Congenital muscle disorders with cores: the ryanodine receptor calcium channel paradigm.

Susan Treves[1,2], Heinz Jungbluth[3], Francesco Muntoni[4] and Francesco Zorzato[1,2].

[1]Dipartimento di Medicina Sperimentale e Diagnostica, Universita` di Ferrara, 44100 Ferrara, Italy. [2]Departments of Anaesthesia and Biomedicine, Basel University Hospital, 4031 Basel, Switzerland.[3]Department of Paediatric Neurology, Neuromuscular Service, Evelina Children's Hospital, St. Thomas' Hospital, London, SE1 7EH, U.K. [4]The Dubowitz Neuromuscular Centre, Imperial College, Hammersmith Hospital, London W120NN, U.K.

Summary

Dysregulation of calcium signals due to defects of the skeletal muscle sarcoplasmic reticulum calcium release channel (ryanodine receptor; RyR1) is causative of several congenital muscle disorders including malignant hyperthermia (MH; MIM #145600), Central Core Disease (CCD; MIM #11700), specific forms of Multi-minicore Disease (MmD; MIM # 255320) and Centronuclear myopathy (CNM). Experimental data have shown that *RYR1* mutations result mainly in four types of channel defects: one class of *RYR1* mutations (MH) cause the channels to become hypersensitive to activation by electrical and pharmacological stimuli. The second class of *RYR1* mutations (CCD) result in leaky channels leading to depletion of Ca^{2+} from SR stores. A third class of *RYR1* mutations linked to CCD causes excitation-contraction uncoupling, whereby activation of the voltage sensor Cav1.1 is unable to release calcium from the SR. The fourth class of mutations are unveiled by wild type allele silencing, and cause a decrease of mutant RyR1 channels expression on SR membranes. In this review, we discuss the classes of *RYR1* mutations which have been associated with CCD, MmD and related neuromuscular phenotypes.

Introduction

Although 5 decades of research into the mechanisms involved in cytosolic Ca^{2+} regulation have advanced our understanding of fundamental cellular processes ranging from

muscle contraction to gene expression [1], the precise impact of altered Ca^{2+} signalling on human disease has remained elusive for a long time. The discovery of genes encoding key proteins involved in Ca^{2+} homeostasis was fundamental in bridging the gap between understanding the role of Ca^{2+} in basic physiological processes and the pathophysiology of human diseases. The identification of the Ca^{2+} release channel protein (ryanodine receptor, RyR1) of striated muscle [2] and the identification of mutations in its gene, *RYR1* [3], allowed for the first time a direct correlation between altered Ca^{2+} homeostasis and muscle disease (Fig.1), in particular Malignant Hyperthermia (MH) [4-6], Central Core Disease (CDD) [7], specific forms of Multi-minicore disease (MmD) [8-10] and centronuclear myopathy (CNM) [11] (Tab.1). The overall population frequency of *RYR1* mutations (about 1:50000) is likely to have been underestimated as suggested by the finding of compound heterozygosity or homozygosity for *RYR1* mutations in some patients within extensively analyzed MH and CCD pedigrees and other rare disorders including MmD [8-10], exercise-induced rhabdomyolysis [12], and some forms of exercise-induced hyperthermia [13].

The Ryanodine Receptor calcium channels

Ryanodine receptors are members of a family of intracellular Ca^{2+} release channel proteins present on ER/SR membranes [14]. Type 1 RyR is encoded by a gene on human chromosome 19q13.1 [15], and is mainly expressed in skeletal muscle and to a lower level in Purkinje cells [16], human B-lymphocytes [17,18], and dendritic cells [19-22]; this implies that mutations in the *RYR1* might affect not only excitable cells but also the immune system and other tissues. The functional calcium release channel is made up of four protomers forming a large macromolecular complex of approx. 2,500,000 Da. The complex contains a large hydrophilic domain and a relatively small hydrophobic COOH-terminal domain containing several transmembrane (TM) segments [23-25]. The *RYR1* displays three mutation hot spot regions, of which two are localised in the large hydrophilic region and the third in the COOH-terminal TM domain [5,26] (Fig. 2).

Most of the CCD linked mutations are localised within and around a short hydrophobic segment between the last two transmembrane segments, a region which is thought to form the pore helix and the selectivity filter of the RyR channels [25,27,28], whereas MmD (and CNM) linked *RYR1*-mutations are distributed along the entire coding sequence.

