Familial RYR 1 Mutation Associated with Mild and Severe Central Core Disease

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Ryanodine receptor type 1, encoded by the *RYR1* gene, is the skeletal muscle receptor that serves as a calcium release channel for the sarcoplasmic reticulum. Mutations in this gene are associated with Malignant Hyperthermia (MH), Central Core Disease (CCD) and Multi-minicore Disease (MmD). Malignant Hyperthermia is an autosomal dominant pharmacogenetic disease of calcium homeostasis in skeletal muscle brought on by exposure to volatile general anesthetics. Susceptible individuals develop a hypermetabolic state with generalized contractures[1]. Multi-minicore Disease caused by RYR1 mutations is an autosomal recessive congenital myopathy with distinct histopathological features and variable clinical presentation[2]. Central Core Disease is a congenital myopathy that is usually inherited in an autosomal dominant manner. It is classically considered a non-progressive neuromuscular disorder and is characterized by proximal weakness and hypotonia with frequent hip dislocation and scoliosis. Bulbar and respiratory muscles are frequently spared, but there can be considerable variability in the severity and age of onset of CCD. The diagnosis is made from histopathological findings on muscle biopsy when central cores are identified in type 1 muscle fibers[3].

The ryanodine receptor protein consists of three domains[4]. Most mutations causing CCD are found in exons 90-104 within domain 3 in the C-terminal region of the protein[3,5]. This domain is considered to be the pore-forming part of the channel[6]. Mutations causing MmD and MH are distributed throughout the *RYR1* gene[7].

We describe a family with two maternal half-brothers affected with CCD. The older sibling has a mild CCD phenotype, while the younger brother presented with severe symptoms at birth. The boys' DNA testing determined that they both carried the same *RYR1* gene mutation. The mother, who is the common parent to these half-siblings, was not a carrier of the RYR1 mutation suggesting that she is a gonadal mosaic. We report on the very different clinical presentations of the two half-siblings with CCD. To our knowledge, this is the first such report for CCD where two individuals are harboring the same familial mutation as a result of germline mosaicism.

Case Report

Patient 1

Patient 1 was born vaginally to a 21-year-old Gravida 2, Para 1 female at term following prolonged premature rupture of the membranes. Delivery and newborn period were uneventful and both he and his mother were discharged after two days.

He first came to medical attention at age 18 months because of delayed motor development. He had generalized hypotonia and was still not walking. Tendon reflexes were absent in his lower extremities. His cranial nerves examination was normal and there was no myotonia. He began walking at age 30 months. He demonstrated a positive Gowers' sign. At age 35 months, a biopsy from the right vastus medialis demonstrated some variation in fiber diameter, with a range of 10 to 50 microns, and minimal patchy endomysial fibrosis. The majority of fibers contained a single core; these were evident with the Gomori trichrome stain (Figure 1a) and, in particular, the NADH-TR reaction (Figure 1b). The myofibrillar ATPase reacted sections revealed reaction product within the cores, and virtually all fibers were type I in appearance. Electron microscopy revealed mild myofibrillar disorganization in the core regions, with mild Z line irregularities and sparse to absent mitochondria (Figure 1c). A diagnosis of CCD was made.

Throughout childhood, the hypotonia and proximal weakness persisted. He tired on long walks and was not able to run. Muscle strength was assessed as 4 out of 5 (World Health Organization scale). By age five years, he had had two surgeries (dental extraction and muscle biopsy) under general anesthesia without evidence of malignant hyperthermia. The muscle biopsy was done with sevofloran, nitrous oxide and fentanyl; information on the type of anesthesia used for the dental extraction was not available.

Presently, at age 11 years, Patient 1 is functioning relatively well from a motor standpoint. He participates in recreational sports and is able to ride a bicycle. On physical assessment, he demonstrates proximal muscle atrophy. There is no evidence of involvement of the facial musculature nor does he have a scoliosis. Muscle strength is 4+/5 at the shoulders and hips. His gait is normal except for his tendency to toe walk. Tendon reflexes are absent in both upper and lower extremities.

