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COMPOUND HETEROZYGOSITY IN CEREBELLAR ATAXIA, MENTAL RETARDATION, AND DISEQUILIBRIUM SYNDROME TYPE 4

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ABSTRACT

Cerebellar ataxia, mental retardation, and disequilibrium syndrome (CAMRQ) is a genetically and clinically heterogeneous disorder with four described subtypes. Autosomal recessive syndrome of cerebellar ataxia, mental retardation, and disequilibrium type 4 (CAMRQ4) is caused by mutations in the *ATP8A2* gene. We report an 8-year-old boy with choreoathetosis, hypotonia, without the ability to keep his head up and profound mental retardation. There was quadrupedal locomotion, as well. MRI of the brain revealed a hypotrophy of the corpus callosum, diffuse white matter reduction, widespread delayed myelination and ventriculomegaly. Trio whole-exome sequencing revealed compound heterozygosity in the *ATP8A2* gene consisting of a known variant c.1756C>T (p.Arg586*) inherited from the mother and a novel variant c.691_701delCTGATGAAGTT (p.Leu231fs) inherited from the father. CAMRQ type 4 has been found in about 50 patients. To the best of our knowledge, this is the first reported patient with CAMRQ4 with these gene variants. The clinical presentation is severe.

Keywords: Cerebellar ataxia, mental retardation, disequilibrium, quadrupedal locomotion, CAMRQ4, ATP8A2

INTRODUCTION

Cerebellar ataxia, mental retardation, and disequilibrium syndrome (CAMRQ) is a complex, rare and heterogeneous disorder [1]. Four subtypes of autosomal recessive CAMRQ syndrome have been identified (CAMRQ1-CAMRQ4) [1, 2]. CAMRQ1-3 subtypes are caused by mutations in very low-density lipoprotein receptor (VLDLR) gene, WD repeat domain 81 (WDR81) gene and carbonic anhydrase 8 (CA8) gene, respectively [1, 3-5]. CAMRQ4 is caused by mutations in the ATPase class I type 8A member 2 (ATP8A2) gene [2]. *ATP8A2* encodes a member of the P4 ATPase subfamily that is involved in the transport of amino-phospholipids [6]. Evidence suggests that *ATP8A2* is critically involved in the development of the cerebro-cerebellar structures required for posture and gait [2].

We report a patient with compound heterozygosity in the *ATP8A2* gene consisting of a known gene variant and a novel, so far not described variant. This variant combination conferred severe clinical expression. In addition, we analyze the phenotype-genotype relationship in about 50 reported patients.

PATIENT AND METHOD

The proband is an 8-years old male patient, son of non-consanguineous healthy parents of Macedonian descent, who was referred to the University Children's Hospital in Skopje, North Macedonia. He has two siblings, an older brother and an older sister, both healthy. There is no record of similar diseases in the family. Whole blood was drawn from the proband and his parents for analysis. The siblings were not included in this study. Informed consent was obtained from both parents.

The patient and his parents had whole-exome sequencing carried out at Zan Mitrev Clinic in Skopje, North Macedonia. Genomic DNA was extracted from whole blood and libraries were prepared with the Twist Human Core Exome kit. Next-generation sequencing was carried out on an Illumina NovaSeq 6000 instrument with an average variant depth of 53x. The entire workflow of sequence trimming, demultiplexing, sequence alignment to Hg19, variant calling and annotation and filtering analysis were carried out on the GENOOX platform. Additional annotation was done with Annovar [7]. Pathogenicity assessment of candidate variants was done by in-silico analysis following the American College of Medical Genetics and Genomics (ACMG) criteria [8].

RESULTS

Following an uncomplicated pregnancy, the patient was born at full term with a birth weight of 3,200g, birth length of 50cm and Apgar score of 9/10. The first noticeable clinical sign was hypotonia in early infancy, feeding difficulties and failure to thrive. Afterward, he demonstrated a deficiency in all psychomotor and developmental milestones. At the age of 8 years, he had profound mental retardation, hypotonia, inability to keep the head upright and choreoathetosis. There was quadrupedal locomotion, as well. Deep tendon reflexes were diminished. The patient was nonverbal but was able to recognize his parents. He still suffered from feeding difficulty, being able to eat pureed food only.

