NEW PATHOGENIC VARIANTS IN A PATIENT WITH CENTRAL CORE DISEASE AND MALIGNANT HYPERTHERMIA SUSCEPTIBILITY

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ABSTRACT

PURPOSE: Central Core Disease and malignant hyperthermia susceptibility results from *RYR1* gene mutations. More than 200 different mutations in *RYR1* have been reported, and they are also associated with other conditions, such as Malignant Hyperthermia (MH) susceptibility. Report new pathogenic variants in a patient with Central Core Disease and Malignant Hyperthermia susceptibility. Medical record research of the index case and data obtained from the patient's mother (legal guardian). CASE DESCRIPTION: The index case is a seven years old Caucasian presented marked generalized hypotonia and failed to achieve motor milestone. Investigation was not a muscle biopsy, but whole exome sequencing identified three variants in *RYR1*, two inherited in block from the mother, and one from the father: pathogenic c.7373G>A (p.Arg2458His) in exon 46 (associated with MH), likely pathogenic c.7093G>A (p.Gly2365Arg) in exon 44 and c.13746+4C>G in intron 94 (variant of uncertain significance), respectively. CONCLUSION: The described variants of maternal origin corroborated the clinical diagnosis of central core disease of autosomal recessive inheritance and heterozygous manifestation. Patient and his mother are predisposed to malignant hyperthermia episodes. Failure of the genetic test to detect the second mutation, or a synergistic effect of *cis* mutations on the protein, may explain the genotype-phenotype correlation.

Keywords: Central core disease; Malignant hyperthermia susceptibility; RYR1; Whole exome sequencing.

NOVAS VARIANTES PATOGÊNICAS EM PACIENTE COM MIOPATIA DO CERNE CENTRAL E SUSCEPTIBILIDADE À HIPERTERMIA MALIGNA

RESUMO

OBJETIVO: A Miopatia do Cerne Central e a susceptibilidade à hipertermia maligna são resultado de mutações no gene RYR1. Mais de 200 mutações diferentes no RYR1 foram relatadas e também estão associadas a outras condições, como a suscetibilidade à Hipertermia Maligna (MH). O relato de caso tem por objetivo demonstrar novas variantes patogênicas em um paciente com Miopatia do Cerne Central e susceptibilidade à Hipertermia Maligna, através de Pesquisa de prontuário e dados obtidos da mãe do paciente (responsável legal). DESCRIÇÃO DO CASO: O caso-índice é um caucasiano de sete anos de idade que apresentou hipotonia generalizada acentuada e não conseguiu atingir o marco motor. Não foi realizada biópsia muscular, mas o sequenciamento completo do exoma identificou três variantes no RYR1, duas herdadas em bloco da mãe e uma do pai: variante patogênica c.7373G> A (p.Arg2458His) no exon 46 (associada à HM), variante provavelmente patogênica c.7093G> A (p.Gly2365Arg) no exon 44 e variante de significado incerto c.13746 + 4C> G no intron 94, respectivamente. CONCLUSÃO: As variantes descritas de origem materna corroboraram o diagnóstico clínico de Miopatia do Cerne Central, de herança autossômica recessiva e manifestação heterozigótica. O paciente e sua mãe estão predispostos a episódios de hipertermia maligna. A falha no teste genético para detectar a segunda mutação, ou um efeito sinérgico das mutações em cis na proteína, podem explicar a correlação genótipo-fenótipo.

Palavras-chave: Miopatia do Cerne Central; Hipertermia Maligna; RYR1; Sequenciamento de Exoma.

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INTRODUCTION

Central Core Disease (CCD, MIM #117000) is a hereditary congenital myopathy, usually inherited as an autosomal dominant (AD) trait (1), and less frequently as autosomal recessive (AR) or due to *de novo* mutations. It is characterized by muscular hypotonia and delayed motor development in childhood, which may manifest itself in different degrees. CCD is probably the most common congenital myopathy (2). A regional England study indicated a CCD frequency of 1 in 250,000 individuals (3). In Brazil, epidemiological data on CCD are unknown (4).

CCD results from mutations in the skeletal muscle ryanodine receptor 1 (*RYR1*) gene, on chromosome 19q13.1. The *RYR1* gene comprises 106 exons and encodes the skeletal muscle sarcoplasmic reticulum calcium (Ca^{2+}) release channel receptor, a protein of 5,038 amino acids that is fundamental for the excitation-contraction coupling (ECC) and Ca^{2+} homeostasis (5). More than 200 different mutations in the *RYR1* gene have been reported (6), and they are also associated with other conditions besides CCD, such as Malignant Hyperthermia (MH) susceptibility (1,7). Some of the mutations are exclusively associated with CCD, others with MH, or both (2). The *RYR1* mutations linked to CCD and MH are located mainly in three "hotspots" of the protein: the N-terminal (residues p.M1-R614), central (p.R2163–p.R2458) and C-terminal (p.R4136- p.P4973).

