Functional effects of mutations identified in patients with multiminicore disease

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SUMMARY

Multiminicore disease is a recessive congenital myopathy characterized by the presence of small cores or areas lacking oxidative enzymes, in skeletal muscle fibres. From a clinical point of view, the condition is widely heterogeneous and at least four phenotypes have been identified; genetic analysis has revealed that most patients with the classical form of multiminicore characterized by rigidity of the spine, early onset and respiratory impairment harbour recessive mutations in the *SEPN1* gene, whereas the majority of patients belonging to the other categories, including patients with ophthalmoplegia or patients with a phenotype similar to central core disease, carry recessive mutations in the *RYR1*. In the present review we discuss the most recent findings on the functional effect of mutations in *SEPN1* and *RYR1* and discuss how they may adversely affect muscle function and lead to the clinical phenotype.

INTRODUCTION

Multi-minicore disease (MmD; MIM # 255320) is an early onset congenital myopathy usually inherited in an autosomal recessive mode (1). Histochemical and electron microscopic studies on biopsies from patients affected by MmD show small cores, which are areas of sarcomeric disorganization lacking oxidative enzymes and mitochondria (fig. 1, panels C and D). Multiple small cores are present in most muscle fibres, affecting few sarcomeres and typically not running the length of the muscle fibre; this feature distinguishes MmD from central core disease (CCD) in which cores are larger and run the entire length of the fibre (fig.1 panels A and B). There are typically no signs of muscle regeneration/degeneration.

Clinical features associated with the histopathologic appearance of multiminicores are markedly heterogeneous and to date four clinical phenotypes have been recognized. The “classic” and most common phenotype (2-4) is characterized by spinal rigidity, axial weakness, scoliosis and early respiratory impairment. In a second variant of MmD, in addition to a distribution of weakness and wasting similar to the classic form, patients also exhibit extraocular muscle involvement (2,5,6). In a third variant, patients show proximal weakness pronounced in the hip girdle similar to CCD with little or no respiratory impairment, whilst other patients may feature marked distal weakness, wasting and arthrogryposis (2,3).

The clinical variability seen in MmD partially corresponds to underlying genetic heterogeneity: the first molecular defect identified in patients affected by the classical form of MmD, were mutations in the selenoprotein 1 (*SEPN1*) gene (4,7). Further genetic investigations revealed that patients affected by forms of MmD with ocular involvement, carried mutations in the skeletal muscle ryanodine receptor gene (*RYR1*) (8-10), as did those with predominant pelvic girdle weakness. The present review will focus on the molecular defects underlying MmD and in particular on recent advancements on the functional consequences of newly identified *SEPN1* and *RYR1* mutations. For more in-depth clinical information, the readers are referred to several articles in this field (2,3,9).

Mutations in the Selenoprotein N (*SEPN1*) gene:

Selenium is an essential element whose deficiency can interfere with normal embryonic development and fertility. The major biological form of selenium in bacteria and eukaryotes is the 21st amino acid selenocysteine, which is found in the active site of selenoproteins and is directly involved in catalytic reactions (11). Selenocysteine is encoded by an in-frame UGA codon. In order to read through the UGA translational stop codon, the 3'-untranslated region of the selenoprotein mRNA must have a hairpin sequence called the selenocysteine insertion sequence (SECIS) which, together with a SECIS binding protein, a special elongation factor (EFsec) and a selenocysteyl-tRNA, form a functional complex. Disruption of this complex (or limiting selenium availability in the diet) results in premature termination at the UGA codon, giving rise to selenoprotein mRNAs which are susceptible to degradation via the "nonsense-mediated decay pathway" (12).

At least 26 selenoproteins have been identified in humans and shown to mediate a number of important functions, including the regulation of thyroid hormone metabolism, maintenance of the redox status of the cell and scavenging reactive oxygen species (13,14). Selenoprotein N, one of the last members of selenoproteins to be identified, is a 70 kDa integral membrane glycoprotein localized in the endoplasmic reticulum of many tissues, including pancreas, kidney, heart, liver, lung, brain and skeletal muscle. Though its function remains unknown, its expression is developmentally regulated as demonstrated by its reduced expression during human myoblast differentiation (15,16).