Patient 2

Patient 2, a maternal half-sibling to Patient 1, was born at term by caesarean section because of breech presentation to a 32-year old multigravida. The pregnancy was uneventful until

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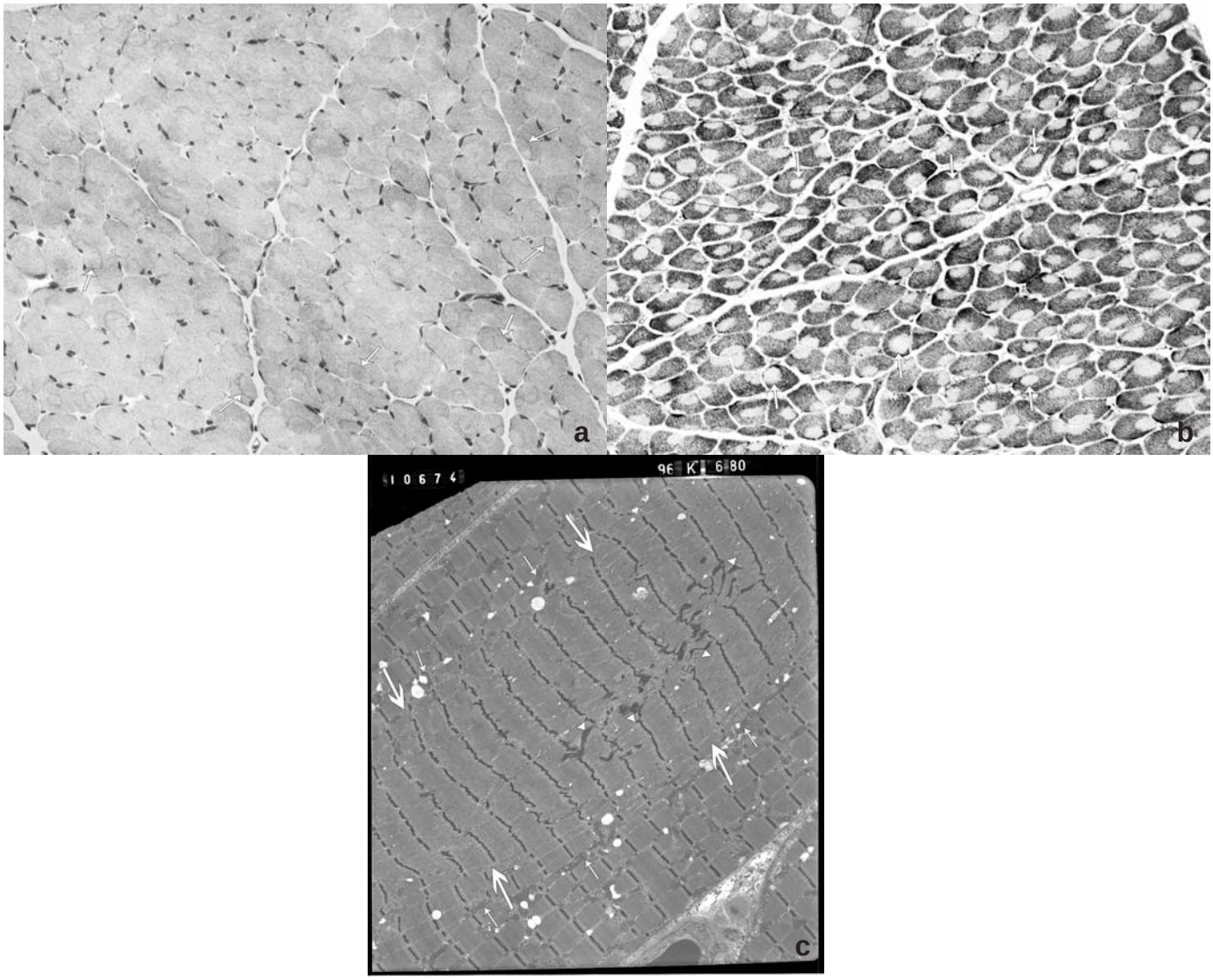

Figure 1: *Right vastus medialis biopsy from Patient 1, age 35 months. (a) Cores (arrows) are seen in many fibers with Gomori trichrome staining (frozen section, original magnification x 200). (b) Cores (arrows) are seen as areas lacking reaction product with NADH-TR histochemistry (frozen section, original magnification x 200). (c) Electron micrograph of a core (between large arrows) surrounded by normal myofibrils. Note the mild myofibrillar disorganization in the core, including mild Z line streaming (arrow heads). Mitochondria are scarce in the core, while they are present in adjacent areas of normal myofibrils (small arrows) (longitudinal orientation, original magnification x 2500).*

oligohydramnios was noted in the third trimester. At delivery, the unusually short umbilical cord resulted in a cord tear while extracting the baby. He required positive pressure ventilation and free flow oxygen. He was found to have multiple joint contractures involving all four limbs. He was discharged from the Neonatal Intensive Cae Unit on supplemental oxygen at age three weeks.