Central Core Disease

CCD is usually inherited as an autosomal dominant (AD) trait [7] but recessive inheritance has been recently described in few families [29-32]. The clinical phenotype of dominantly inherited CCD is variable but usually mild and non-progressive; however, more severe forms including the foetal akinesia syndrome have also been reported [33] associated with recessive or *de novo* dominant mutations. Type I fibre predominance and hypotrophy are common; cores are typically centrally located, cover a considerable length of the fibre and lack mitochondria and oxidative enzymes. Electron microscopy reveals variable degrees of disintegration of the contractile apparatus within the core region, from Z line streaming to total loss of myofibrillar structure. In most cases, patients with dominant CCD carry mutations in the *RYR1* gene; with few exceptions these are clustered in the hydrophobic COOH-terminal pore-forming region of the molecule (domain 3) [5,26,30].

Functional effects of RYR1 mutations linked to CCD.

Two hypotheses have been suggested to explain the functional effect of CCD-linked *RYR1* mutations: the first one suggests that these mutations lead to leaky channels, depletion of SR Ca^{2+} stores and consequently muscle weakness [26, 34-37]. This "leaky channel" hypothesis has been challenged by data obtained using myotubes from the RyR1 knock out animals reconstituted with recombinant RyR1 cDNA carrying mutations in the COOH-terminal domain which have suggested an alternative "E-C uncoupling" hypothesis [34,38]. According to the latter hypothesis, CCD mutations in the hot spot domain #3 lead to functional uncoupling of sarcolemma depolarisation from release of Ca^{2+} from the SR Ca^{2+} stores [34]. The kernel of the two hypothesis concerns the extent of the Ca^{2+} load in the lumen of the SR from CCD muscles

[26]: the uncompensated Ca^{2+} leak hypothesis predicts a decrease of the SR Ca^{2+} load, while the E-C uncoupling hypothesis predicts that the muscle weakness does not result from major changes in the SR Ca^{2+} levels, but rather is due to a defect in excitation contraction coupling (ECC), the molecular mechanism underlying the transmission of the signal from sarcolemma depolarisation to the RyR. To discriminate between these two pathogenetic hypotheses it is crucial to define (i) SR Ca^{2+} content in muscle cells from CCD patients carrying mutation in the COOH-terminal domain, and (ii) clearly establish the role of SR Ca^{2+} load in the regulation of Ca^{2+} release in mammalian fibres [39]. If the Ca^{2+} leak hypothesis is correct, the prevalent involvement of type I fibres suggests that the extent of Ca^{2+} leak via mutated RyR is higher in slow fibres compared to fast fibres, and likely reflects larger SR Ca^{2+} load of slow twitch muscles [40]. A knock-in CCD mouse model carrying the heterozygous RyR1 I4895T mutation does not reconstitute the severe CCD skeletal muscle phenotype of humans harbouring homologous heterozygous *RYR1* mutations [41]. It appears that the CCD knock-in mice phenotype is somehow different from its human counterpart since, in addition to dysfunctional RyR1, homozygous mice display perinatal death, embryonic developmental defect of the skin, bones and cardiovascular system.

Multi-minicore Disease

MmD disease is an autosomal recessive early onset congenital myopathy [42]. Muscle biopsies from MmD patients show multiple small areas (cores) of sarcomeric disorganization lacking oxidative enzymes and mitochondria, which typically do not run the length of the muscle fibre. MmD is clinically highly variable (Tab. 1) and genetically heterogeneous having been linked to recessive mutations both in the selenoprotein 1 (*SEPN1*)(Tab.2) gene and the skeletal muscle *RYR1* gene [43-46]. Although genotype-phenotype correlations have not been fully established, it appears that extra-ocular muscle involvement is exclusive to the *RYR1*-related form of MmD, whilst severe scoliosis and respiratory impairment requiring ventilatory support are more prevalent in *SEPN1*-related “classical” MmD [44,45,47].

Selenoprotein N (SEPN1) mutations

The selenoproteins are a family of proteins characterised by the presence of the 21st amino acid selenocysteine in their primary structure. Selenocysteine represents the main biological form of selenium [48]. The selenoprotein SEPN1, which is encoded by a gene (*SEPN1*; MIM#606210) on human chromosome 1p36 (RSMD1 locus), 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 [49]. Approximately 20 mutations have been identified within the coding sequence of the *SEPN1* gene (Tab. 2), and half of the mutations identified in patients with the classical form of MmD give rise to a truncated SEPN1 protein. It has recently been shown that selective down regulation of SEPN-1 in zebra fish muscles causes alterations (i) in the organization and attachment of the myofibrils and (ii) in the formation of myoseptum, a structure homologous to the human myotendineuos junction [50]. The decrease of SEPN1 synthesis might be associated with an intracellular accumulation of selenite, a precursor of the selenocysteine synthetase [51]; the intracellular accumulation of selenite might in turn alter the redox modulation of the RyR1 [52,53].

