families. 154 mutations were ‘private’ mutations and were found only in one family [3], [4].

Eighty five percent of the mothers that were tested were mutation's carriers, a fact that is important for genetic counselling. In addition, germline mosaicism has been described in several cases, therefore prenatal diagnosis should be proposed to each mother, even if she does not carry the mutation.

Most XLMTM patients were ventilator dependent while 16% of them were classified as having either a moderate or a mild phenotype based on the level of the ventilatory support required [5], that is to say with no chronic ventilator dependence.

In most cases mutation leads to the inactivation or absence of myotubularin. Out of the 196 mutations the percentage of mutation types observed was 29% missense mutations, 21% nonsense mutations, 23% small insertion/deletion; 20% splice site mutations and 7% large deletion. These are widespread through the gene but nearly half of the patients have mutations in exons 12, 8 and 4. These 3 exons should then be tested in first intention in a diagnostic laboratory (with exon 9 in case of a patient with a mild phenotype).

In fact, 7 mutations account for 25% of all MTM1 mutations. In the order of frequency in the total set of mutations there are the splice site mutation c.1261-10A>G ensuing in the insertion of three amino acid FIQ at position 420 (7.3%), R241C (3.8%), c.141-144 delAGAA resulting in a frameshift at amino acid 48 (3.8%), R37X (2.7%), P205L (2.4%), R421Q (2.4%) and R421 X (2.4%). They are mainly associated with a severe form of the disease, except R241C, which is often found associated with a mild or intermediate phenotype.

Myotubularins all share the same protein domains core, which consists of the following four domains: GRAM, RID, PTP/DSP active site and SID. The 100 disease causing missense mutations have been found in each of the domains, suggesting that they all participate in the function of myotubularin. Missense mutations are, however, predominant in the RID and in the PTP/DSP homology region.

Wolfram Kress summarized his experience on molecular genetic diagnosis in Germany. The number of new confirmed diagnosis of patients with congenital MTM1 is about 7–8/year and this reflects very roughly the German incidence of severe MTM1, because routine molecular diagnosis in Germany is being performed only in his lab. Mutation analysis is performed by direct sequencing of all fifteen exons of the myotubularin gene, and this method should be considered as the gold standard because it improves molecular diagnosis in relation to other screening methods. However, the impression is that the number of mutations found in severe MTM1 patients has not decisively increased and do not get beyond 60–70%. Probably some mutations localized in the promoter or intron regions of the MTM1 gene are still missing. Kress reported that he had analysed about the same number (7–8/year) of new mild sporadic cases with a centronuclear myopathy (older than two years), and one third of them were females. The number of mutations found in the mild cases is low and never exceeds 20%.

Norma Romero from Paris presented a particular three-generation family with XLMTM. The grandfather (67 years) and his brother (48 years) were considered as affected by centronuclear myopathy (CNM) until his two grandsons (13 and 5 years) presenting with severe hypotonia at birth, respiratory mechanic assistance, and latter muscle weakness, long thin face, ptosis and opthalmoplegia were diagnosed as having X-linked myotubular myopathy (their mothers, obligated carriers, were healthy). A common MTM gene mutation (N180K) was identified in all patients [3]. These patients are the oldest alive patients with myotubular myopathy known so far.

Manifesting carriers in XLMTM

Manifesting carriers of MTM1 are rare (3 out of 43 studied). To find out whether clinical manifestations in a proportion of carriers was due to skewed X-inactivation, a recent study was presented by Carina Wallgren-Pettersson who reported the X-inactivation pattern in blood DNA compared with the clinical phenotype in 43 carriers of X-linked myotubular myopathy [6]. Three overtly affected carriers had skewed X inactivation with the mutated X as the predominantly active X for at least two of them. Four females with mild symptoms had random X inactivation. The unaffected XLMTM carriers had either skewed X inactivation in favor of expression from the normal X or random X inactivation. Thus, there appeared to be a correlation between overt manifestations and skewed X inactivation, but the correlation did not reach significance, possibly due to the small patient numbers in this series, or to the tissue under study being blood and not the muscle tissue itself. Skewing of X inactivation in XLMTM is likely to be due to genetic factors influencing the X-inactivation process rather than to chance or to a selection against cells expressing the X chromosome carrying the mutated MTM1 gene.

Wolfram Kress also presented his experience on one symptomatic female patient with a MTM1 mutation. In a five years old congenitally affected girl showing muscle hypotonia, proximal weakness, a waddling gait, joint laxity and a myopathic face a frameshift mutation in exon 8 was found. She had a skewed X-inactivation [7]. There were at least two families in the last few years with manifesting carrier mothers and grandmothers (weakness in early adulthood, myopathic face, ptosis). X-inactivation was not enlightening in any case.

Functional studies of myotubularin on MTM1 defects

In addition to mutation analysis, Jocelyn Laporte presented results on his studies with protein analysis to verify if this method can improve the diagnostic workup of XLMTM [8] The protein status can be studied by immunoprecipitation but not yet routinely. He characterized antibodies against myotubularin and monitored the level of the protein in XLMTM patients cell lines. The vast majority of mutations, including some missenses, lead to a decrease in protein level. All suspected autosomal cases had normal level suggesting that CNM is not due to an indirect decrease in MTM1 protein level [8]. Laporte also used a probe specific to PI3P provided by Harald Stenmark [9]. While overexpression of myotubularin clearly remove the PI3P from early endosomes, he failed to detect any differences in PI3P (and EEA1, an early endosome marker) in fibroblasts and myoblasts cell lines from patients with XLMTM or suspected autosomal CNM (Tronchere et al., submitted) [10].

Myotubularin function and myotubularin related proteins

 [return to article outline](#)

Metabolic pathways and subcellular localization

X-linked myotubular myopathy is a muscle disorder caused by mutations on the MTM-1 gene, coding for myotubularin—a 65 kDa polypeptide similar to protein phosphatases. Myotubularin is a dual-specific phosphatase that dephosphorylates phosphatidylinositol 3-phosphate (PtdIns(3)P), and phosphatidylinositol (3,5)-bisphosphate (PtdIns(3,4)P₂).

The establishment of a causal relation between mutations on the MTM-1 gene and the development of myotubular myopathy, prompted the search for a biochemical function of myotubularin [1].

Phosphoinositide 3-kinase (PI3-kinase)-derived [11], [12] membrane-anchored phosphoinositides regulate diverse cellular processes, including proliferation, cell survival, vesicular trafficking, cytoskeletal remodeling, and metabolism [13].