CCD clinical features are usually proximal weakness with prominent involvement of the hip and axial muscles and orthopedic complications (8). Marked facial and extra ocular and respiratory involvement are uncommon features. Another clinical condition that may be associated with CCD is MH, a pharmacogenetic disorder related to the administration of inhalation anesthetics, depolarizing muscle relaxants or extreme physical activity practice. In these situations, an immense accumulation of Ca^{2+} may occur, leading to a hyper metabolic state and consequent hypoxemia, metabolic acidosis, rhabdomyolysis and a rapid increase in body temperature, that can be fatal (7,9). The incidence of the allelic MH trait is estimated at 1 in 3,000 - 10,000; and the clinical prevalence is at 1 in 60,000 - 100,000 (10).

The diagnosis of CCD occurs through a combination of clinical features and complementary tests, such as muscle biopsy with histopathological analysis, electron microscopy and muscle magnetic resonance imaging (MRI), which are capable to exclude other neuromotor diseases (3). Currently, the gold standard for CCD diagnosis is muscle biopsy with well-defined, single or multiple, central or eccentric cores running a significant extent along the longitudinal muscle fiber axis (11). Genetic identification for *RYR1*

mutations, especially by next generation sequencing (NGS) methods, can differentiate the diagnosis of patients with clinical features of a congenital myopathy and histopathological findings suggestive of CCD (12). Besides allowing the diagnosis and identification of CCD family inheritance, NGS, like whole exome sequencing (WES), also allows the identification of individuals predisposing to MH (13). Nowadays, these tests are usually requested when no specific alterations are found in muscle biopsy and muscle MRI, or when the clinical findings are nonspecific (14).

We present a case of a new pathogenic variants in a patient diagnosed with CCD through WES, and Malignant Hyperthermia susceptibility.

MATERIALS AND METHODS

Clinical data and complementary exams were obtained through research in medical records, in addition to complementary data, through interviews with the mother (legal guardian). Informed consents were obtained from the patient's mother and from the responsible physician. This study was approved by the local Ethics Committee (Federal University of Fronteira Sul, Chapecó, SC, Brasil).

CASE REPORT

The index case is a seven years old caucasian male, with no prenatal history of complications. His mother referred muscle weakness within three months of postnatal life. At age of six months, he presented marked generalized hypotonia and failed to achieve motor milestone. Present evaluation reveals mild facial and proximal muscle weakness, scoliosis, generalized hypotonia, sialorrhea, low orofacial motor control, hyporreflexia, strabismus, ametropia and focal epileptogenic seizures. He has no cardiac or respiratory abnormalities and had no family history of similar phenotype. The initial clinical hypotheses were cerebral palsy, genetic unknown syndrome, neuromuscular disease and inborn errors of metabolism (IEM).

Cranial MRI indicated a volumetric reduction of the periventricular and supratentorial white matter, with right temporal predominance and hypoplasia of the corpus callosum. Serum creatine kinase (CK) was normal. Electroencephalogram showed severe, bilateral and independent epileptogenic paroxysmal activity in temporal regions, with left predominance. Brainstem Evoked Response Audiometry (BERA) was normal. IEM investigation showed no

abnormalities. After this initial investigation, neuromuscular disease was considered as the first diagnostic hypothesis.

Although the gold standard for neuromuscular disease or myopathy diagnosis is muscle biopsy, this technique was not available at the time. Therefore, genetic evaluation was suggested. The karyotype was normal, so WES was performed. In the first analysis, genes associated with neurological and musculoskeletal diseases, and corpus callosum malformations were included. Two pathogenic variants of maternal origin, inherited in block (in *cis*), were described in the *RYR1* gene: c.7373G>A (p.Arg2458His) in exon 46 (already described in families with MH episodes), and c.7093G>A (p.Gly2365Arg) in exon 44. Such variants were initially considered without clinical significance to the presented phenotype but suggested that the patient should be considered at risk for an advent of MH during procedures that involve general anesthesia. Eight months after the initial report, a reanalysis was performed with new bioinformatic techniques, that allowed a better study of the genetic variants associated with the phenotype. In this second analysis, the same variants c.7373G>A (p.Arg2458His) and c.7093G>A (p.Gly2365Arg) were described as pathogenic and likely pathogenic, respectively. In addition, a new variant of paternal origin was described: c.13746 + 4C>G in intron 94. This variant had not previously been described in the *RYR1* gene and has uncertain clinical significance (VUS). The genetic details of the patient are presented in Table 1.