RYR1 mutations

A consistent number of MmD patients, particularly those with associated extra-ocular involvement, harbour recessive mutations in *RYR1* [45,54]. The first functional study of MmD-related *RYR1* mutations demonstrated that the p.P3527S and p.V4849I substitutions are associated with a slightly elevated resting Ca^{2+} concentration, but not depleted intracellular stores [55]. Interestingly, cells carrying the homozygous P3527S *RYR1* mutation were found to release significantly less Ca^{2+}after pharmacological activation. Similarly, in another study it was shown that RyR macromolecular complexes carrying the p.S71Y+p.N2283H compound heterozygous mutations did not exhibit reduced Ca^{2+} release after pharmacological stimulation [31]. On the other hand, one of the mutations (p-N2283H) actually increased the sensitivity of the RyR1 activation by KCl and caffeine and is probably also linked to the MHS phenotype. In

some MmD patients, particularly those with ophthalmoplegia, the clinical phenotype may be at least partly explained by a decrease of the RyR1 channel density in the junctional sarcoplasmic reticulum membrane, as demonstrated by Western blot analysis with anti-RyR Ab of muscles obtained from patients carrying the p.R109W+p.M485V as well as other recessive mutations [31,32,56], and the homozygous 14646+2.99 kb intronic splicing variant [47]. In some of these patients with recessive core myopathies, heterozygosity for the *RYR1* mutation at the genomic level but apparent monoallelic expression in muscle (mimicking homozygosity), suggested that the RyR1 decrease resulted from muscle specific *RYR1* gene silencing [56]. In fact, in these patients, the only allele transcribed in skeletal muscle carried the mutation and was paternally inherited [56]. One of the families where wild type allele silencing occurred carried the p.R109W+p.M485V substitutions. The p.M485V substitution did not affect the function of the RyR in a discernable way [31], whereas the p.R109W homozygous mutation was associated with (i) very low levels of RyR1 in the muscle from the affected patient, and (ii) a significant decrease of ryanodine binding and inability to transport calcium by the recombinant mutant channel expressed in HEK293 cells.

Centronuclear myopathy

Centronuclear myopathy (CNM) is a rare, genetically heterogeneous congenital myopathy characterized by numerous centrally located nuclei; additional but inconsistent histopathological features comprise a central zone either devoid of oxidative enzyme activity or with oxidative enzyme accumulation, radial strands surrounding the central area, type 1 fibre predominance and hypotrophy. CNM exists in X-linked, autosomal-dominant and recessive forms and mutations in several genes whose products play a role in membrane trafficking have been identified (Tab.3). The most severe and often fatal X-linked form ("myotubular myopathy") is associated with mutations in the myotubularin *MTM1* gene [57,58], whereas autosomal dominant and autosomal recessive forms have been attributed to mutations in a number of genes including *DNM2* and amphiphysin (*BIN1*) (Tab. 3) [59-61]. In a recent report, a dominant *de*

novo RYR1 mutation was identified in a patient with histopathologic and clinical features of CNM and a pattern of selective muscle involvement on MR imaging suggestive of *RYR1* involvement [11]. Nevertheless, the overall frequency of *RYR1* mutations in this phenotype is currently unknown. Mutation analysis identified the p.S4112L *RYR1* substitution (c12335C>T) which lies within MH/CCD domain 3 in a region lying on the myoplasmic loop adjacent to the first putative transmembrane domain.

Imaging of Ca^{2+} fluxes in primary cultured myotubes from the CNM patient carrying the p.S4112L *RYR1* mutation in the COOH domain revealed an increased sensitivity to depolarization-induced Ca^{2+} release [11], suggesting that this mutation might affect long range interactions between the transmembrane COOH terminal domain and the region involved in the RyR1/Cav.1.1 intermolecular signalling [62]. Alterations of such long range interactions would result in a more sensitive calcium release induced by the activation of the Cav1.1 voltage sensor, but it is unclear how these alterations would determine the clinical phenotype of CNM.

These data suggest that in the future, *RYR1* screening ought to be considered in CNM patients with suggestive clinical and radiological features, in whom other genetic defects have been excluded.

Conclusions.

During the last few years the general understanding of congenital muscle disorders has greatly improved thanks to the identification of causative mutations in the *RYR1* gene. However, the development of therapeutic treatments for affected patients has been hampered by the poor understanding of the molecular pathological mechanisms of the RyR1 defects. Understanding the mechanism(s) responsible for *RYR1* allele silencing, discriminating between Ca^{2+} leak and EC uncoupling are important not only to improve our basic knowledge of the “ryanodinopathies”, but also to develop effective therapeutic strategies aimed at treating the muscle weakness in patients with these conditions.

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References

1 Berridge MJ: **Unlocking the secrets of cell signalling.** *Annu Rev Physiol* 2005, **67**:1-21.

2 Lai FA, Erickson HP, Rosseau E, Liu QY, Meissner G: **Purification and reconstitution of the calcium release channel from skeletal muscle.** *Nature* 1988, **331**:315-319.