Lipid products produced by PI 3-kinase activity include the following: [PtdIns(3)P], [PtdIns(3,4)P₂], phosphatidylinositol 3,5 bisphosphate [PtdIns(3,5)P₂], and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P₃).

Diverse proteins are regulated by binding to the D3-phosphorylated phosphoinositides PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂, including the serine/threonine kinase Akt, a potent suppressor of apoptosis [14]. PtdIns(3)P, the most abundant product of PI 3-kinase activity in mammalian cells, specifically interacts with a protein module designated the 'FYVE domain' and functions as a regulator of endosomal trafficking [15].

Initial experiments by using synthetic substrates suggested a function for myotubularin as a protein phosphatase.

However, more compelling evidence from both biochemical and in vivo studies now define myotubularin as a lipid phosphatase capable of hydrolysing the 3' inositol position of phosphatidylinositol 3-phosphate [PtdIns(3)P] and [PtdIns(3,5)P₂] [2]. Although there is now information on the biochemical function of myotubularin, the rationale whereby mutation on MTM-1 leads to myotubular myopathy is still elusive.

A whole morning was focused on studies that try to unravel the subcellular localization and the function of MTM1 and myotubularin related proteins (MTMRs), highlighting the specific substrates, the catalytic activities and the crystallographic structure of MTMR2. Using a bio-informatic approach, Laporte characterized the 14 human members which include 6 inactive phosphatases [2]. He reported the chromosomal localisation for all of them as there are candidate for human diseases. Indeed mutations in MTMR2 and MTMR13 have recently been found, respectively, in CMT4B1 [16], [17] and CMT4B2 [18], [19]. There is no co-evolution of catalytically active and inactive myotubularins looking at the sequenced genomes [2]. Although all myotubularins tested localized as a dense cytoplasmic network and are recruited to plasma membrane upon Rac1 GTPase induction, differences exist in the gradient of localisation from the nucleus membrane to the plasma membrane suggesting different substrate pools [20].

The group from Australia conducted by Harshal Nandurkar had previously described the purification of a rat [PtdIns(3)P] 3-phosphatase, comprising a heterodimer of a 78-kDa adapter subunit in complex with a 65-kDa catalytic subunit [20], [21]. In addition, they cloned and characterized the cDNA encoding the human 3-phosphatase adapter subunit (3-PAP) [22]. Sequence alignment showed that 3-PAP shares significant sequence similarity with the protein and lipid 3-phosphatase myotubularin, and with several other members of the myotubularin gene family [22]. Nandurkar's group also had previously described that unlike myotubularin, 3-PAP does not contain a consensus H₃CX₅R catalytic motif, and is thus a catalytically inactive member of the myotubularin gene family, which coprecipitates lipid phosphatidylinositol 3-phosphate-3-phosphatase activity from lysates of human platelets [22].

Nandurkar reported that he recently identified myotubularin as the catalytically active 3-phosphatase subunit interacting with 3-PAP [23]. A 65-kDa polypeptide, coprecipitating with endogenous 3-PAP, was purified from SDS/PAGE,

subjected to trypsin digestion, and analysed by collision-induced dissociation tandem MS. Three peptides derived from human myotubularin were identified. Association between 3-PAP and myotubularin was confirmed by reciprocal co-immunoprecipitation of both endogenous and recombinant proteins expressed in the human K562 cell line. Recombinant myotubularin localized to the plasma membrane, causing extensive filopodia formation. However, co-expression of 3-PAP with myotubularin led to attenuation of the plasma membrane phenotype, associated with myotubularin relocalisation to the cytosol. Collectively these studies indicate that 3-PAP functions as an 'adapter' for myotubularin, regulating myotubularin intracellular location and thereby altering the phenotype resulting from myotubularin overexpression. In collaboration with Jocelyn Laporte of Professor Mandel's group (Strasbourg, France), Nandurkar has demonstrated that certain myotubularin mutations that are associated with disease phenotype, but retain enzyme activity, do not interact with 3-PAP. This observation supports his hypothesis that any perturbation of interaction between 3-PAP and myotubularin may be sufficient to cause myopathy. Nandurkar is generating mice with tissue-specific targeted null mutation of the 3-PAP gene. The phenotype of these mice will contribute to the understanding of 3-PAP in muscle development and maturation.

The X-ray crystal structure of human MTMR2 was presented by Matthew Wishart. The structure revealed that the previously described GRAM sequence is actually a part of larger pleckstrin-homology domain, and that SID and RID are, in fact, sequence motifs within a larger lipid phosphatase domain. Moreover, the so-called 'substrate-trapping' aspartic acid does not play a role in catalysis, but rather is important for maintaining the structural integrity of the phosphatase domain. One of the aspartic acids within the active site CX5R loop likely functions as the catalytic acid. The structure also provided a framework for understanding the functional consequences of mutations associated with human disease.

Helene Tronchére focused her presentation on myotubularin and phosphoinositides pathways. Phosphoinositides represent 10% of the total phospholipids pool of cell membranes, where they do not play a structural role but have been shown to be essential component of the signal transduction machinery. Their role of second messenger controls many key cellular functions like proliferation, apoptosis, vesicular trafficking and cytoskeleton remodeling. Phosphoinositides levels in cells are tightly controlled by a set of lipid phosphatases and kinases, some of them have been linked to human diseases, like the tumor suppressor PTEN. Tronchere's laboratory has previously shown that myotubularin, encoded by the MTM1 gene, which is mutated in the X-linked genetic muscular disorder myotubular myopathy (XLMTM), is a lipid phosphatase hydrolyzing PtdIns(3)P. PtdIns(3)P is localized in the membranes of internal vesicles, the early endosomes and is implicated in vesicular trafficking regulation. Tronchere's group has therefore looked for the implication of MTM1 in intracellular vesicular trafficking by comparing PtdIns(3)P levels and localization in XLMTM patients or control derived myoblasts and fibroblasts. They did not see any differences between the patients and control cell lines suggesting that MTM1 could act on small pools of PtdIns(3)P, but also that the phosphatase could have other(s) function(s) in the cell. Therefore, they pushed further the in vitro characterization of its phosphatase activity by using fluorescent lipids. Thus, they could show that MTM1 and also other members of the MTM family (MTMR1, MTMR2, MTMR3) can hydrolyze, in addition to PtdIns(3)P, PtdIns(3,5)P₂, leading to the production of phosphatidylinositol 5-phosphate (PtdIns(5)P). It was confirmed that myotubularin hydrolyzes phosphatidylinositol (3,5)P₂ in vivo and induces an increase of PtdIns(5)P. Future developments are devoted to studying the function of PtdIns(5)P whose function in the cell is still not known and trying to determine the importance of the lack of production of PtdIns(5)P in the etiology of

XLMTM [10].