SEPN1 is encoded by a gene (*SEPN1*; MIM#606210) present on human chromosome 1p36 (RSMD1 locus); *SEPN1* is made up of 13 exons spanning 18.5 Kb giving rise to a transcript of 4.5 Kb encoding for a protein of 590 amino acids (17). Two isoforms are predicted to exist: isoform 1 corresponds to the full length transcript, whereas in isoform 2, exon 3 is spliced out. Mutations in the *SEPN1* gene were first identified by Moghadaszadeh et al. (17) in 10 families whose members were affected by a congenital form of rigid spine muscular dystrophy (MIM #602771). In one of these families, the mutation (1385G->A) transformed the selenocysteine specific codon TGA into the stop codon TAA, thus preventing selenocysteine incorporation and leading to nonsense-mediated decay. In the other families, novel mostly private *SEPN1* mutations were found. To date, approximately 20 mutations have been identified within the coding

sequence of the *SEPN1* gene (Table 1)(4,18,19). Among these are a nonsense mutation (1370C->T), a homozygous 20 bp duplication in exon 1 and a 1 nucleotide insertion at position 1, that are all predicted to result in a truncated protein which should be degraded via the nonsense-mediated decay pathway. More recently, a point mutation within the SECIS domain of the hairpin-containing 3' untranslated region of SEPN1 mRNA was found (20). The latter mutation, together with mutations affecting the initiation codon or insertions within exon 1, were shown to significantly diminish the level of SEPN1 expressed in cultured fibroblasts (20), confirming that mRNA degradation occurs when the transcript is terminated prematurely or when the SECIS complex can not properly assemble and direct read-through the internal UGA selenocysteine –encoding, termination codon.

It is noteworthy that at least half of the mutations identified in patients with the classical form of MmD give rise to truncated selenoprotein N. Thus, though the functional role of *SEPN1* remains largely speculative, one can not exclude the possibility that the selenocysteine residues of *SEPN1* might participate in redox reactions, as has been observed for other members of the selenoprotein family. If this is so, the lack of *SEPN1* could be associated with alterations of redox activity of skeletal muscle membranes. Behne et al. (13) reported the content of selenium in various rat tissues and organs and found that tissue containing most selenium appears to be the testes (6600 µg/kg dry tissue), followed by liver, adrenal gland, pituitary gland and skeletal muscle (440 µg/kg dry tissue). If one considers that skeletal muscle represents approximately 40% of the human body weight, then muscle is the body organ with the highest selenium content. But what is the role of selenium in skeletal muscle and how may truncation *SEPN1* affect muscle function? And in particular, how can one link *SENP1* deficiency to the multiminicores in skeletal muscles? One interesting observation is that patients with classical MmD share a very similar clinical phenotype, indicating that mutations in the *SEPN1* gene result in a "all or nothing" effect. Selenium compounds have been shown to cause (i) calcium release from isolated rabbit sarcoplasmic reticulum vesicles and (ii) affect ryanodine binding in a biphasic manner, whereby low concentrations (high nM to sub- µM) open the RyR channel and high concentrations (100 µM), inhibit channel opening (21). The mechanism of action of selenium is thought to involve oxidation of

functional thiol groups on the RyR1 (21). Since the RyR1 contains 100 cysteine residues per monomer and their state of oxidation strongly influences channel activity (22), depletion of *SEPN1* might be associated with an alteration of the *SEPN1*/selenium ratio which may result in a moderate oxidative stress leading to an abnormal function of the RyR calcium channel and/or of other integral SR proteins and even in those patients in whom *SEPN1* mutations do not result in truncated proteins, the mutations may affect SEPN1-dependent redox activity.

