SHORT REPORT

ABSTRACT: Central core disease (CCD) is an autosomal-dominant congenital myopathy, with muscle weakness and malignant hyperthermia (MH) susceptibility. We identified two of nine Brazilian CCD families carrying two mutations in the RYR1 gene. The heterozygous parents were clinically asymptomatic, and patients were mildly affected, differing from the few autosomal-recessive cases described previously. Recessive inheritance in CCD may therefore be more common than previously appreciated, which has important implications for genetic counseling and MH prevention in affected families.

CENTRAL CORE DISEASE DUE TO RECESSIVE MUTATIONS IN RYR1 GENE: IS IT MORE COMMON THAN DESCRIBED?

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Central core disease (CCD) is a rare congenital myopathy, with high inter- and intrafamilial phenotype variability ranging from asymptomatic to severely affected individuals. Clinical signals include hypotonia, delayed motor milestones, proximal muscle weakness, and skeletal anomalies such as hip dislocation, scoliosis, and foot deformities.1,2

The diagnosis of CCD is confirmed by the presence within muscle fibers of cores, which are lesions of the sarcomeres with sarcomeric disorganization, lack of mitochondria, and lack of oxidative activity.13 These cores, mainly in type I muscle fibers, may be single or multiple, central or peripheral, and occasionally associated with rods.13,20

The main gene associated with this disorder is the ryanodine receptor (RYR1) gene at chromosome 19q13,15 which is also linked to malignant hyperthermia (MH). Both MH and CCD are disorders of Ca2+ release channels, and patients with CCD are at risk for MH because there are mutations that cause both phenotypes.9

The RYR1 gene is a very large gene, with 106 exons. However, RYR1 mutations are essentially clustered in three regions: regions 1 and 2 reside in the myoplasmatic foot domain of the protein, and region 3 is located in the transmembrane/luminal region of the highly conserved C-terminal domain. Recently, the majority of the novel mutations linked to CCD was found to be concentrated in region 3, particularly between exons 95 and 103.3

The human RYR1 gene encodes a 5037 amino-acid protein, the functional skeletal-muscle calcium release channel, which is composed of four RYR1 subunits of 565 kDa each and a number of accessory proteins that are thought to regulate and stabilize the calcium channel.2,6,11,22,23 This protein is located in the junctional terminal cisternae of the sarcoplasmatic reticulum membrane.
CCD is usually transmitted through autosomal-dominant inheritance with variable penetrance but a few reported cases have autosomal-recessive inheritance.\textsuperscript{5,8,10,18} The pattern of inheritance is important for genetic counseling and prevention of malignant hyperthermia.

Here, we screened the hot spot C-terminal region of the \textit{RYR1} gene (encompassing exons 94 to 106) for mutation, and identified two of nine Brazilian CCD families with autosomal-recessive mutations, suggesting that this pattern of inheritance is more frequent than expected.

**PATIENTS AND METHODS**

Thirteen exons from the C-terminal region of the \textit{RYR1} gene were screened for mutations in 15 patients, from 9 unrelated families. A total of 200 chromosomes, from 100 control individuals, matched for ethnic background, age, and sex were used to confirm the frequency of the novel mutations.

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood lymphocytes.\textsuperscript{12} The C-terminal region (exons 94 to 106) was analyzed using polymerase chain reaction (PCR) amplification with described primers.\textsuperscript{14} The mutation screening was performed by single-strand conformation polymorphism (SSCP) analysis\textsuperscript{16} through electrophoresis on MDE gel (FMC Bioproducts, Rockland, ME). Bands with altered migration were sequenced using a sequencing kit on a MegaBACE 1000 automated DNA sequencer (Amershan Biosciences, Piscataway, NJ).

Muscle biopsy was taken from the biceps muscle in the first family and from the quadriceps muscle in the second family, for diagnostic purpose, cryoprotected, and snap-frozen in liquid nitrogen. Routine histological and histochemical analyses included staining for hematoxylin and eosin (HE), modified Gomori trichrome, succinate dehydrogenase (SDH), nicotinamide adenine dinucleotide (NADH), acid phosphatases, and adenosine triphosphatase (AT-Pase) at pH 9.4 and 4.3.\textsuperscript{1}

Informed consent was obtained from each subject.

**RESULTS**

Seven different mutations were identified in six of the nine families (Table 1).

Three mutations were new ones (families 2 and 4), whereas the other four have been described previously (families 1, 3, 5, and 6). In two sporadic cases (families 3 and 6), the mutation was not present in the parents, and were therefore new mutations. In the other two sporadic cases (families 5 and 9), the parents were not available for analysis.

In two families (1 and 2), mutations were identified in the two alleles, confirming an autosomal-recessive disease (Fig. 1).

**Autosomal-Recessive Families: Family 1.** In a 9-year-old affected girl, we identified the homozygous V4849I mutation in exon 101 (a previously described mutation). The patient had muscle weakness and difficulties in running, jumping, and climbing stairs. Her serum creatine kinase (CK) was normal, and electromyography (EMG) showed myopathic features. Histological analysis of the muscle biopsy showed discrete myopathic alterations, a total type I predominance, and a pattern of several diffuse large and mini-cores within muscle fibers (Fig. 2A). The parents were clinically asymptomatic, consanguineous, and carriers of the same mutation in a heterozygous state. No previous history of neuromuscular diseases was present in this large family with many probable heterozygote relatives (Fig. 2A).