Molecular function of MTM1 and related proteins

Michael Clague presented data demonstrating that several members of the myotubularin family utilize PtdIns(3,5)P₂ in addition to PtdIns3P as substrate [24]. Expression of MTM1 and MTMR3 in yeast demonstrated that hydrolysis of PtdIns(3,5)P₂ provides a biosynthetic pathway to PtdIns5P[25]. PtdIns5P is an allosteric activator of MTM1 and MTMR3 and a mutation in MTM1 (R69C), which leads to a moderate disease phenotype, shows both reduced enzyme activity and dampened response to PtdIns5P. Enzyme activity of MTM is non-linear within a narrow concentration range. Incubation of a catalytic site mutant of MTM1 with PtdIns5P and substrate (PtdIns3P) leads to the formation of a 12.5 nm diameter heptameric ring structure, which can be visualised by negative stain electron microscopy. It is proposed that this structure represents a stabilised form of an otherwise unstable intermediate that dissociates upon hydrolysis. This may explain the observed co-operativity of enzyme activation.

Luciano Pirola investigated the function of myotubularin in its most physiologically relevant context, i.e. in differentiated muscle cell lines. As differentiated muscle cell lines are hardly transfectable, he used an adenoviral vector to efficiently express wild type myotubularin and the two catalytically inactive mutants C375S and D278A. Metabolic labelling of L6 and C2C12 myotubes followed by purification of total phosphoinositides and profiling of the differently phosphorylated forms by HPLC demonstrated an increase of PtdIns(3)P upon expression of catalytically inactive myotubularins, suggesting they may have a dominant negative action. In keep with this observation, overexpression of GFP-myotubularin disrupted the endosomal punctuated staining of early endosomal antigen 1 (EEA1), a FYVE-domain-containing PtdIns(3)P binding protein.


The modulation of PtdIns(3)P levels by myotubularin associated to its capability to displace EEA1 from an endosomal localisation prompted Luciano Pirola to search for a cell function affected by PtdIns(3)P. As this lipid is involved in the trafficking of internal membranes [9], he enquired whether ectopic expression of myotubularin might affect insulin-induced glucose uptake, which is mediated by the translocation of the glucose transporter GLUT4 from intracellular insulin-responsive membranous compartments to the plasma membrane [12], a fusion process possibly requiring PtdIns(3)P. Indeed, expression of wt myotubularin-while not affecting the activation of proximal insulin signalling targets such as PKB and MAPK-induced a decrease in insulin-induced glucose uptake. Moreover, overexpression in 3T3-L1 adipocytes of myotubularin impaired insulin-induced translocation at the plasma membrane of GFP tagged GLUT4. Together, his observations indicate that PtdIns(3)P is required for the proper trafficking of GLUT4 and thus myotubularin might intervene in the regulation of this cellular process [26]. An important question to be further addressed is whether the impact of myotubularin on glucose transport has a significant physiological link to the development of myotubular myopathy.

The myotubularin deficient mouse

A mouse model for X-linked myotubular myopathy has been generated by homologous recombination in order to understand the physiopathological mechanism of the disease. Anna Buj-Bello showed that absence of myotubularin in mouse leads to a generalized and progressive myopathy starting at around 4 weeks of age [27]. The life span of knockout mice is severely reduced and death occurs at 59±19 days, probably due to cachexia and respiratory difficulties. The histopathological analysis of Mtm1 deficient mice reveals lesions only in the skeletal muscle with a progressive accumulation of paracentral and central nuclei in myofibres, fibre size variation and atrophy. Degenerative changes

appear progressively in fibres but signs of necrosis are very limited. The analysis of these mice shows that myotubularin is essential for skeletal muscle maintenance but not for the differentiation of myotubes into mature myofibres, contrary to prior hypothesis on human XLMTM pathogenesis. Anna Buj-Bello used a mouse conditional targeting strategy to determine whether a defect in proper innervation or nerve trophic supply to developing skeletal muscle could play a role in the pathogenesis of the disease. Two tissue specific knockout lines were generated by crossing an *Mtm1* conditional line with transgenic mouse lines overexpressing the Cre recombinase under either an HSA (human skeletal muscle α -actin) or NSE (human neuron specific enolase) promoter. The analysis of these mutant mice reveals that deletion of *Mtm1* specifically in skeletal muscle is sufficient to induce a severe centronuclear myopathy with clinical and pathological signs similar to that observed in constitutive knockout animals. *Mtm1* knockout mice will be used to dissect the molecular events leading to the myopathy and represent an important tool for developing therapeutic approaches.

Charcot-marie-tooth and MTMR related proteins

 [return to article outline](#)

Mutations in the gene on chromosome 11p15 encoding myotubularin-related protein 2 (MTMR2) are linked to autosomal recessive Charcot-Marie-Tooth disease type 4B1 (CMT4B1), a severe hereditary motor and sensory neuropathy characterized by focally folded myelin sheaths and demyelination [16], [17]. More recently, mutations in the MTMR13/SBF2 gene were identified by two different groups [18], [19] in patients with CMT4B2 showing myelin outfoldings in the sural nerve biopsy, and occasionally associated with infantile glaucoma. Both MTMR2 and MTMR13 belong to the Myotubularin-related (MTMR) family of dual specificity PTP-like protein phosphatases [2]. MTMR2 shows specific activity towards PI(3)P, both in vitro and in vivo as well as PI(3,5)P₂, as recently demonstrated in vitro. However, how abrogation of this lipid phosphatase activity is leading to the specific disease phenotype has not yet been demonstrated.