Fundoscopy did not reveal optic atrophy. Hearing impairment tests were not performed. All relevant laboratory investigations were in the reference range. Metabolic disorders were excluded. Multiplex ligation-dependent probe amplification test excluded some of the most common microdeletion syndromes.

The brain magnetic resonance imaging revealed a hypotrophy of the corpus callosum, widespread delayed myelination for the age, global loss of white matter and ventriculomegaly.

Trio whole-exome sequencing was carried out, analyzing exons and adjacent regions of 19 497 genes of the proband and his parents. Trio whole-exome sequencing revealed compound heterozygous gene alterations in the *ATP8A2* gene; the c.1756C>T (p.Arg586*) variant was inherited from the mother and the c.691_701delCTGATGAAGTT (P.Leu231fs) variant was inherited from the father.

The c.1756C>T (p.Arg586*) variant is annotated as a likely pathogenic variant, with ultra-rare population frequency in ClinVar. According to ACMG, it meets a few pathogenicity criteria, including PVS1, PM2, PP3 and PP5. According to GeneDx, it was predicted to result in loss of function, either due to stop gain or nonsense elimination of the mRNA. c.691_701delCTGATGAAGTT (p.Leu231fs) is a variant with ultra-rare population frequency, it is not annotated in ClinVar and a unique rsID code has not been yet designated. According to ACMG, it meets a few pathogenicity criteria, including PVS1, PM2 and PP3. It was predicted to result in a frameshift, incomplete protein and loss of function.

According to our knowledge, the variant c.1756C>T (p.Arg586*) has been previously reported in the literature, whilst the variant c.691_701delCTGATGAAGTT (p.Leu231fs) is novel and reported in this study for the first time.

DISCUSSION

So far, eleven studies have reported 45 CAMRQ4 patients from 26 families with various ethnic backgrounds [2, 9-19]. Five were deceased and three were not available for examination at the time of the report [13, 14]. The phenotype varies from extremely severe occurring in early childhood, to relatively moderate manifested in adult life. There is a preponderance of female versus male sex (26:19). Consanguinity was found in 16 families, six families denied consanguinity, while

information was not reported for four families [2, 9-19].

There were patients with a severe phenotype, developmental delay, severe hypotonia and intellectual disability [9, 11-14, 16, 17, 19]. CAMRQ4 has only recently been associated with a less severe phenotype, with cerebellar ataxia without encephalopathy and mild to borderline intellectual disability [15]. Another mild phenotype was reported in two siblings with congenital ataxia, independent gait and near to normal cognition [18]. We report a non-verbal patient with profound mental retardation.

Ataxic symptoms were reported in many of the patients [10, 11, 13, 15-19]. The majority of the described patients were non-ambulatory [9, 11-13, 15-17, 19]. Non-ambulatory patients had involuntary movements, mainly choreoathetosis, dystonia or dyskinesia [9, 11-13, 15, 17, 19]. One non-ambulatory patient had opisthotonos [17]. It is of note, that only four patients, with or without ataxia and without involuntary movements, acquired independent gait [15, 18]. Three patients had impaired ambulation, needing assistance, while two of them had choreoathetosis [14, 15]. Various severity and modes of ambulation were found in a same family. Two members were quadrupedal, one was quadrupedal in childhood but became bipedal in adulthood and one was quadrupedal in childhood but became non-ambulatory in adulthood. There were no involuntary movements in all of them [2, 10]. Our patient is only the fifth reported patient with quadrupedal locomotion. In contrast to the previous report, he had choreoathetosis. Hypotonia of various degrees was present in all patients, except the patients with a mild phenotype [15]. Our patient had ataxia and pronounced hypotonia non-responsive to continuous physical therapy.

Optic atrophy was the most common eye abnormality, reported in 18 patients, in some accompanied by ophthalmoplegia, ptosis, nystagmus, or other abnormalities [11-15, 17, 19]. Two of the patients with mild phenotypes also had optic atrophy [15]. Our patient had a normal fundoscopic examination and normal MRI presentation of the optic nerves. Hearing impairment was reported in seven patients [13-15, 19]. Eight patients with CAMRQ4 had seizures [14, 15, 17, 19]. Microcephaly was rarely reported, only in severe phenotype patients [11, 13, 15]. Some of the patients had minor dysmorphic features including elongated face, low set ears, high arched palate and/or teeth abnormalities [17, 19]. One patient had lordoscoliosis, pectus excavatum, gum hypertrophy, upturned nose and triangular face [13].