3 Fujii J, Otsu K, Zorzato F, deLeon S, Khanna VK, Weiler JE, O'Brien PJ, MacLennan DH: **Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia**. *Science* 1991, **253**:448-451.

4 Rosenberg H, Davis M, James D, Pollock N, Stowell K: **Malignant hyperthermia** *Orphanet J Rare Dis* 2007, **24**: 2-21.

5 Robinson R, Carpenter D, Shaw MA, Halsall J, Hopkins P: **Mutations in RYR1 in malignant hyperthermia and central core disease.** *Hum Mutat* 2006, **27**:977-989.

6 Dulhunty AF, Beard NA, Pouliquin P, Kimura T: **Novel regulators of RyR Ca2+ release channels: insight into molecular changes in genetically-linked myopathies.** *J Muscle Res Cell Motil* 2006, **27**:351–365.

7 Jungbluth H: **Central core disease** *Orphanet J Rare Dis* 2007, **15**:2-25.

8 Jungbluth H: **Multi-minicore Disease** *Orphanet J Rare Dis* 2007, **13**:2-31.

9 Mathews KD, Moore SA: **Multiminicore myopathy, central core disease, malignant hyperthermia susceptibility, and RYR1 mutations: one disease with many faces?** *Arch Neurol* 2004, **61**: 27-29.

10 Zorzato F, Jungbluth H, Zhou H, Muntoni F, Treves S: **Functional effects of mutations identified in patients with multiminicore disease**. *IUBMB Life* 2007, **59**:14-20.

11 Jungbluth H, Zhou H, Sewry CA, Robb S, Treves S, Bitoun M, Guicheney P, Buj-Bello A, Bönnemann C, Muntoni F**: Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene.** *Neuromuscul Disord* 2007, **17:**338-345.

12 Davis M, Brown R, Dickson A, Horton H, James D, Laing N, Marston R, Norgate M, Perlman D, Pollock N, Stowell K: **Malignant hyperthermia associated with exercise-induced rhabdomyolysis or congenital abnormalities and a novel RYR1 mutation in New Zealand and Australian pedigrees**. *Br J Anaesth* 2002, **88**:508-515.

13 Wappler F, Fiege M, Steinfath M, Agarwal K, Scholz J, Singh S, Matschke J, Schulte AM, Esch J: **Evidence for susceptibility to malignant hyperthermia in patients with exercise-induced rhabdomyolysis.** *Anesthesiology* 2001, **94**: 95-100.

14 Hamilton S: **Ryanodine receptors.** *Cell Calcium* 2005, **38**: 253-260.

15 Phillips MS, Fujii J, Khanna VK, DeLeon S, Yokobata K, de Jong PJ, MacLennan DH: **The structural organization of the human skeletal muscle ryanodine receptor (RYR1) gene.** *Genomics* 1996, **34**: 24-41.

16 Kakizawa S, Kishimoto Y, Hashimoto K, Miyazaki T, Furutani K, Shimizu H, Fukaya M, Nishi M, Sakagami H, Ikeda A, Kondo H, Kano M, Watanabe M, Iino M, Takeshima H: **Junctophilin-mediated channel crosstalk essential for cerebellar synaptic plasticity.** *EMBO J* 2007, **26**:1924-1933.

17 Sei Y, Gallagher KL, Basile AS: **Skeletal muscle type ryanodine receptor is involved in calcium signaling in human B lymphocytes.** *J Biol Chem* 1999, **274**: 5995-6002.

18 Girard T, Cavagna D, Padovan E, Spagnoli G, Urwyler A, Zorzato F, Treves S: **B-lymphocytes from malignant hyperthermia-susceptible patients have an increased sensitivity to skeletal muscle ryanodine receptor activators.** *J Biol Chem* 2001, **276**: 48077-48082.

19 O'Connell PJ, Klyachko VA, Ahern GP: **Identification of functional type 1 ryanodine receptors in mouse dendritic cells** *FEBS Lett* 2002, **512**: 67-70.

20 Goth SR, Chu RA, Gregg JP, Cherednichenko G, Pessah IN: **Uncoupling of ATP-Mediated Calcium Signaling and Dysregulated Interleukin-6 Secretion in Dendritic Cells by Nanomolar Thimerosal.** *Environ Health Perspect* 2006, **114**: 1083-1091.

21 Bracci L, Vukevich M, Spagnoli G, Ducreux S, Zorzato F, Treves S: **Ca2+ signaling through ryanodine receptor 1 enhances maturation and activation of human dendritic cells.** *J Cell Sci* 2007, **120**: 2232-2240.

22 Uemura Y, Liu T-Y, Narita Y, Suzuki M, Ohshima S, Mizukami S, Ichihara, Kikuchi H, Matsushita S: **Identification of functional type 1 ryanodine receptors in human dendritic cells.** *Biochem Biophys Res Commun* 2007, **362**:510-515.