The group of Ueli Sutter analyzed the biochemical properties of the MTMR2 protein. Both, phosphatidylinositol-3-phosphate (PI(3)P) and PI(3,5)P₂, are substrates for MTMR2 and are dephosphorylated by this enzyme at the D3 position with high efficiency and peak activity at neutral pH [17]. Furthermore, disease-associated MTMR2 mutations lead to dramatically reduced phosphatase activity, indicating that the MTMR2 phosphatase activity is crucial for the proper function of peripheral nerves in CMT4B1. Functional dissection of different domains of the MTMR2 protein revealed that its membrane association is mediated by a pleckstrin homology- GRAM domain and a coiled-coil dimerization module [28]. In hypoosmotically stressed COS cells with increased levels of PI(3,5)P₂, MTMR2 is recruited to the membrane of vacuoles. The pleckstrin homology-GRAM and the coiled-coil domains are crucial mediators of this membrane targeting. The pleckstrin homology-GRAM domain binds directly to PI(3,5)P₂ and PI(5)P, a substrate and a product of the MTMR2 enzyme, respectively. In addition, MTMR2 forms dimers and the coiled-coil is responsible for homodimerization. Thus, phosphoinositide-protein interactions together with protein-protein interactions, are required for correct regulation of MTMR2.

On the cellular level, expression analysis of MTMR2 suggests particularly high levels in neurons with some expression also by myelinating Schwann cells. Thus, the demyelinating neuropathy CMT4B1 might be triggered by the malfunction of neural membrane recycling, membrane trafficking, and/or endocytic or exocytotic processes, combined with altered

axon-Schwann cell interactions [17], [29].

Alessandra Bolino and her group analysed the cellular and subcellular distribution of Mtmr2 (mouse MTMR2) in nerve. Mtmr2 was detected in all cytoplasmic compartments of myelin-forming Schwann cells, as well as in the cytoplasm of non-myelin forming Schwann cells and both sensory and motoneurons. In contrast, Mtmr2 was detected in the nucleus of Schwann cells and motoneurons, but not in the nucleus of sensory neurons [30]. As MTMR2 is diffusely present also within the nerve, a specific function could derive instead from nerve-specific interacting proteins. We performed two yeast two-hybrid screenings, using either fetal brain or peripheral nerve cDNA libraries. The neurofilament light chain protein, NF-L, was identified repeatedly in both screenings, and found to interact with MTMR2 in both Schwann cells and neurons. Interestingly, NF-L, encoding NF-L, is mutated in CMT2E and in severe forms of CMT often diagnosed as Dejerine-Sottas syndrome. These data may provide a basis for the nerve-specific pathogenesis of CMT4B1, and further support for the notion that hereditary demyelinating and axonal neuropathies may represent different clinical manifestations of a common pathological mechanism.

Jan Senderek reviewed in detail CMT4B2. So far, six families with SBF2/MTMR13 mutations were identified. Affected individuals have early-onset severe sensorimotor neuropathy with slow nerve conduction velocities. In some families, patients had congenital glaucoma or elevated intraocular pressure. Nerve biopsies showed signs of demyelination and characteristic myelin outfoldings. Preliminary expression studies suggest ubiquitous expression in mouse and human adult tissues, which is similar to other MTMRs. This clearly awaits more detailed studies, at different developmental stages, especially in neural and ocular tissues. The encoded SBF2/MTMR13 protein belongs to the catalytically inactive antiphosphatase branch of the myotubularin group. As shown for other active and inactive members of this family [23], [31], [32], SBF2/MTMR13 may physically interact with MTMR2. This would be a plausible explanation of how mutations in an active phosphatase (MTMR2) and an inactive phosphatase (SBF2/MTMR13) result in the same phenotype.

Centronuclear myopathy

 [return to article outline](#)

A whole session was dedicated to autosomal CNM [33].

First of all Caroline Sewry discussed the pathology of a variety of cases with an abundance of central nuclei. These included severe and infantile cases of X-linked myotubular myopathy, 2 fetuses with a mutation in the MTM1 gene and 5 females and one male in whom mutations in MTM1 and expansions of the gene for myotonic dystrophy had been excluded. A young female case presented at the previous workshop had been identified as a manifesting MTM1 carrier [34]. Severe male, X-linked cases showed large, well spaced central nuclei and peripheral nuclei were rare. Necrosis and endomysial fibrosis were not present and oxidative enzymes were abundant in the centre of fibres and were surrounded by a pale peripheral halo. In infantile cases central nuclei were often confined to small type 1 fibres and some nuclei were internal rather than central. Again, nuclei at the periphery of the fibre were rare. Some fibres were hypertrophic and type 1 atrophy/hypotrophy was also apparent. Oxidative enzyme stains showed pale peripheral halos but the dark centres were less obvious than in the severe cases. The possibility that this difference relates to age could not be excluded. Infantile cases can show lines of glycogen radiating from the centre of the fibre but this was not a universal feature in the

cases reviewed. Immunolabelling of myosin isoforms showed that the maturational switch occurs in myotubular myopathy and that fibres with central nuclei lack neonatal myosin with most fibres expressing the mature slow or fast isoform. Fibres with high levels of desmin and vimentin were not a universal feature. Electron microscopy of severe X-linked cases often showed clusters of dense tubules. Analysis of skeletal muscle from 2 fetuses, aged 13 weeks of gestation, with an MTM1 mutation, showed that myotube formation occurs and that there was a possible increase in the number of central nuclei. Myofibril and sarcomere formation had also occurred but myofibrils were sparse in some fibres. Some fibres also showed non-membrane bound spaces of unknown origin. An artefact of processing could not be excluded. The mutation therefore seems to relate to maintenance of the myofibres rather than to myogenesis. The 5 female and one male case without MTM1 mutations showed both peripheral and central nuclei and some showed mild endomysial fibrosis. Core-like lesions devoid of oxidative enzymes were a common feature in these cases. In view of these pathological findings and the clinical features, that included facial involvement and neck weakness, Dr Sewry suggested that the gene for the ryanodine receptor 1 (RYR1) should be screened before considering them as autosomal cases of myotubular/centronuclear myopathy. The pathological spectrum associated with defects in RYR1 is now known to be broad and includes the features found in these cases, and it was agreed that at least the 'hot spot' mutations in RYR1 should be excluded.

Heinz Jungbluth from London presented clinical, radiological and genetic data from 5 sporadic cases with histopathological features of myotubular (centronuclear) myopathy. Four of these cases were female and one was male. Age range was 4–14 years. All were cytogenetically normal.

In addition to a full clinical assessment, T1-weighted muscle MR images were obtained from the pelvis, thigh and lower legs in 4 patients. Mutational screening of the MTM1 gene (Dr Biancalana, Strasbourg) was performed following exclusion of the CTG repeat expansion associated with myotonic dystrophy. All affected females had X-inactivation studies in lymphocytes (Dr Kristiansen, Oslo).