Since two of the variants described are located in exons and are of maternal origin, such genetic findings, associated with the clinical presentation, corroborated the diagnosis of CCD of AR inheritance with heterozygous manifestation. Since the beginning of the clinical manifestations, the patient was referred to physical and speech therapy services, and he continues in rehabilitation. After the CCD diagnosis, the patient started Pediasuit physical therapy, which enabled him to walk with support. In addition, he developed epilepsy and currently is under treatment with primidone 250 mg BID and clobazam 10 mg once a day. His prognosis is uncertain.

Table 1 - Genetic details of patient *RYR1* variants

	Exon/ intron	Nucleotide change	Aminoacid change	Mode of inheritance	Variant classification	Reported in
1	E 46	c.7373G>A	p.Arg2458His	AR	Pathogenic	[28,29]
2	E 44	c.7093G>A	p.Gly2365Arg	AR	Likely pathogenic	-
3	I 94	c.13746 + 4C>G	(intronic)	AR	VUS	-

Legend: *E* exon number, *I* intron number, AR autosomal recessive, *VUS* variant of uncertain significance.

DISCUSSION

CCD is a rare neuromuscular disease associated with different mutations in the *RYR1* gene. These mutations are usually located in the three "hotspots" domains of the protein: N-terminal, central and C-terminal (4). According to the affected part of the protein, the clinical phenotype is variably presented, as *RYR1*-associated genotype-phenotype correlations are complex and can be partially explained by the degree of functional differentiation conferred to the protein. Patients with mutations on the C-terminal region have classical clinical features, and patients with non-C-terminal mutations have mild musculoskeletal abnormalities (joint contracture and scoliosis) and are more likely to have an MH episode (2). MH is considered an allelic disease of *RYR1* gene and should be considered a risk in all cases of CCD (1,7).

Genetic inheritance in myopathies related to the *RYR1* gene may be AD, AR or due to *de novo* mutations. In those cases, a failure to detect a second mutation (as a major deletion not detected by sequencing, or intronic or promoter mutations) cannot be excluded. This inheritance is caused by combinations of null with missense mutations, or of two or more missense mutations in both alleles (1). CCD diagnosis, in combination with suggestive clinical features, depends on the presence of typical histopathological findings on muscle biopsy, as nuclear areas with a lack of mitochondria and stains of oxidative enzymes. However, genetic tests are important for the correct classification of the disease (2). Thus, molecular genetic testing for *RYR1* mutations, as WES, are extremely useful in CCD diagnosis, in addition to identifying and classifying the variants involved (6).

The described patient presented generalized hypotonia and failed to achieve motor milestone, and a congenital myopathy was suggested. However, muscle biopsy was not available at the time, therefore genetic evaluation was accomplished. The WES analysis indicated three variants in *RYR1* gene, heterozygous composed (Table 1). Two exonic variants were inherited in block from the mother, and the other one, intronic, from the father. The exonic variants are located in the second "hotspot" mutational domain (4). According to the WES, the variant c.7373G>A (NM_000540.2) has pathogenic characteristics. Li et al. (15) identified this variant in a family from Singapore that satisfied the criteria of MH susceptibility. In the presented case, both the patient and his mother are predisposed to MH episodes if exposed to general anesthesia, mainly succinylcholine and halothane. During the genetic counseling, it was suggested that the mother and other relatives of maternal origin should be tested for the allele c.7373G>A (NM_000540.2), that predisposes to MH. The other variant of maternal origin, c.7093G>A, is likely pathogenic. It has not been described in more than six thousand normal patients in the NHLBI Exome Sequencing Project. The variant c.13746 +4C>G has not previously been described in the *RYR1* gene, and it is a VUS. The pathogenic variants identified in the *RYR1* gene were not related to the cranial MRI alterations and focal epileptogenic seizures of the patient, but maybe those characteristics are associated to hypoxemia due to hypotony during childbirth.

This case report presents new genetic variants in *RYR1* gene related to non-classic CCD with earlier presentation, and MH susceptibility. The described variants inherited in block from the mother accounted to the clinical presentation, with an AR inheritance and heterozygous manifestation. Usually, AR mutations are located throughout the entire *RYR1* gene, and are characterized by a more severe and progressive clinical presentation with neonatal-onset and significant generalized muscle weakness (1). Once the described variant in *trans* is localized in an intronic domain, it cannot be considered as the second mutation of the recessive inheritance. Some hypothesis to explain the disease presentation and the genotype-phenotype correlation are failure of the technic to detect a second mutation (major deletions, promoter mutations), or a synergistic effect on protein function of the *cis* mutations from maternal origin.