23 Ludtke SJ, Serysheva II, Hamilton SL, Chiu W: **The Pore Structure of the Closed RyR1 Channel.** *Structure* 2005, **13**: 1203-1211.

24 Samsó M, Wagenknecht T, Allen PD: **Internal structure and visualization of transmembrane domains of the RyR1 calcium release channel by cryo-EM.** *Nature Structural Molecular Biol* 2005, **12**: 539-544.

25 Williams AJ, West DJ, Sitsapesan R: **Light at the end of the Ca(2+)-release channel tunnel: structures and mechanisms involved in ion translocation in ryanodine receptor channels.** *Q Rev Biophys* 2001, **34**:61-104.

26 Treves S, Anderson AA, Ducreux S, Divet A, Bleunven C, Grasso C, Paesante S, Zorzato F**: Ryanodine receptor 1 mutations, dysregulation of calcium**

homeostasis and neuromuscular disorders. *Neuromuscul Disorders* 2005, **15**: 577-587.

27 Chen SR, Li P, Zhao M, Li X, Zhang L: **Role of the proposed pore-forming segment of the Ca2+ release channel (ryanodine receptor) in ryanodine interaction.** *Biophys J* 2002, **82:** 2436–2447.

28 Balshaw D, Gao L, Meissner G: **Luminal loop of the ryanodine receptor: A pore-forming segment?** *Proc Natl Acad Sci U S A* 1999, **96**:3345-3347.

29 Kossugue PM, Paim JF, Navarro MM, Silva HC, Pavanello RCM, Gurgel-Giannetti J, Zatz M, Vainzof M: **Central core disease due to recessive mutations in RYR1 gene: is it more common than described?** *Muscle Nerve* 2007, **35**:670-672.

30 Wu S, Ibarra MCA, Malicdan MCV, Murayama K, Ichihara Y, Kikuchi H, Nonaka I, Noguchi S, Hayashi YK, Nishino I: **Central core disease is due to RYR1 mutations in more than 90% of patients.** *Brain* 2006, **129**:1470–1480.

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

32 Zhou H, Jungbluth H, Sewry CA, Feng L, Bertini E, Bushby K, Straub V, Roper H, Rose MR, Brockington M, Kinali M, Manzur A, Robb S, Appleton R, Messina S, D'Amico A, Quinlivan R, Swash M, Müller CR, Brown S, Treves S, Muntoni F: **Molecular mechanisms and phenotypic variation in RYR1-related congenital myopathies**. *Brain* 2007, **130:** 2024-2036.

33 Romero NB, Monnier N, Viollet L, Cortey A, Chevallay M, Leroy JP, Lunardi J, Fardeau M: **Dominant and recessive central core disease associated with RYR1 mutations and fetal akinesia.** *Brain* 2003, **126**: 2341-2349.

34 Rossi AE, Dirksen RT: **Sarcoplasmic reticulum: The dynamic calcium governor of muscle.** *Muscle Nerve* 2006, **33**: 715-731.

35 Lynch PJ, Tong J, Lehane M, Mallet A, Giblin L, Heffron JJ, Vaughan P, Zafra G, MacLennan DH, McCarthy TV: **A mutation in the transmembrane/luminal domain of the ryanodine receptor is associated with abnormal Ca2+ release channel function and severe central core disease**. *Proc Natl Acad Sci USA* 1999, **96**:4164-4169.

36 Tilgen N, Zorzato F, Halliger-Keller B, Muntoni F, Sewry C, Palmucci LM, Schneider C, Hauser E, Lehmann-Horn F, Müller CR, Treves S: **Identification of four novel mutations in the C-terminal membrane spanning domain of the ryanodine receptor 1: association with central core disease and alteration of calcium homeostasis.** *Hum Mol Genet* 2001, **10**:2879-2887.

37 Zorzato F, Yamaguchi N, Xu L, Meissner G, Müller CR, Pouliquin P, Muntoni F, Sewry C, Girard T, Treves S: **Clinical and functional effects of a deletion in a COOH-terminal lumenal loop of the skeletal muscle ryanodine receptor.** *Hum Mol Genet* 2003, **12**: 379-388.

38 Dirksen RT, Avila G: **Distinct effects on Ca2+ handling caused by malignant hyperthermia and central core disease mutations in RyR1.** *Biophys J* 2004, **87**:3193-3204.

39 Rios E, Launikonis BS, Royer L, Brum G, Zhou J: **The elusive role of store depletion in the control of intracellular calcium release.** *J Muscle Res Cell Motil* 2006, **27**:335-350.

40 Lamb GD, Cellini MA, Stephenson DG: **Different Ca2+ releasing action of caffeine and depolarisation in skeletal muscle fibres of the rat.** *J Physiol* 2001, **531**:715-728.