A heterozygous mutation C1315T in exon 12 of the MTM1 gene was identified in this patient resulting in a R421X substitution [34], and previously reported in severely affected males. This patient had presented at birth with marked hypotonia. She did not achieve independent walking and required a gastrostomy and nighttime ventilation because of persistent feeding and respiratory difficulties. Urinary and stool incontinence were additional features, suggesting smooth muscle involvement. Marked facial weakness with bilateral ptosis and external ophthalmoplegia, a mild scoliosis and severe axial and proximal weakness were prominent clinical findings. Height and weight were above the 90th centile. The clinical phenotype of this female was of moderate severity and associated with an extremely skewed X-inactivation pattern (97:3) in favour of the X-chromosome harbouring the mutation. This case emphasizes that investigation of the MTM1 gene and X-inactivation studies are indicated in isolated females with histopathological and clinical findings suggestive of myotubular myopathy.

MTM1 mutations could not be identified in three female patients and one male patient. The only male patient without confirmed MTM1 mutation was more severely affected than other patients in this cohort and had presented antenatally with reduced fetal movements. There was a marked chest deformity and severe respiratory impairment. There were no axial antigravity movements with associated severe wasting of neck muscles. Feeding and respiratory difficulties were progressive and he died from respiratory failure in his second year of life.

All female patients without confirmed mutation had a normal X-inactivation pattern, providing further evidence against

involvement of the MTM1 gene. These patients presented at birth with marked hypotonia and two girls each required gastrostomy insertion and nocturnal ventilation for persisting feeding and respiratory difficulties. Motor development was delayed but all achieved independent ambulation. Delayed maturation of bladder control was a consistent finding, suggesting smooth muscle involvement. In two patients myasthenia gravis had been initially suspected because of a positive Tensilon test, consistent improvement following a trial of pyridostigmine and a suggestive single fibre EMG in one girl. Marked axial weakness and wasting of the lower leg with associated foot deformities were prominent findings on examination. Eye movements were preserved in all but one patient. All female patients shared a consistent appearance on muscle MRI characterized by marked and diffuse signal increase within the thigh and severe and diffuse involvement of the lower leg with relative sparing of the gastrocnemii. Muscle bulk within the thigh was better preserved compared to the X-linked form and muscle involvement was more diffuse compared to other congenital myopathies. Muscle MRI may therefore provide an additional tool in the assessment of patients with myotubular (centronuclear) myopathy. Norma Romero from Paris presented the clinical and morphological data from a group of 29 patients from 12 unrelated families affected by autosomal centronuclear myopathy [35]. The clinical course of CNM is nonspecific and the diagnosis is based on the morphological findings: centrally located nuclei in a large portion of muscle fibers, most often associated with fiber type 1 predominance and hypotrophy. The typical radial arrangement of sarcoplasmic strands around the central nuclei on NADH-tetrazolium reductase staining, initially described by Goulon et al. in 1976 [36], is an hallmark of CNM. It was always found in our series of muscle biopsies. The aim of our study is to better define the clinical and morphological characteristics of autosomal CNM and identify potentially homogeneous subgroups for genetic analysis.

Pascale Guicheney from Paris presented molecular studies performed on CNM. A canine autosomal recessive centronuclear-like myopathy has recently been reported [37]. It involves Labrador Retrievers, which exhibit early occurrence of muscle weakness, hypotonia, proximal muscle wasting and abnormal neck posture, mimicking the clinical evolution of the human centronuclear myopathy. The muscle pathology is also similar to that seen in human CNM, and consists in with centralized myofiber nuclei associated with predominance and hypotrophy of type 1 fibers. By homozygosity mapping, a locus has been identified on canine chromosome 2, in a 18cM interval including the VIM gene encoding vimentine [38]. The human locus, syntenic to canine *cnm*, is located in 10p13-14.


The group of Pascale Guicheney used four microsatellite markers, D10S547, D10S1653, D10S548, and D10S197, to screen this human locus in two large autosomal dominant (AD) CNM families and one putative autosomal recessive family by linkage analysis. In one AD family, an haplotype was cosegregating with most of the affected subjects. In this interval, PTPLA gene was thus considered as a putative candidate gene. This gene encoded a Tyrosine Phosphatase-Like Protein with a proline instead of arginine in its catalytic site, presenting an early expression at embryonic day 8.5 in murin muscle progenitors and later in differentiated muscle types. In murin and human adult tissues it is expressed in heart, skeletal muscle, liver, testis and kidney, but its exact role in myogenesis or cardiogenesis is not known [39], [40]. The seven exons corresponding to the coding sequence were studied in 6 CNM probands but no mutation was found [41].

Besides, a genome screening was performed in the two French AD CNM families by the French National Center of Genotyping (CNG, Evry) with 400 microsatellite markers set with an average spacing of 10cM, and a putative locus common to these two families was found. Confirmation of this locus will be undertaken by the analysis of additional

markers and family members and by analysis of additional CNM families.

Jocelyn Laporte used a candidate gene approach testing MTMRs for mutation in autosomal CNM. He searched for mutations in an interacting partner of MTM1. No mutations were found in 17 patients with suspected autosomal CNM (dominant, recessive and sporadic cases), nor in 11 patients with suspected XLMTM where no MTM1 mutations have been found. Other functional candidate genes implicated in the same functions as myotubularin are currently being sequenced and additional CNM patients are collected.

X-linked myotubular myopathy and trophic factors

 [return to article outline](#)

Insulin related trophic factors (IGF1 and IGFII) in patients with myotubular myopathy

Finally the last session was devoted to recent findings emerged on the reported growth abnormalities in XLMTM. It is well known that the majority of the patients with XLMTM have linear growth that remains above the 50th percentile [5]. Moreover, in the large series of 55 males reported by Herman et al. [5] five patients, ranging in age from 3 to 9 years, had documented bone ages >1 standard deviation above their chronologic age in comparison with radiographic standards. Enrico Bertini reported the detailed study on 2 patients affected by a severe XLMTM confirmed by 2 missense mutations (F438S and R69C). These 2 patients had typical clinical signs of XLMTM, showed respiratory failure from birth and were still ventilator dependent at the age of 1 year. In both babies serum levels of IGF1 were not quantifiable at the age of 6 months, while the serum levels of IGFII, IGFBP2, and GH were in normal ranges for age. These 2 patients had no signs of overgrowth and the bone age corresponded to the anagraphic age by the age of 12 months. Another patient with a mild form of XLMTM and a de novo missense mutation (T197I), who is still walking at the age of 23 years, had an overgrowth syndrome with accelerated bone age [42]. In this patient IGF1 serum levels were increased (345 ng/ml, normal ranges: 50–150), IGFII were at the higher limits (1622 ng/ml; normal range: 1100–1700), and IGFBP3 was markedly increased (7.5 ng/ml; nv: 1.9–4.5). Anna Buj-Bello reported a study on 2 patients with severe XLMTM carrying MTM1 deletions (a 46 bp intronic deletion encompassing exon 7 splice site and a large deletion from exon 4 to 15). One patient had an overgrowth syndrome and the other had an accelerated bone age. The bone age of the patient with overgrowth has not been calculated yet. IGF1 serum level was increased in both cases and IGFBP3 was at the upper limit in one patient (2.6 mg/ml, normal range: 1.1–2.5 mg/ml). GH blood level was normal for the age and IGF2 was not analysed in these patients. From this work we can demonstrate that by measuring systematically insulin related trophic factors in serum we may subdivide XLMTM patients in 2 groups: one with markedly reduced IGF1 in serum and a second group with overgrowth and increased serum IGF1. Further confirmation studies are needed in a larger series of patients.