Mutations in the Ryanodine Receptor 1 (*RYR1*) gene:

After the initial identification of *SEPN1* mutations, it became clear that not all patients with MmD harbour such mutations and search for other candidate genes, revealed that some patients harbour mutations in the gene encoding the skeletal muscle ryanodine receptor (*RYR1*)(6,8,9). In the present review we will only focus on patients affected by myopathy with minicores not affected by the "classical" form of MmD. The mode of inheritance of this condition is usually autosomal recessive (whereas CCD is considered dominantly inherited), presence of multiple small cores in fibre types 1 and 2, which are short and do not run the fibre length and weakness of the hands.

Ryanodine receptors (RyR) are intracellular channels mediating calcium release from intracellular stores of excitable cells (23). Three isoforms encoded by different genes have been identified at the molecular level: type 1 RyRs are predominantly expressed in skeletal muscle and in humans are encoded by a gene on chromosome 19q13.1 (*RYR1*) (24). Type 2 RyRs are mainly expressed in the heart and cerebellum and are encoded by a gene present on human chromosome 1 (25). Type 3 RyRs are expressed throughout the central nervous system as well as in a variety of other tissues and their expression level appears to be developmentally regulated; the gene encoding RyR3 is located on human chromosome 15 (26). The three isoforms share an overall amino acid identity of approximately 60% and experimental evidence suggests that they are structurally similar, with a large hydrophilic NH_2-terminal domain and a hydrophobic COOH-terminal domain containing several transmembrane domains as well as the channel pore.

RYR1 is composed of 106 exons and encodes for a protein of 5038 amino acids, which assembles as a tetramer of >2 MDa and constitutes the functional skeletal muscle sarcoplasmic reticulum calcium release channel (24,27), a key player in excitation-contraction coupling i.e. the process linking plasma membrane depolarization to Ca^{2+} release from the lumen of the sarcoplasmic reticulum (28-30). In addition to the RyR protein, the functional channel binds a number of accessory proteins (calmodulin, S100, FK506BP, sorcin, homer, calcineurin , PKA) which regulate its activity (for review see 31-32).

More than 100 dominant mutations in the *RYR1* have been identified to date in patients and found to associate with dominantly inherited conditions (for recent reviews see 33,34): Malignant Hyperthermia (MH; OMIM #145600) and Central Core Disease (CCD; OMIM # 117000). In these disorders, disease-causing mutations appear to cluster in 3 defined regions of the *RYR1*. In MH, a pharmacological disorder triggered by exposure to volatile anaesthetics and/or muscle relaxants (35,36), disease-linked mutations predominantly cluster in domains within the hydrophilic portion of the protomer, whereas in CCD most *RYR1* substitutions occur in the hydrophobic pore-forming region. As shown in Table 2, recessive *RYR1* mutations identified so far in a subgroup of MmD patients, do not cluster in particular hot spots, but rather are distributed throughout the whole *RYR1* gene.

As to the functional effect of mutations in this protein, a number of studies have demonstrated that most *RYR1* mutations associated with MH and CCD affect calcium homeostasis either because they render the channel hypersensitive to activation (MH-linked mutations), or because they decrease the amount of calcium released after activation (CCD-linked mutations) (33,37). Few studies so far have appeared on the functional effect of recessive *RYR1* mutations linked to minicore myopathies. In the first functional study (38,39) we showed that the P3527S and V4849I *RYR1* substitutions are associated with a slightly elevated resting calcium concentration, but not depleted intracellular stores. Furthermore, upon pharmacological RyR1 activation, cells harbouring the homozygous P3527S mutation were found to release significantly less calcium. We hypothesised that since this mutation lies in the vicinity of calmodulin and S100 binding sites on the RyR1 and since the αhelix breaking amino acid proline is

substituted by serine, the mutation may interfere with the formation of a stable macromolecular complex, whereby proteins which normally bind to the RyR macromolecular complex and regulate its activity, do so in an altered way (38). A similar conclusion concerning instability of the RyR macromolecular complex carrying the S71Y+N2283H mutations was also recently reached (39), though in the latter case, (i) no reduction in calcium released was observed after pharmacological RyR stimulation, and (ii) one of the mutations (N2283H) actually increased the sensitivity of the RyR1 activation by KCl and caffeine, and is probably also linked to the MHS phenotype.