He was readmitted to hospital at age six weeks when he was found to be hypotonic and to have evidence of a chronic respiratory acidosis. He required continuous positive airway pressure while in hospital and it was difficult to wean him from ventilatory support. He had no underlying cardiac pathology to explain his oxygen dependence. Multiple joint contractures involving both upper and lower limbs remained apparent, and a mid-thoracic dextroconvex scoliosis was found. There was no dislocation of the hips. The patient was not moving his extremities but was able to turn his head, to fix and follow, and to occasionally vocalize. His cranial nerve examination was normal. A severe form of CCD was entertained in this patient given that he had an affected maternal half-brother. A muscle biopsy of his left vastus medialis was performed at age six months. This revealed findings similar to those of Patient 1, with cores present in many fibers (Figure 2), variation in fiber diameter (5 to 30 microns), and mild patchy endomysial fibrosis. The myofibrillar ATPase reaction revealed a predominance of type I fibers and reaction product within cores. Tissue was not

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available for electron microscopy. A diagnosis of CCD was made. He remained in hospital on CPAP until age 12 months when he succumbed to an H1N1 influenza infection.

Family History:

Patients 1 and 2 are maternal half-siblings, each the product of healthy non-consanguineous parents (Figure 3). Each has a healthy older full sister. The family history was otherwise unremarkable with no other reported history of CCD or any other neuromuscular disease.

Genetic Testing

Mutation analysis of the *RYR1* gene was performed on Patient 1. This involved bi-directional sequencing of exons 2, 6, 8, 9, 11, 12, 14, 15, 17, 39, 40-41, 44-47, 95 and 100-104. These 22 exons contain the great majority of conclusively documented MH and CCD causative mutations (www.emhg.org). The full coding region of each exon plus ~ 50 bp of flanking non-coding DNA on either side were sequenced. He was found to have a heterozygous 27-base pair duplication in exon 101 of the *RYR1* gene (c.14545_14571 dupCGTCTACCTGTACACCGTGGTG GC CTTC) resulting in the duplication of amino acids p.4849_4857 (dupValTyrLeuTyrThrValValAlaPhe). This alteration has not been reported previously in over 500 *RYR1* genes analyzed and was interpreted as being pathogenic because of the patient's clinical and muscle biopsy findings and because of the nature of the change (duplication) occurring in the region of the *RYR1* gene known to harbour severe CCD-causing mutations. Mutation specific DNA testing was subsequently initiated in Patient 2 and the mother. Patient 2 was found to carry the same 27 nucleotide duplication in exon 101 as his maternal half-brother, thus confirming a diagnosis of CCD. Surprisingly, the boys' mother did not carry the exon 101 duplication thereby suggesting gonadal mosaicism. The fathers were not tested because the common parent was the mother. Also, it would be unusual for two unrelated fathers to each to be a carrier for a rare disease like CCD.

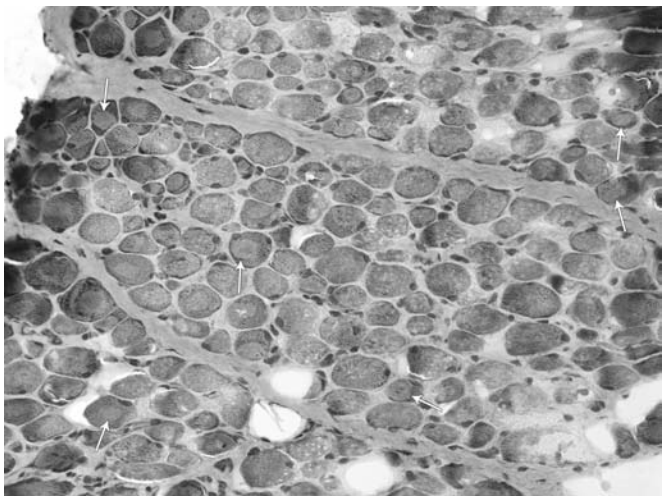

Figure 2: *Left vastus medialis biopsy from Patient 2, age 6 months. Cores are present in most fibers (arrows) (Gomori trichrome stained frozen section, original magnification x 200).*

Discussion

Central Core Disease is a common, non-progressive congenital myopathy which classically presents with proximal muscle weakness and orthopedic complications such as congenitally dislocated hips and/or scoliosis. Clinical presentations may vary greatly. While some individuals with CCD are only very mildly affected, with almost no clinical symptoms, others may have an infantile onset with profound hypotonia and respiratory dysfunction, including ventilator-dependence. The precise underlying pathophysiological basis of CCD remains unknown, but is thought to involve a calcium ion disturbance in skeletal muscle[4].