41 Zvaritch E, Depreux F, Kraeva N, Loy RE, Goonasekera SA, Boncompagi S, Kraev A, Gramolini AO, DirksenRT, Franzini-Armstrong C, Seidman CE, Seidman JE, MacLennan DH: **An *Ryr1*^{I4895T} mutation abolishes Ca^{2+} release channel function**

and delays development in homozygous offspring of a mutant mouse line 2007**, 104:** 18537-18542.

42 Engel AG, Gomez MR, Groover RV: **Multicore disease.** *Mayo Clin Proc* 1971, **10**: 666-681.

43 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, Guicheney P: **Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome.** *Nat Genet* 2001, **29**:17-18.

44 Ferreiro A, Monnier N, Romero NB, Leroy JP, Bönnemann C, Haenggeli CA, Straub V, Voss WD, Nivoche Y, Jungbluth H, Lemainque A, Voit T, Lunardi J, Fardeau M, Guicheney PA: **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* 2002, **51**:750-759.

45 Jungbluth H, Müller CR, Halliger-Keller B, Brockington M, Brown SC, Feng L, Chattopadhyay A, Mercuri E, Manzur AY, Ferreiro A, Laing NG, Davis MR, Roper HP, Dubowitz V, Bydder G, Sewry CA, Muntoni F.: **Autosomal recessive inheritance of RYR1 mutations in a congenital myopathy with cores**. *Neurology* 2002, **59**: 284-287.

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

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

48 Moghadaszadeh B. Beggs AH: **Selenoprotein and their impact on human health through diverse physiological pathways.** *Physiology* 2006, **21**: 307-315.

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

50 Deniziaka M, Thisseb C, Rederstorffa M, Hindelangb C, Thisseb B, Lescurea A.: **Loss of selenoprotein N function causes disruption of muscle architecture in the zebrafish embryo** (2007) *Exp. Cell Res.* **313**: 156-167

51 Xu XM, Carlson BA, Mix H, Zhang Y, Saira K, Glass R S, Bery MJ, Gladyshev VN, Hatfield DL: **Biosynthesis of selenocysteine on its tRNA in eukaryotes.** *PloS Biology* 2007, **5** e4: 0096-0105.

52 Xia R, Ganther HE, Egge A, Abramson JJ: **Selenium compounds modulate the calcium release channel/ryanodine receptor of rabbit skeletal muscle by oxidizing functional thiols.** *Biochemical Pharmacol* (2004), **67**: 2971-2079.

53 Sun J, Xu L, Stamler JS, Meissner G: **Classes of thiols that influence the activity of the skeletal muscle calcium release channel.** *J Biol Chem* 2001, **276**: 15625-156230.

54 Jungbluth H, Davis MR, Muller C, Counsell S, Allsop J, Chattopadhyay A, Messina S, Mercuri E, Laing NG, Sewry CA, Bydder G., Muntoni F: **Magnetic resonance imaging of muscle in congenital myopathies associated with RYR1 mutations**. (2004). *Neuromuscul. Disord.* **14**: 785-790.

55 Ducreux S, Zorzato F, Ferreiro A, Jungbluth H, Muntoni F, Monnier N, Müller CR, Treves S: **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* 2006, **395**: 259-266.

56 Zhou H, Brockington M, Jungbluth H, Monk D, Stanier P, Sewry CA, Moore GE, Muntoni F: **Epigenetic Allele Silencing Unveils Recessive *RYR1* Mutations in Core Myopathies.** *Am J Hum Gen* 2006, **79**: 859-868.

57 Laporte J, Hu LJ, Kretz C, Mandel JL, Kioschis P, Coy JF, Klauck SM, Poustka A, Dahl N: **A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphates family conserved in yeast**. *Nat Genet* 1996, **13**:175–82.

58 Laporte J, Guiraud-Chaumeil C, Vincent MC, Mandel JL, Tanner SM, Liechti-Gallati S, Wallgren-Pettersson C, Dahl N, Kress W, Bolhuis PA, Fardeau M, Samson F, Bertini E: **Mutations in the MTM1 gene implicated in X-linked myotubular myopathy.** *Hum Mol Genet* 1997, **6**:1505–1511.

59 Echaniz-Laguna A, Nicot AS, Carré S, Franques J, Tranchant C, Dondaine N, Biancalana V, Mandel JL, Laporte J: **Subtle central and peripheral nervous system abnormalities in a family with centronuclear myopathy and a novel dynamin 2 gene mutation**. *Neuromuscul Disord* 2007, **17**:955-959.