As to other MmD-linked *RYR1* mutations, Western blot analysis on muscle biopsies from patients characterised by muscle weakness and ophthalmoplegia, carrying the R109W+M485V and the homozygous 14646+2.99 kb intronic splicing variant, revealed very low endogenous RyR protein levels (39,40). Thus, in some MmD patients, particularly those with ophthalmoplegia, part of the clinical phenotype may be caused by a decrease in the amount of calcium release channels present in the sarcoplasmic reticulum. In this case, depolarization of the plasma membrane will be accompanied by a reduction of calcium release not because the mutations affect the properties of the RyR channel *per se* or the intracellular stores, but simply because fewer channels are present. One aspect which remains to be investigated is whether ophthalmoplegia is due to a decrease in RyR1 content which can not be compensated by the expression of other RyR isoforms, such as RyR3; little is known about the expression of this isoform in the ocular rectus muscles. In a study on non-mammalian vertebrate RyR isoform expression, O'Brien et al. reported that extraocular muscles of fish and birds only express the αRyR isoform, whereas most other muscles such as the swimming muscles of fish or pectoral muscle of birds, express the α and ß RyR isoforms (41). Some adult skeletal muscles, including abdominal muscles, diaphragm and soleus have been shown to express type 3 RyR (42). One plausible explanation for the partial muscle function in these MmD patients, is that in those muscles in which type 3 RyR is expressed, the latter isoform is functionally compensating for the diminished levels of RyR1, maybe by forming heteroduplex channels of RyR1and RyR3. Thus, the distinctive ocular involvement in this subset of patients may reflect the absence of compensation by type 3 RyR in the eye.

Finally, a recent in investigation aimed at uncovering novel mutations, has revealed yet another novel mechanism whereby apparently recessive *RYR1* mutations may be causative of MmD. In a recent paper, Zhou et al. report that in a number of patients with recessive core myopathies (6/11), *RYR1* is transcribed from only one allele in skeletal muscle and that the transcribed allele is usually paternally inherited and carries the mutation (43). Thus, such patients are heterozygous for the mutation at the genomic level, but only express the mutant allele in their skeletal muscles, mimicking a homozygous state (43); interestingly, silencing of the normal maternally inherited allele, is tissue specific and does not occur in B-cells which also express low levels of type 1 RyR. One of the families where wild type allele silencing occurred carried the R109W+M485V substitutions. The M485V substitution did not affect the function of the RyR in a discernable way (39), whereas R109W expressed at the homozygous level (as found in the patient's muscle), was accompanied by a significant decrease of ryanodine binding and inability to transport calcium. In addition, in this patient, the level of RyR detected in the muscle biopsy was very low (39).

As more mutations are identified, it will be important to verify how often they are accompanied by a decrease in the RyR1 protein level and whether up-regulation of RyR3 does indeed occur in the skeletal muscle of patients.

CONCLUSIONS AND FUTURE OUTLOOKS

The identification of disease causing human mutations is of fundamental importance from a diagnostic point of view. For some diseases where accurate diagnosis will allow the patient to live a normal life by avoiding certain medications or foods and anticipation of severe complications such as respiratory insufficiency, the quality and duration of life have undoubtedly improved. For others however, the fact that they carry an identified mutation has little positive influence unless the defective gene or its gene product can be replaced by a wild type one. Advancing our understanding of the role of SEPN1 in muscle and whether its lack is indeed affecting the oxidised status of sarcoplasmic reticulum proteins, may help to develop pharmacological therapeutic strategies aimed at improving the quality of life of these patients. On the same line, reversal of gene silencing in the skeletal muscle of patients in which monoallelic

expression leads to the expression of a mutated *RYR1* gene, may similarly improve muscle function. Finally identifying how mutations affect the function of a protein is of fundamental importance if one needs to identify novel drugs which may partially reverse the negative effects of a mutation.