Insulin signaling pathways and related trophic factors in skeletal muscle. Lessons from the transgenic mouse for IGF1. Antonio Musaro' presented an overview on IGF-I and muscle. Among growth factors, the insulin-like growth factor-1 (IGF-1) has been implicated in the control of skeletal muscle growth and differentiation in embryonic development, while in the adult it plays a central role during muscle regeneration [43]. Since IGF-1 levels decline with age in both rodents and human muscle, this growth factor has been considered a promising therapeutic agent in staving off advancing muscle weakness during ageing and promoting regeneration.

Skeletal muscle regeneration involves the activation of quiescent satellite cells, which participate in the reconstitution of damaged tissue. However, in several muscle pathologies the regenerative process is compromised, leading to a progressive loss of muscle mass and function. In this context the goal of our research was to characterize the role of factors involved in muscle regeneration and repair.

His group reported that muscle-specific expression of a local IGF-1 isoform (mIGF-1) promotes and improves regeneration in senescent muscle [44]. In addition they have recently reported that high levels of mIGF-1 transgene expression in the mdx mouse model of muscular dystrophy also preserves muscle function in the absence of dystrophin, inducing significant hypertrophy and hyperplasia at all ages observed, reducing fibrosis and myonecrosis, and elevating signaling pathways associated with muscle survival and regeneration [45]. Proliferation of desmin-expressing cells in the injured area of mIGF-1 transgenic muscle, suggested that mIGF-1 induces a local increase in the myoblast population in response to damage. In addition, they analyzed whether muscle regeneration involved the recruitment of uncommitted cell populations, and they have been able to demonstrate that the capacity of the transgenic muscle to regenerate is also associated to an increase in the recruitment of circulating stem cells expressing Sca1 and c-Kit, general markers of hematopoietic stem cells. The recruited uncommitted cells homing the damage muscle, contribute to muscle regeneration and guarantee a reserve of muscle stem cells.

IGF-1 can therefore act, in combination with other regenerative factors, as a homing signal attracting circulating stem cells to repair injured muscle.

Conclusions

 [return to article outline](#)

This workshop has been particularly useful in giving some guidelines for the molecular diagnosis of XLMTM and to start clarifying some points on the function of myotubularin and myotubularin-related proteins. The presentations related to unravel the function of myotubularin have given prerequisites for accelerating future therapeutic directions. The workshop has also been fruitful in describing different strategies directed to solve the genetic characterization of several forms of autosomal centronuclear myopathies, and will surely lead to new collaborations in this field.

Acknowledgements

 [return to article outline](#)

This workshop was made possible thanks to the financial support of the European Neuromuscular Centre (ENMC) and the ENMC main sponsors: Association Française contre les Myopathies (France); Deutsche Gesellschaft für Muskelkranke (Germany); Telethon Foundation (Italy); Muscular Dystrophy Group of Great Britain and Northern Ireland (UK); Muskelvindfonden (Denmark); Prinses Beatrix Fonds (Netherlands); Schweizerische Stiftung für die erforschung der Muskelkrankheiten (Switzerland); Verein Zur Erforschung von Muskelkrankheiten bei Kindern (Austria); Vereniging Spierziekten Nederland (Netherlands); and ENMC associated member: Muscular Dystrophy Association of Finland

appendix a List of participants
Enrico Bertini (Rome, Italy)

Valerie Biancalana (Strasbourg, France)
Alessandra Bolino (Milan, Italy)
Anna Buj Bello (Strasbourg, France)
Michael J. Clague (Liverpool, UK)
Pascale Guicheney (Paris, France)
Heinz Jungbluth (London, UK)
Wolfram Kress (Wurzburg, Germany)
Antonio Musaro (Rome, Italy)
Harshal Nandurkar (Melbourne, Australia)
Luciano Pirola (Nice, France)
Norma Romero (Paris, France)
Jan Senderek (Aachen Germany)
Ueli Suter (Zurich, Switzerland)
Caroline Sewry (London, UK)
Helene Tronchere (Toulouse, France)
Carina Wallgren-Pettersson (Helsinki, Finland)
Matthew J. Wishart (La Jolla, CA, USA)
Jocelyn Laporte (Strasbourg, France)

References

 [return to article outline](#)

- [1]. Laporte J, Hu LJ, Kretz C, et al. A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat Genet.* 1996;13:175-182 [MEDLINE](#) | [CrossRef](#)
- [2]. Laporte J, Bedez F, Bolino A, Mandel JL. Myotubularins, a large disease-associated family of cooperating catalytically active and inactive phosphoinositides phosphatases. *Hum Mol Genet.* 2003;12:285-292 [Full Text](#) | [PDF \(131 KB\)](#)
- [3]. Biancalana V, Caron O, Gallati S, et al. Characterisation of mutations in 77 patients with X-linked myotubular myopathy, including a family with a very mild phenotype. *Hum Genet.* 2003;112:135-142 [Abstract](#) | [Full Text](#) | [PDF \(504 KB\)](#)
- [4]. Herman GE, Kopacz K, Zhao W, Mills PL, Metzenberg A, Das S. Characterization of mutations in fifty North American patients with X-linked myotubular myopathy. *Hum Mut.* 2002;19:114-121
- [5]. Herman GE, Finegold M, Zhao W, de Gouyon B, Metzenberg A. Medical complications in long-term survivors with X-linked myotubular myopathy. *J Pediatr.* 1999;134:206-214 [CrossRef](#)
- [6]. Kristiansen M, Knudsen GP, Tanner SM, et al. X-inactivation patterns in carriers of X-linked myotubular myopathy. *Neuromuscul Disord.* 2003;13:468-471 [Abstract](#) | [Full Text](#) | [PDF \(76 KB\)](#) | [CrossRef](#)
- [7]. Schara U, Kress W, Tucke J, Mortier W. X-linked myotubular myopathy in a female infant caused by a new MTM1 gene mutation. *Neurology.* 2003;60:1363-1365 [Full Text](#) | [PDF \(131 KB\)](#)
- [8]. Laporte J, Kress W, Mandel JL. Diagnosis of X-linked myotubular myopathy by detection of myotubularin. *Ann Neurol.* 2001;50:42-46 [CrossRef](#)
- [9]. Simonsen A, Wurmser AE, Emr SD, Stenmark H. The role of phosphoinositides in membrane transport. *Curr Opin*