The authors reported many *ATP8A2* gene variants (Table 1). All of the reported patients were homozygous or compound heterozygous, except for the patient reported by Cacciagli et al. (2010) who was the only heterozygous individual with CAMRQ4 phenotype. He had balanced translocation t(10;13), disrupting the coding sequence of one allele of the *ATP8A2* gene, while the second allele was normal and the translocation did not modify another gene(s). This patient demonstrated moderate mental retardation, but severe hypotonia [9].

It is of note that despite the broad variability in type and position of the detected gene alterations, the phenotypic presentation in the majority was similar [2, 9-17, 19]. There are just two reports of patients with a milder phenotype [15, 18]. The variants detected in these patients are missense mutations c.1754G>T, (p.Gly585Val) and c.1762C>T, (p.Arg588Trp) and splicing mutation c.1580-18C>G-intron 17, which have not been reported in any other known patients [15, 18]. Many of the patients, who expressed severe phenotype, also had missense or splicing variants in the *ATP8A2* gene. Hence, it is impossible to conclude whether they are solely responsible for the severity of the phenotype.

ATP8A2 variants detected in multiple family members resulted in a similar phenotype [13-16, 18, 19]. Only a few variants were reported in different families. c.1312A>G, (p.Met438Val) was detected in two homozygous non-related male patients from different ethnicities, sharing a similar severe phenotype [15, 17]. c.1741C>T, (p.Arg581*) was found in multiple members of a consanguine Saudi family and two compound heterozygous Japanese siblings. The phenotype did not differ significantly [13, 19].

Our patient was compound heterozygous, carrying a previously observed variant c.1756C>T (p.Arg586*) and a novel variant c.691_701delCTGATGAAGTT (p.Leu231fs). The variant c.1756C>T (p.Arg586*) was previously detected in a homozygous female patient from consanguineous parents of Turkish descent, who presented with severe hypotonia, choreoathetosis, dystonia and dysarthria [14]. Those clinical characteristics were similar to those of our patient. In contrast to our patient, the previously reported patient also had optic atrophy and ptosis. The major difference

Table 1. *Ethnicity, consanguinity and gene variant(s) in all reported CAMRQ4 patients*

	Ethnicity	Consanguinity	Alleles	Gene variant(s)
Current patient	Macedonian	No	Compound heterozygous	c.1756C>T, (p.Arg586*) and c.691_701delCTGATGAAGTT, (p.Leu231fs)
Cacciagli et al, 2010	French	No	Heterozygous	De novo t(10;13)(p12.1;q12.13)dn balanced translocation
Tan et al, 2006 and Onat et al, 2013	Turkish	Yes	Homozygous	c.1128C>G, (p.I376M)
Martin-Hernandez et al, 2016	Spanish	Yes	Homozygous	c.1287G>T, (p.K429N)
	Spanish/Argentinian	No	Compound heterozygous	c.1630G>C, (p.A544P) and c.1873C>T, (p.R625W)
Quintas et al, 2017	Portuguese	No	Compound heterozygous	c.1761dup, (p.Arg588Serfs*5) and Chr13(GRCh37):g.26151255dup
Alsahli et al, 2018	Saudi	Yes	Homozygous	c.1741C>T, (p.Arg581X)
	Saudi	Yes		intronic pathogenic variant [G > C at position 26273310]
	Saudi	Yes		c.2749A>G (p.Asn917Asp)
McMillan et al, 2018	French	No	Compound heterozygous	c.1185+5G>A and Deletion of exons 28-33
	European Ashkenazi/ Native American	No		c.1787delA, (p.Asn596MetfsX) and c.321+3_321+8 delAATGGT
	Turkish	Yes	Homozygous	c.1756C>T, (p.Arg586*)
	Moroccan	Yes		c.2104T>C, (p.Trp702Arg)
	Sri Lankan	Yes		c.1286A>T (p.Lys429Met)
	Iranian	Yes		c.1474_1662del, (p.Pro492_Ala554del)
	Lebanese	Yes		c.3188_3196delCTATGGTC insGAAGAAG, (p.Thr1063fs)
Guissart et al, 2020	Turkish	Yes	Homozygous	c.1754G>T, (p.Gly585Val)
	Algerian	N/A		c.1762C>T, (p.Arg588Trp)
	Caucasien (Unknown)	N/A		c.1057+5G>C
	Italian	N/A	Compound heterozygous	643A > T, (p.Ile215Leu) and c.1916A > G, (p.Tyr639Cys)
	Turkish	N/A	Homozygous	c.1312A>G, (p.Met438Val)
Mohamadian et al, 2020	Arab	Yes	Homozygous	c.1339G>A, (p.G447R)
Heidari et al, 2021	Iranian	Yes	Homozygous	c.1868-2A>G
	Iranian	Yes		c.2473G>C, (p.Asp825His)
	Iranian	Yes		c.1312A>G, (p.Met438Val)
Damasio et al, 2021	N/A	Yes	Homozygous	c.1580-18C>G-intron 17
Narishige et al, 2022	Japanese	No	Compound heterozygous	c.1741C>T, (p.Arg581*) and c.2158C>T, (p.Arg720*)