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REFERENCES

1. Engel, A.G., Gomez, M.R. and Groover, R.V. (1971). Multicore disease. Mayo. Clin. Proc. 10, 666-681.
2. Jungbluth, H., Sewry, C., Brown, S.C., Manzur, A.Y., Mercuri, E., Bushby, K., Rowe, P., Johnson, M.A., Hughes, I., Kelsey, A., Dubowitz, V. and Muntoni, F. (2000). Minicore myopathy in children – A clinical and histopathological study of 19 cases. Neuromuscul. Disord. 10, 264-273.
3. Ferreiro, A, Estournet, B., Chateau, D. Romero, N.B., Laroche, C., Odent, S., Toutain, A., Cabello, A., Fontan, D., dos Santos, H.G.,, Haenggeli, C.A., Bertini, E., Urtizberera, J.A., Guichery, P. and Fardeau, M. (2000). Multi-minicore disease – searching for boundaries: phenotype analysis of 38 cases. Ann. Neurol. 48, 745-757.
4. Ferreiro, A., Quijano-Roy, S., Pichereau, C., Moghadaszadeh, B., Goemans, N., Bönnemann, C., Jungbluth, H., Straub, V., Villanova, M., Leroy, J.P., Romero, N.B., Martin, J.J., Muntoni, F., Voit, T., Estournet, B., Richard, P., Fardeau, M. and Guicheney, P. (2002). Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multi-minicore disease: reassessing the nosology of early-onset myopathies. Am. J. Hum. Genet. 71, 739-749.
5. Swash, M. and Schwartz, M.S. (1981). Familial multicore disease with focal loss of cross-striations and ophthalmoplegia. J. Neurol. Sci. 52,1-10.
6. Jungbluth, H., Davis, M.R., Muller, C., Counsell, S., Allsop, J., Chattopadhyay, A., Messina, S., Mercuri, E,, Laing, N.G., Sewry, C.A., Bydder, G. and Muntoni, F. (2004). Magnetic resonance imaging of muscle in congenital myopathies associated with RYR1 mutations. Neuromuscul. Disord. 14, 785-790.
7. Ferreiro, A., Ceuterick-de Groote, C., Marks, J.J., Goemans, N., Schreiber, G., Hanefeld, F., Fardeau, M., Martin, J.J., Goebel, H.H., Richard, P., Guicheney, P. and Bonnemann, C.G. (2004) Desmin-related myopathy with Mallory

body-like inclusions is caused by mutations of the selenoprotein N gene. Ann. Neurol. 55, 676-686.

8. Jungbluth, H., Müller, C.R., Halliger-Keller, B., Brockington, M., Brown, S.C., Feng, L., Chattopadhyay, A., Mercuri, E., Manzur, A.Y., Ferreiro, A., Laing, N.G., Davis, M.R., Roper, H.P., Dubowitz, V., Bydder, G., Sewry, C.A. and Muntoni, F. (2002). Autosomal recessive inheritance of RYR1 mutations in a congenital myopathy with cores. Neurology 59, 284-287.
9. Jungbluth, H., Beggs, A., Bonnemann, C., Bushby, K., Ceuterick-de Groote, C., Estournet-Mathiaud, B., Goemans, N., Guicheney, P., Lescure, A., Lunardi, J., Muntoni, F., Quinlivan, R., Sewry, C., Straub, V., Treves, S. and Ferreiro, A. (2004). 111th ENMC International Workshop on Multi-minicore Disease. 2nd International MmD Workshop, 9-11 November 2002, Naarden, The Netherlands. Neuromuscul. Disord. 14,754-766.
10. Ferreiro, A., Monnier, N., Romero, N.B., Leroy, J.P., Bönnemann, C., Haenggeli, C.A., Straub, V., Voss, W.D., Nivoche, Y., Jungbluth, H., Lemainque, A., Voit, T., Lunardi, J., Fardeau, M. and Guicheney, P. (2002). A recessive form of central core disease, transiently presenting as multi-minicore disease, is associated with a homozygous mutation in the ryanodine receptor type 1 gene. Ann. Neurol. 51, 750-759.
11. Burk, R.F. and Hill, K.E. (1999) Orphan selenoproteins. Bioessays 21, 231-237.
12. Low, S.C. and Berry, M.J. (1996), Knowing when not to stop: selenocysteine incorporation in eukaryotes. TIBS 21, 203-208.
13. Behne, D., Hilmert, H., Scheid, S., Gessner, H., and Elger, W. (1988). Evidence for specific selenium target tissues and new biologically important selenoproteins. Biochim. Biophys. Acta. 966, 12-21.
14. Kryukov, G.V., Castellano, S., Novoselov, S.V., Lobanov, A.V., Zehtab, O., Guigo, R. and Gladyshev, V.N. (2003). Science 300, 1439-1443.
15. Lescure, A., Gautheret, D., Carbon, P. and Krol, A. (1999) Novel selenoproteins identified in Silico and in Vivo by using a conserved RNA structural motif. J. Biol. Chem. 274, 38147-38154.