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CONTRIBUTORS

Sarah Franco Vieira de Oliveira Maciel: interviewed the patient's legal guardian and the attending physician; collected clinical information from the medical records and the complementary tests; assisted during article writing and submission; coordinated the research group during article writing and submission.

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REFERENCES

1 Robinson R, Carpenter D, Shaw MA, Halsall J, Hopkins P. Mutations in RYR1 in malignant hyperthermia and central core disease. Hum Mutat. 2006; 27:977–89. doi: 10.1002 / humu.20356

2 Jungbluth, H. Central core disease. Orphanet journal of rare diseases 2007; 2: 25. doi: 10.1186 / 1750-1172-2-25

3 Norwood FL, Harling C, Chinnery PF, Eagle M, Bushby K, Straub V. Prevalence of genetic muscle disease in Northern England: in-depth analysis of a muscle clinic population. Brain 2009; 132: 3175–86. doi: 10.1093 /brain/ awp236

4 Cuperman T, Fernandes SA, Lourenço NC, Yamamoto LU, Silva HC, Pavanello RC, et al. Silent polymorphisms in the RYR1 gene do not modify the phenotype of the p. 4898 I> T pathogenic mutation in central core disease: a case report. BMC research notes 2014; 7: 487. doi: 10.1186 / 1756-0500-7-487

5 Jungbluth H, Müller CR, Halliger–Keller B, Brockington M, Brown SC, Feng L, et al. Autosomal recessive inheritance of RYR1 mutations in a congenital myopathy with cores. Neurology 2002; 59: 284-87. doi: 10.1212 / wnl.59.2.284

6 Quinlivan RM, Muller CR, Davis M, Laing NG, Evans GA, Dwyer J, et al. Central core disease: clinical, pathological, and genetic features. Archives of disease in childhood 2003; 88:1051-55. doi: 10.1136 / adc.88.12.1051

7 Carpenter D, Robinson RL, Quinnell RJ, Ringrose C, Hogg M, Casson F, et al. Genetic variation in RYR1 and malignant hyperthermia phenotypes. British journal of anaesthesia 2009; 103: 538-48. doi: 10.1093 / bja / aep204

8 Ryan MM, Schnell C, Strickland CD, Shield LK, Morgan G, Iannaccone ST, et al. Nemaline myopathy: a clinical study of 143 cases. Annals of neurology 2001; 50:312-20. doi: 10.1002 / ana.1080

9 Dlamini N, Voermans NC, Lillis S, Stewart K, Kamsteeg EJ, Drost G, et al. Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. Neuromuscular Disorders 2013; 23: 540-48.

10 Monnier N, Krivosic-Horber R, Payen JF, Kozak-Ribbens G, Nivoche Y, Adnet P, et al. Presence of two different genetic traits in malignant hyperthermia families: implication for genetic analysis, diagnosis, and incidence of malignant hyperthermia susceptibility. Anesthesiology 2002; 97: 1067-74. doi: 10.1097 / 00000542-200211000-00007

11 Sewry CA, Müller C, Davis M, Dwyer JSM, Dove J, Evans G. The spectrum of pathology in central core disease. Neuromuscul Disord 2002; 12: 930–38. doi: 10.1016 / s0960-8966 (02) 00135-9
12 Lillis S, Abbs S, Ferreiro A, Muntoni F, Jungbluth H. Clinical utility gene card for: Central core disease. European Journal of Human Genetics 2012; 20. doi: 10.1038 / ejhg.2011.179

13 Jung NY, Park YE, Shin JH, Lee CH, Jung DS, Kim DS. Mild Clinical Features and Histopathologically Atypical Cores in Two Korean Families with Central Core Disease Harboring RYR1 Mutations at the C-Terminal Region. J Clin Neurol 2015; 11:97-01. doi: 10.3988 / jcn.2015.11.1.97.

14 Ellard S, Kivuva E, Turnpenny P, Stals K, Johnson M, Xie W, et al. An exome sequencing strategy to diagnose lethal autosomal recessive disorders. European Journal of Human Genetics 2015; 23: 401–04. doi: 10.1038 / ejhg.2014.120

15 Li DW, Lai PS, Lee DW, Yong RY, Lee TL. Malignant Hyperthermia and Ryanodine Receptor Type 1 Gene (RyR1) Mutation in a Family in Singapore. Annals of the Academy of Medicine, Singapore 2017; 46: 455-60.