Cell Biol. 2001;13:485-492 Full Text | PDF (131 KB) | CrossRef

[10]. Tronchere H, Laporte J, Pendaries C, Chaussade C, Liaubet L, Pirola L, et al. Production of phosphatidylinositol 5-phosphate by the phosphoinositide 3-phosphatase myotubularin in mammalian cells. *J Biol Chem.* 2004;279:7304-7312 Abstract | Full Text | PDF (109 KB) | CrossRef

[11]. Vanhaesebroeck B, Leever SJ, Panayotou G, Waterfield MD. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem Sci.* 1997;22:267-272 Abstract | Full Text | PDF (119 KB) | CrossRef

[12]. Mora S, Pessin JE. An adipocentric view of signaling and intracellular trafficking. *Diab Metab Res Rev.* 2002;18:345-356

[13]. Czech MP. PIP2 and PIP3: complex roles at the cell surface. *Cell.* 2000;100:603-606 Abstract | Full Text | PDF (381 KB) | CrossRef

[14]. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. *Genes Dev.* 1999;13:2905-2927 Abstract | Full Text | PDF (930 KB) | CrossRef

[15]. Corvera S, D'Arrigo A, Stenmark H. Phosphoinositides in membrane traffic. *Curr Opin Cell Biol.* 1999;11:460-465 Abstract | Full Text | PDF (111 KB) | CrossRef

[16]. Bolino A, Muglia M, Conforti FL, et al. Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. *Nat Genet.* 2000;25:17-19 CrossRef

[17]. Berger P, Bonneick S, Willi S, Wymann M, Suter U. Loss of phosphatase activity in Myotubularin-Related-Protein-2 is associated with Charcot-Marie-Tooth disease type 4B1. *Hum Mol Genet.* 2002;11:1569-1579 Abstract | Full Text | PDF (231 KB) | CrossRef

[18]. Senderek J, Bergmann C, Weber S, et al. Mutation of the SBF2 gene, encoding a novel member of the myotubularin family, in Charcot-Marie-Tooth neuropathy type 4B2/11p15. *Hum Mol Genet.* 2003;12:349-356 Abstract | Full Text | PDF (231 KB) | CrossRef

[19]. Azzedine H, Bolino A, Taieb T, et al. Mutations in MTMR13, a new pseudophosphatase homologue of MTMR2 and Sbf1, in two families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease associated with early-onset glaucoma. *Am J Hum Genet.* 2003;72:1141-1153 Abstract | Full Text | PDF (306 KB) | CrossRef

[20]. Laporte J, Blondeau F, Gansmuller A, Lutz Y, Vonesch JL, Mandel JL. The PtdIns3P phosphatase myotubularin is a cytoplasmic protein that also localizes to Rac1-inducible plasma membrane ruffles. *J Cell Sci.* 2002;115:3105-3117 Full Text | PDF (131 KB)

[21]. Caldwell KK, Lips DL, Bansal VS, Majerus PW. Isolation and characterization of two 3-phosphatases that hydrolyze both phosphatidylinositol 3-phosphate and inositol 1,3-bisphosphate. *J Biol Chem.* 1991;266:18378-18386 Abstract | Full Text | PDF (292 KB) | MEDLINE

[22]. Nandurkar HH, Caldwell KK, Whisstock JC, et al. Characterization of an adapter subunit to a phosphatidylinositol (3)P 3-phosphatase: identification of a myotubularin-related protein lacking catalytic activity. *Proc Natl Acad Sci USA.* 2001;98:9499-9504 Full Text | PDF (131 KB) | CrossRef

[23]. Nandurkar HH, Layton M, Laporte J, et al. Identification of myotubularin as the lipid phosphatase catalytic subunit associated with the 3-phosphatase adapter protein, 3-PAP. *Proc Natl Acad Sci USA.* 2003;100:8660-8665 Full Text | PDF (131 KB) | CrossRef

[24]. Schaletzky J, Dove SK, Short B, Lorenzo O, Clague MJ, Barr FA. Phosphatidylinositol-5-phosphate activation and conserved substrate specificity of the myotubularin phosphatidylinositol 3-phosphatases. *Curr Biol.* 2003;13:504-509

CrossRef

[25]. Walker DM, Urbe S, Dove SK, Tenza D, Raposo G, Clague MJ. Characterization of MTMR3. an inositol lipid 3-phosphatase with novel substrate specificity. *Curr Biol.* 2001;11:1600-1605 Abstract | Full Text | PDF (756 KB) |

CrossRef

[26]. Chaussade C, Pirola L, Bonnafous S, et al. Expression of myotubularin by an adenoviral vector demonstrates its function as a PtdIns(3)P phosphatase in muscle cell lines. Involvement of PtdIns(3)P in insulin-stimulated glucose transport. *Mol Endocrinol.* 2003;17:2448-2460 Full Text | PDF (131 KB) | CrossRef

[27]. Buj-Bello A, Laugel V, Messaddeq N, Zahreddine H, Laporte J, Pellissier JF, et al. The lipid phosphatase myotubularin is essential for skeletal muscle maintenance but not for myogenesis in mice. *Proc Natl Acad Sci USA.* 2002;99:15060-15065 Abstract | Full Text | PDF (231 KB) | CrossRef

[28]. Berger P, Schaffitzel C, Berger I, Ban N, Suter U. Membrane association of myotubularin-related-protein-2 is mediated by a pleckstrin homology-GRAM domain and a coiled-coil dimerization module. *Proc Natl Acad Sci USA.* 2003;100:12177-12182 Full Text | PDF (131 KB) | CrossRef