16. Petit, N., Lescure, A., Rederstorff, M., Krol, A., Moghadaszadeh, B., Wewer, A.M. and Guicheney, P. (2003). Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern. Hum. Mol. Genet. 12, 1045-1053.

17. Moghadaszadeh, B., Petit, N., Jaillard, C., Brockington, M., Quijano-Roy, S., Merlini, L., Romero, N., Estournet, B., Desguerre, I., Chaigne, D., Muntoni, F., Topaloglu, H. and Guicheney, P. (2001). Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. Nature Genetics 29, 17-18.

18. Okamoto, Y., Takashima, H., Higuchi, I., Matsuyama, W., Suehara, M., Nishihira, Y., Hashiguchi, A., Hirano, R., Ng, A.R., Nakagawa, M., Izumo, S., Osame, M. and Arimura, K. (2006). Molecular mechanism of rigid spine with muscular dystrophy type 1 caused by novel mutations of selenoprotein N gene. Neurogenetics 7, 175-183.

19. Tajsharghi, H., Darin, N., Tulinius, M. and Oldfors, A. (2005). Early onset myopathy with a novel mutation in the selenoprotein N gene. Neuromuscul. Disorders. 15, 299-302.

20. Allamand, V., Richard, P., Lescure, A., Ledeuil, C., Desjardin, D., Petit, N., Gartioux, C., Ferreiro, A., Krol, A., Pellegrini, N., Urtizberera, A. and Guicherney, P. (2006). A single homozygous point mutation in a 3'untranslated region motif of selenoprotein N mRNA causes SEPN1-related myopathy. EMBO reports. 7, 450-454.

21. Xia, R., Ganther, H.E., Egge, A., and Abramson, J.J. (2004). Selenium compounds modulate the calcium release channel/ryanodine receptor of rabbit skeletal muscle by oxidizing functional thiols. Biochemical Pharmacol. 67, 2971-2079.

22. Sun, J., Xu, L., Stamler, J.S. and Meissner, G. (2001). Classes of thiols that influence the activity of the skeletal muscle calcium release channel. J. Biol. Chem. 276, 15625-156230.

23. Sutko, J.L. and Airey, J.A. (1996). Ryanodine receptor Ca2+ release channels: does diversity in form equal diversity in function? Physiol Rev 76: 1027-1071.

24. Phillips, M.S., Fujii, J., Khanna, V.K., DeLeon, S., Yokobata, K., de Jong, P.J. and MacLennan, D.H. (1996). The structural organization of the human skeletal muscle ryanodine receptor (RYR1) gene. Genomics 34, 24-41.

25. Otsu, K., Willard, H.F., Khanna, V.K., Zorzato, F., Green, N.M. and MacLennan D.H. (1990). Molecular cloning of cDNA encoding the Ca2+ release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. J. Biol. Chem. 265, 13472-13483.

26. Sorrentino, V., Giannini, G., Malzac, P. and Mattei, M.G. (1993). Localization of a novel ryanodine receptor gene (RYR3) to human chromosome 15q14-q15 by in situ hybridization. Genomics 18, 163-165.

27. Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N.M., Lai, A., Meissner, G. and MacLennan, D.H. (1990). Molecular cloning of cDNA encoding human and rabbit forms of the Ca2+ release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. J. Biol. Chem. 265, 2244-2256.