60 Bitoun M, Maugenre S, Jeannet PY, Lacène E, Ferrer X, Laforêt P, Martin JJ, Laporte J, Lochmüller H, Beggs AH, Fardeau M, Eymard B, Romero NB, Guicheney P: **Mutations in dynamin 2 cause dominant centronuclear myopathy**. *Nat Genet* 2005, **37**:1207-1209.

61 Tosch V, Rhode HM, Tronchère H, Zanoteli E, Monroy N, Kretz C, Dondaine N, Payrastre B, Mandel JL, Laporte J: **A novel PtdIns3P and PtdIns(3,5)P2 phospahtase with an inactivating variant in centronuclear myopathy.** *Hum Mol Genet* 2006, **15**:3098-3106.

62 Proenza C, O'Brien J, Nakai J, Mukherjee S, Allen PD, Beam KG**: Identification of a region of RyR1 that participates in allosteric coupling with the alpha(1S) (Cav1.1) II-III loop.** *J Biol Chem* 2002, **277:**6530-6535.

63 Ferreiro A, Quijano-Roy S, Pichereau C, Moghadaszadeh B, Goemans N, Bönnemann C, Jungbluth H, Straub V, Villanova M, Leroy JP, Romero NB, Martin JJ, Muntoni F, Voit T, Estournet B, Richard P, Fardeau M, Guicheney P: **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* 2002, **71**: 739-749.

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

65 Schara U, Kress W, Bönnemann CG, Breitbach-Faller N, Korenke CG, Schreiber G, Stoetter M, Ferreiro A, von der Hagen M: **The phenotype and long term follow up in 11 patients with juvenile selenoprotein N1-related myopathy**. *Eur J Paediat Neurol* 2007, doi:10.1016/ejpn.2007.08.011.

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

67 Bitoun M, Bevilacqua JA, Prudhon B, Maugenre S, Taratuto AL, Monges S, Lubieniecki F, Cances C, Uro-Coste E, Mayer M, Fardeau M, Romero NB, Guicheney P: **Dynamin 2 mutations cause sporadic centronuclear myopathy with neonatal onset.** *Ann Neurol* 2007, doi 10.1002/ana.21235.

68 Nicot AS, Touissant A, Tosch V, Kretz C, Wallgren-Pettersson C, Iwarsson E, Kingston H, Garnier JM, Biancalana V, Oldfors A, Mandel JL, Laprote J: **Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy**. *Nat Genet* 2007, **39**: 1134-1139.

Figure Legends

Figure 1: Classification of RyR1 channelopathies based on the functional effect of mutations.

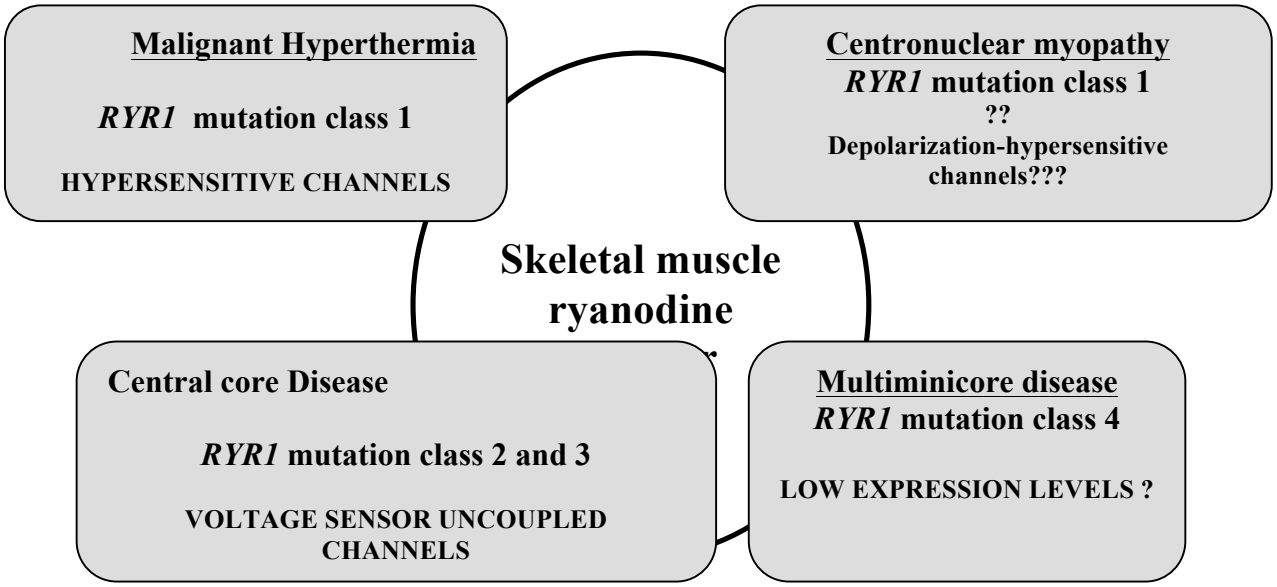
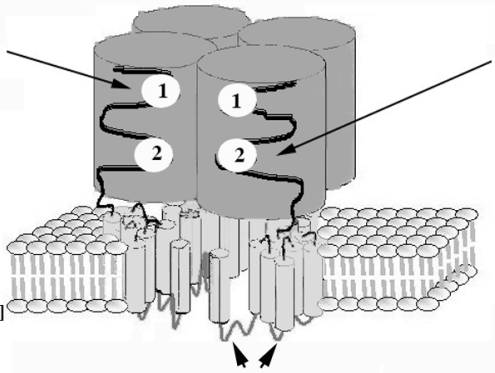