**Family 2.** Two different and novel mutations (R4558Q and A4846V) were identified in two af-
fected sibs: a 46-year-old woman and a 42-year-old man. The R4558Q mutation (c.13673 G>A) in exon 94 was inherited from the sibs’ mother and the A4846V mutation (c.14537 C>T) in exon 101 from the father, both clinically unaffected (Fig. 1). These mutations were confirmed by automatic sequencing

FIGURE 1. Molecular analysis of the RYR1 gene in the patients from family 1 (A) and family 2 (B). ○, female; □, male; ◇, unidentified member of the family. Filled symbols are affected members.

FIGURE 2. Histological findings in the patients from family 1 (A) and family 2 (B). In the patient from family 1, hematoxylin and eosin (HE) shows variability in fiber size and some degeneration; nicotinamide adenine dinucleotide (NADH) reaction illustrates the pattern of the cores, and a total predominance of type I fibers is observed on the adenosine triphosphatase (ATPase) 9.4 reaction. In patient II-2 from family 2, HE shows large hypertrophied fibers, splitting and several internal nuclei, and one or two large and well-defined cores can be observed both in NADH and succinate dehydrogenase (SDH) reactions.
and were not found in 200 chromosomes from normal controls. The two affected sibs reported symptoms and weakness since childhood. The oldest sister had foot deformities, surgically repaired during childhood, and her younger brother has also facial weakness and muscular hypotrophy. Complementary studies in the affected male disclosed normal serum CK levels. The EMG showed myopathic features, with small-amplitude, short-duration, polyphasic motor unit potentials. There was a rapid recruitment of motor units, and long-duration, high-amplitude motor unit potentials were present in both tibialis anterior and right gastrocnemius muscles. Motor and sensory conduction velocities were normal. Muscle biopsy showed fiber-size variation, with the presence of some very large fibers, and very discrete perimysial fibrosis. A large number of internally located nuclei were observed. A type I fiber predominance was present and in the oxidative reaction, one or two large cores were identified in almost all muscle fibers (Fig. 2B).

**DISCUSSION**

Since the finding of the association of the RYR1 gene with CCD, several groups have been searching for mutations in this gene in large cohorts of families with affected patients. However, in the last 15 years, only isolated mutations were identified in scattered families. Recently, with the identification of a hot spot of mutations in the C-terminal region of the RYR1 gene, several new and recurrent mutations have been identified in many families. Mutations in this region were found in six of our nine families with CCD, confirming this hot spot for mutation also in Brazilian patients.

CCD has been classified mainly as an autosomal-dominant disease, and three among our nine families showed a typical AD pattern of inheritance. The recessive form of CCD is rare, with only four reported cases. However, we identified mutations with this pattern of inheritance in at least two of our nine analyzed families. It is possible that several sporadic cases will now be identified as having mutations in the two alleles, showing that an AR pattern is in fact not rare. Additionally, more studies of this region of the RYR1 gene may result in the identification of mutations in the second allele in cases that were believed to have dominant inheritance. This could explain the reduced penetrance and variable clinical phenotype found in some families in which individuals with one affected allele have only a susceptibility to MH whereas others, with two affected alleles, have a severe CCD phenotype. In accordance with this hypothesis, Romero et al. described CCD patients with a severe clinical course who were compound heterozygotes for the mutations G215E and R614C in the RYR1 gene. Each mutation had already been described as pathogenic, causing MH when present in only one of the alleles. Furthermore, some mutations that are now characterized as polymorphisms could contribute to the phenotype when associated with other mutations.

The V4849I mutation, identified in one of our patients, has previously been described as pathogenic in a consanguineous family with no history of neuromuscular disorders. This mutation affects a highly conserved and functionally important domain of the RYR1 protein. The biopsy analysis in the patient described by Jungbluth et al. showed the presence of both mini-cores and single central cores, and also a few rod-like structures. Although no rods were identified in our patient with the same mutation, the pattern of the cores seems to be similar, suggesting an association between the type of mutation and its consequence in muscle. Additionally, the clinical picture of our patient is also similar to that in the described family, i.e., moderate proximal weakness since childhood and predominantly axial and proximal weakness with mild thoracolumbar scoliosis in early adulthood.

The R4558Q and A4846V mutations, identified in compound heterozygotes, are both novel mutations and were not found in 200 normal control chromosomes. The alignment of the human RYR1 amino acid sequence to those of mouse, pig, chicken, and fish, and also human RYR2 isoform, demonstrates that the regions affected by these two novel mutations are evolutionary conserved, adding further evidence that they are pathogenic.

The parents of these patients are carriers of each of these mutations in heterozygous states, but do not have any clinical weakness. This suggests that the R4558Q and A4846V mutations are pathogenic only when found together.

The mild clinical course observed in the three patients from our two AR families is surprising, since a severe clinical course has been associated with the AR forms of CCD. There are only two AR families with compound heterozygotes reported in the literature and the patients described showed a severe phenotype, with akinesia and hydramnion during pregnancy. Our patients had clinical signs from childhood and slow progression; both are in their 40s and are still ambulant.

In conclusion, although the classic pattern of inheritance in CCD is autosomal dominant, our data suggests that the autosomal-recessive form may be
more common than expected. It is possible that other mutations, previously found in asymptomatic individuals and considered as nonpathogenic, are responsible for muscle weakness if present in both alleles, in homozygous or compound heterozygous states. Considering that the parents in our two AR families are all clinically asymptomatic heterozygous carriers for one of the mutations, and since a significant clinical variability is observed in this disease, it is recommended that exposure to volatile anesthetics should be avoided in such subjects because of the possible high risk for malignant hyperthermia.

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