was the quadrupedal locomotion observed in our patient and the absence of ambulation in the Turkish patient. The brain MRI finding of mild delay in myelination, subcortical white matter volume loss and thin corpus callosum was also found in our patient [14]. Variant c.691_701delCTGATGAAGTT (p.Leu231fs) is a novel variant, not reported so far. In silico analysis predicted a frameshift resulting in incomplete protein and loss of function.

CONCLUSION

All patients with CAMRQ4 present with hypotonia in the early infancy, developmental delay and failure to thrive, resembling encephalopathy from other causes. However, cerebellar ataxia or a quadrupedal locomotion in association with visual and hearing impairment, which may present later and involuntary movement may be a clue to suspect for CAMRQ4. Until recently, all of the described cases of CAMRQ4 were associated with severe presentation. Only recently, less severe phenotypes were reported with cerebellar ataxia without encephalopathy. *ATP8A2* should be analyzed in all patients of syndromic or non-syndromic ataxia. Whole exome sequencing is a fast and cost-effective technique to identify disease-causing variants, making possible genetic counseling and prenatal diagnosis in families with CAMRQ4 syndrome.

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Резиме

ДВОЈНО ХЕТЕРОЗИГОТСТВО КАЈ ПАЦИЕНТ СО СИНДРОМ НА ЦЕРЕБЕЛАРНА АТАКСИЈА, МЕНТАЛНА РЕТАРДАЦИЈА И ДИЗЕКВИЛИБРИУМ ТИП 4

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Синдромот на церебеларна атаксија, ментална ретардација и дизеквилибриум (CAMRQ) е генетски и клинички хетерогено нарушување со 4 опишани поттипа. Автозомно рецесивниот синдром на церебаларна атаксија, ментална ретардација и дизеквилибриум тип 4 е предизвикан од мутации во генот ATP8A. Ова е случај на 8-годипно момче со хореоатетоза, хипотонија без можност за држење на главата исправена и длабока ментална ретардација. Исто така, пациентот има и квадрипедален од. Магнетната резонанца на мозокот покажа хипотрофија на corpus callosum, дифузна редукција на белата маса, распространета одложена миелинизација и вентрикуломегалија. Секвенционирањето на целиот егзом откри двојно хетерозиготство во генот ATP8A2, сочинето од позната варијанта c.1756C>T (p.Arg586*), наследена од мајката и нова варијанта c.691_701delCTGATGAAGTT (p.Leu231fs), наследена од таткото. CAMRQ тип 4 е пронајден кај околу 50 пациенти. Според нашите сознанија, ова е прв објавен случај на пациент со CAMRQ тип 4 со овие генски варијанти. Клиничката презентација е тешка.

Клучни зборови: церебеларна атаксија, ментална ретардација, дизеквилибриум, квадрипедален од, CAMRQ4, ATP8A2