Figure 2: Cartoon depicting the ryanodine receptor tetramer inserted into a lipid bilayer. The mutations identified in the different domains, their mode of transmission (D= dominant; r= recessive) as well as their association with CCD and MmD are indicated.

Figure 2



MH/CCD domain1

p.D60N, r (compound +/-), CCD [30]
p.S71Y, r (compound +/-), MmD [31]
p.R109W, r (compound +/-), MmD [31]
p.E160G, D (+/-), CCD [26] *
p.R163C, D (+/-), CCD [26] *
p.G215E, D (+/-), CCD [26] *
p.R401C, D (+/-), CCD [26]*
p.M402T, r (compound +/-), MmD [32]
p.I403M, D (+/-), CCD [26]*
p.Q474H, D (+/-), CCD [30]
p.M485V, r (compound +/-), MmD [31]
p.Y522S, D (+/-), CCD [26]*
p.R614C, D (+/-), CCD [26]*
p.A1577T, r (compound +/-), MmD [31]

MH/CCD domain 2

p.G2060C, r (compound +/-), MmD [31]
p.R2163H, D (+/-), CCD [26]*
p.N2283H, r (compound +/-), MmD [32]
p.V2346M, D (+/-), CCD [26]*
p.M2423K, r (monoallelic -/-), MmD [32]
p.R2435H, D (+/-), CCD [26]*
p.R2435L, r (-/-), MmD [32]
p.I2453T, D (+/-), CCD [26]*
p.R2508C/H, D (+/-), CCD [30,32]
p.E2545D, D (+/-), CCD [30]
p.R2939K, r (monoallelic -/-), MmD [32]
p.L3606P, r (compound +/-), CCD [30]
p.K3367R, D (+/-), CCD [30]
p.S3448F, r (compound +/-), MmD [32]
p.P3527S, r (-/-), MmD * [55]
p.R3772Q, r (-/-), MmD [32]

MH/CCD domain 3

p.S4112L, D (+/-), CNM [11]
p.R4214_F4216 del, D (+/-), CCD [26]*
p.A4329D, r (monoallelic -/-), MmD [32]
p.L4568P, D (+/-), CCD [30]
p.Y4631N, D (+/-), CCD [30]
p.E4634K, D (+/-), CCD [30]
p.T4637A/I, D (+/-), CCD [26] *
p.G4638D/S, D (+/-), CCD * [30,32]
p.L4647_S4648 del, D (+/-), CCD [26]*
p.L4650P, D (+/-), CCD [26] *
p.H4651P, D (+/-), CCD [26]*
p.L4665P, D (+/-), CCD [32]
p.T4709M, r (monoallelic -/-), MmD [32]
p.K4724Q, D (+/-) CCD [26]*
p.S4789_K4822 del, r (-/-), MmD [32]
p.L4793P, D (+/-), CCD [26]*
p.Y4796C. D (+/-), CCD [26]*
p.F4808N, D (+/-), CCD [26]*
p. R4825C/P/I, D (+/-), CCD [26,32]*
p.N4858D, D (+/-), CCD [30]
p.V4849I, r (-/-), MmD [26]*
p.P4860del, D (+/-) CCD [26]*
p.R4861H/C, D (+/-), CCD [26] *
p.R4863_D4869delInsT, D (+/-), CCD[32]
p.Y4864C, D (+/-), CCD [26]*
p.F4863_D4869 del, D (+/-), CCD [26]*
p.G4891R, D (+/-), CCD [26] *
p.R4893W/Q, D (+/-), CCD [26]*
p.A4894Q/T/V, D (+/-), CCD [26] *
p.I4898T, D (+/-), CCD [26]*
p.G4899E/R, D (+/-), CCD [26] *
p.T4920N, D (+/-), CCD [30]
p.R4914G/T/Q, D (+/-), CCD [26]*
p.F4921S/T, D (+/-), CCD [26,30]*
p.V4927_I4928del, D (+/-), CCD [26] *
p.A4940T, D (+/-), CCD [26]*
A14646+2.9kb ins, r(-/-) [26] *

* for references of these mutations, see [26]