It has been generally accepted that CCD is an autosomal dominant disorder. Most cases of CCD have identifiable missense or deletion mutations in the *RYR1* gene. Three mutational hotspots (domains 1-3) have been identified. Mutations near the C-terminus (domain 3) are more frequently associated with severe CCD, while cases of mild CCD and the

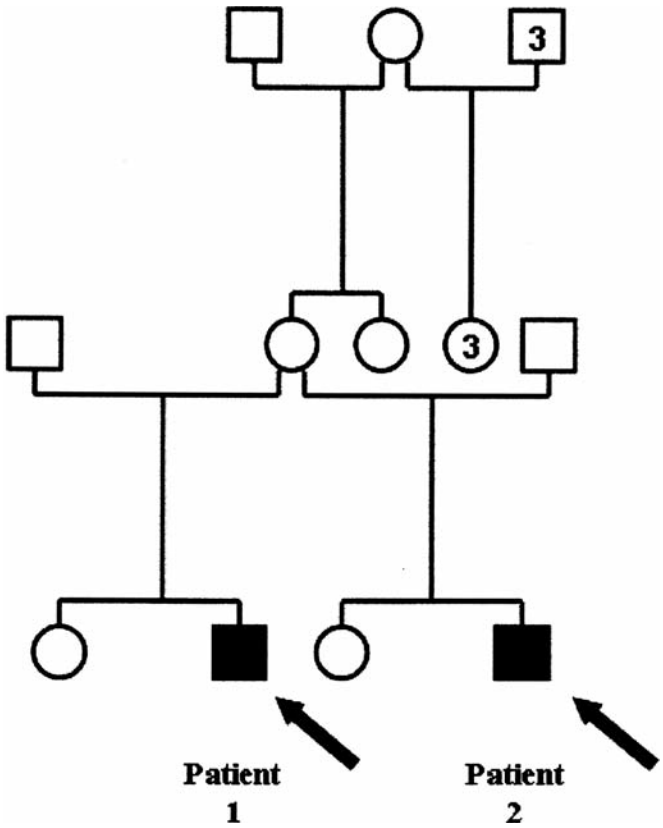

Figure 3: *Partial pedigree. Males and females are indicated by □ and ○, respectively. Filled symbols indicate individuals affected with central core disease. The arrows indicate the two patients.*

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allelic condition MH often have identifiable mutations in domains 1 or 2 of the *RYR1* gene. Zhou et al[8] concluded that the C-terminal domain of the ryanodine receptor was a hot spot for dominant mutations, and that analysis of exons 101 and 102 is an efficient mutation screening strategy.

There have been several reports of an autosomal recessive CCD-like condition caused by mutations in the *RYR1* gene[8-12]. No mutational hotspots for the recessive form were identified and the clinical presentation was quite variable[8]. Jungbluth et al[2] concluded that individuals carrying two recessive *RYR1* mutations have MmD and are clinically distinct from CCD with unique histopathological findings[2].

The nature of the mutation identified in our two cases would be consistent with a severe clinical presentation based on the type of mutation (duplication) and its location (domain 3). Wu et al[3] reviewed 27 unrelated Japanese patients with CCD. Six patients had mutations in exon 101 like our two patients. Four of six had joint contractures and scoliosis, three had joint dislocations and two had generalized muscle weakness. All presented with significant symptoms during the perinatal period. The mutation in our two patients has not been previously reported, so we can only speculate as to its clinical effect.

The very different clinical presentations were unexpected, but clinical variability in carriers of *RYR1* mutations has been previously reported. Romero et al[9] described seven patients from France. All had severe clinical presentations either in utero or after birth. Only two survived beyond infancy to ages five and nine years, respectively. Interestingly, one severely affected newborn had a domain 3 mutation (exon 102) that was transmitted to him by his less severely affected mother. This implies that other genetic and non-genetic factors play a role in determining the clinical presentation of CCD in individuals with an *RYR1* mutation.

Our family with two half-brothers with CCD is unique in that they have such a different clinical presentation from each other. One is mildly affected with only slight impairment of his motor function, whereas the other sibling had an infantile presentation with profound hypotonia and ventilator dependence. Both boys carry the same exon 101 duplication in domain 3. Duplication as a disease causing mutation was reported by Ibarra et al[7] in a Japanese patient (4320-2dup). This was not seen in controls.

Their mother, the common parent, did not carry the mutation in peripheral blood suggesting that she is a gonadal mosaic. We have not found any reports of gonadal mosaicism being associated with CCD in the published literature. It is possible that this may account for some of the autosomal recessive CCD cases that have been reported. The mother has not had a muscle biopsy as she was clinically asymptomatic.

Alternative explanations are possible. We cannot exclude the possibility that Patient 2 carries a second *RYR1* mutation not detectable by sequence analysis that is responsible for his severe clinical presentation. However, if the inheritance pattern is truly autosomal recessive, we would not have expected his carrier half-brother (Patient 1) to demonstrate any clinical symptoms. In addition, the muscle biopsy on Patient 1 demonstrated histopathological findings characteristic of CCD. It has also been suggested that the autosomal recessive form is distinct from CCD and has unique histopathological findings.[2] Further research will be needed to clarify the matter.

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