28. Franzini-Armstrong, C. and Protasi, F. (1997). Ryanodine receptors of striated muscles: a complex channel capable of multiple interactions. Physiol. Rev. 77, 699-729.

29. Fleischer, S. and Inui, M. (1989). Biochemistry and biophysics of excitation-contraction coupling. Annu. Rev. Biophys. Biophys. Chem. 18, 333-364.

30. Rios, E. and Pizarro, G. (1991). Voltage sensor of excitation-contraction coupling in skeletal muscle. Physiol. Rev. 71, 849-908.

31. Rossi A.E. and Dirksen, R.T (2006). Sarcoplasmic reticulum: the dynamic calcium governor of muscle. Muscle Nerve 33, 715-731.

32. Meissner, G (2002). Regulation of mammalian ryanodine receptors. Front. Biosci. 1, d2072-2080.

33. Treves, S., Anderson, A.A., Ducreux, S., Divet, A., Bleunven, C., Grasso, C., Paesante, S. and Zorzato, F. (2005). Ryanodine receptor 1 mutations, dysregulation of calcium homeostasis and neuromuscular disorders. Neuromuscul. Disord. 15:577-587.

34. Robinson, R., Carpenter, D., Shaw, M.A., Halsall, J. and Hopkins, P. (2006). Mutations in RYR1 in malignant hyperthermia and central core disease. Hum. Mutat. 27, 977-989.

35. Denborough, M.A. (1998). Malignant hyperthermia. Lancet, 352, 1131-1136.

36. Gronett, G., Antonigni, J. and Pessah, I. (2000): Anesthesia. Miller R.D. (ed) 5th edition, Churchill Livingstone, New York. pp 1033-1052.

37. Lyfenko, A.D. Goonasekera, S.A. and Dirksen, R.T. (2004). Dynamic alterations in myoplasmic Ca^{2+} in malignant hyperthermia and central core disease. Biochem. Biophys. Res. Commun. 322, 1256-1266.

38. Ducreux, S., Zorzato, F., Ferreiro, A., Jungbluth, H., Muntoni, F., Monnier, N., Müller, C.R. and Treves, S. (2006). Functional properties of ryanodine receptors carrying 3 amino acid substitutions identified in patients affected by multi-minicore disease and central core disease, expressed in immortalised lymphocytes. Biochem J. 395, 259-266.

39. Zhou, H., Yamaguchi, N., Xu, L., Wang, Y., Sewry, C., Jungbluth, H., Zorzato F., Bertini, E., Muntoni, F., Meissner, G. and Treves, S. (2006) Characterization of recessive RYR1 mutations in core myopathies. Hum. Mol. Genet. 15, 2791-2803

40. Monnier, N., Ferreiro, A., Labarre-Vila, A., Mezin, P. and Lunardi, J. (2003). A homozygous splicing mutation causing a depletion of skeletal muscle RyR1 is associated with multi-minicore disease congenital myopathy with ophthalmoplegia. Hum. Mol. Genet. 12, 1171-1178.

41. O'Brien, J., Meissner, G. and Block, B. (1993). The fastest contracting muscles of nonmammalian vertebrates express only one isoform of the ryanodine receptor. Biophys. J. 65, 2418-2427.

42. Conti, Gorza, L. and Sorrentino, V. (1996) Differential distribution of ryanodine receptor type 3 (RyR3) gene product in mammalian skeletal muscles. Biochem. J. 316, 19-23.

43. Zhou, H., Brockington, M., Jungbluth, H., Monk, D., Stanier, P., Sewry, C.A., Moore, G.E. and Muntoni, F. (2006). Epigenetic Allele Silencing Unveils

Recessive *RYR1* Mutations in Core Myopathies. Am. J. Hum. Gen. 79, 859-868.

Figure 1: Histological features of muscle biopsies from patients carrying a mutation in the *RYR1* gene (A and B) and in the *SEPN1* gene (C and D). Hematoxylin and Eosin stain (H&E; A and C) and NADH-TR (B and D).