[29]. Suter U, Scherer SS. Disease mechanisms in inherited neuropathies. *Nat Rev Neurosci.* 2003;4:714-726 Full Text | PDF (71 KB)

[30]. Previtali SC, Zerega B, Sherman DL, et al. Myotubularin-related 2 protein phosphatase and neurofilament light chain protein, both mutated in CMT neuropathies, interact in peripheral nerve. *Hum Mol Genet.* 2003;12:1713-1723 Full Text | PDF (131 KB) | CrossRef

[31]. Kim SA, Vacratsis PO, Firestein R, Cleary ML, Dixon JE. Regulation of myotubularin-related (MTMR)2 phosphatidylinositol phosphatase by MTMR5, a catalytically inactive phosphatase. *Proc Natl Acad Sci USA.* 2003;100:4492-4497 Full Text | PDF (131 KB) | CrossRef

[32]. Mochizuki Y, Majerus PW. Characterization of myotubularin-related protein 7 and its binding partner, myotubularin-related protein 9. *Proc Natl Acad Sci USA.* 2003;100:9768-9773 Full Text | PDF (131 KB) | CrossRef

[33]. Fardeau M, Tomé F. Congenital myopathies. In: Engel AG, Franzini-Armstrong C, eds. *Myology.* (2nd ed.) New York: MacGraw Hill 1994:1500-1504

[34]. Jungbluth H, Sewry CA, Buj-Bello A, et al. Early and severe presentation of X-linked myotubular myopathy in a girl with skewed X-inactivation. *Neuromuscul Disord.* 2002;13:55-59 Full Text | PDF (325 KB) | CrossRef

[35]. Jeannet PY, Bassez G, Eymard B, et al. Clinical and morphological heterogeneity in 29 patients with autosomal centronuclear myopathy. *Neurology.* 2004;62:1484-1490 Abstract | Full Text | PDF (153 KB)

[36]. Goulon M, Fardeau M, Got L, Babinet P, Manko E. Centronuclear myopathy with late clinical manifestations. Clinical, histological and ultrastructural study of a new case. *Rev Neurol.* 1976;132:275-290 Full Text | PDF (131 KB)

[37]. Blot S, Tiret L, Devillaire AC, Fardeau M, Dreyfus PA. Phenotypic description of a canine centronuclear myopathy. *J Neurol Sci.* 2002;9:199(Abstract) Full Text | PDF (131 KB)

[38]. Tiret L, Blot S, Kessler JL, Gaillot H, Breen M, Panthier JJ. The CNM locus, a canine homologue of human autosomal forms of centronuclear myopathy, maps to chromosome 2. *Hum Genet.* 2003;113:297-306 Abstract | Full Text | PDF (153 KB) | CrossRef

[39]. Uwanogho DA, Hardcastle Z, Balogh P, Mirza G, Thornburg KL, Ragoussis J, et al. Molecular cloning, chromosomal mapping, and developmental expression of a novel protein tyrosine phosphatase-like gene. *Genomics.* 1999;62:406-416 Full Text | PDF (131 KB) | CrossRef

- [40]. Li D, Gonzalez O, Bachinski LL, Roberts R. Human protein tyrosine phosphatase-like gene: expression profile, genomic structure, and mutation analysis in families with ARVD. *Gene*. 2000;256:237-243 Full Text | PDF (131 KB) | CrossRef
- [41]. Jeannot PY, Maugendre S, Gouas L, Fardeau M, Romero N, Guicheney P. Five families with centronuclear myopathy (CNM) unlinked to the canine CNM locus. 8th International World Muscle Society Congress, Szeged, Hongrie, 3–6 Septembre. *Neuromuscul Disord*. 2003;13:628(Abstract) Full Text | PDF (131 KB)
- [42]. Flex E, De Luca A, D'Apice MR, Buccino A, Dallapiccola B, Novelli G. Rapid scanning of myotubularin (MTM1) gene by denaturing high-performance liquid chromatography (DHPLC). *Neur Disord*. 2002;12:501-505
- [43]. Musaro A, Rosenthal N. Maturation of the myogenic program is induced by postmitotic expression of insulin-like growth factor I. *Mol Cell Biol*. 1999;19(4):3115-3124 Abstract | Full Text | PDF (311 KB)
- [44]. Musaro A, McCullagh K, Paul A, et al. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet*. 2001;27(2):195-200 Abstract | Full Text | PDF (125 KB) | CrossRef
- [45]. Barton ER, Morris L, Musaro A, Rosenthal N, Sweeney HL. Muscle-specific expression of insulin-like growth factor I counters muscle decline in mdx mice. *J Cell Biol*. 2002;157(1):137-148 Abstract | Full Text | PDF (70 KB) | CrossRef

a Department of Laboratories, Unit of Molecular Medicine, Bambino Gesù' Childrens Hospital, P.za S. Onofrio 4, 00165 Rome, Italy

b Laboratory of Diagnostic Genetics, Faculty of Medicine, University of Strasbourg, Strasbourg, France

c Dulbecco Telethon Institute, DIBIT, San Raffaele Scientific Institute, Milan, Italy

d IGBMC, CNRS/INSERM/Université Louis Pasteur Strasbourg, 67404 Illkirch Cedex, France

e Physiological Laboratory, University of Liverpool, Crown Street, L69 3BX, Liverpool, UK

f INSERM U582, Institut de Myologie, GH Pitié-Salpêtrière, Paris, France

g Dubowitz Neuromuscular Centre, Faculty of Medicine, Hammersmith Hospital, Imperial College, London, UK

h Institut der Humangenetik, Universität Würzburg, Würzburg, Germany

i Department of Histology and Medical Embryology, University of Rome La Sapienza, Rome, Italy

j St. Vincent's Hospital, 3065 Melbourne, Australia

k INSERM Unit 145, Faculty of Medicine, Ave de Valembrose, 06107, Nice Cedex 2, France

l Department of Human Genetics, Aachen University of Technology, Aachen, Germany


m Institute of Cell Biology, Swiss Federal Institute of Technology Zurich, ETH-Honggerberg, Zurich, Switzerland

n Department of Histopathology, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, UK

o Pr. Delsol, INSERM U563-Bat. C, Hopital Purpan, Toulouse 31059, France

p The Folkhalsan Department of Medical Genetics, University of Helsinki, Helsinki, Finland

q Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI, USA

 * Corresponding author. Tel.: +39-06-685-92105; fax: +39-06-685-92024

doi: 10.1016/j.nmd.2004.04.002

