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ORIGINAL ARTICLE

Neurological and extra-neurological clinical spectrum observed in pediatric patients with *EMC1* gene variants identified by whole exome sequencing

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ABSTRACT

Background: The endoplasmic reticulum membrane protein complex 1 (*EMC1*) gene encodes a subunit of the EMC with multiple alternatively spliced transcripts encoding different isoforms. Monoallelic and biallelic mutations of the *EMC1* gene have been reported for cerebellar atrophy, visual impairment, psychomotor retardation, lipoid proteinosis of Urbach and Wiethe, and Alkuraya-Kucinskas syndrome.

Methods and cases: Herein, we present whole exome sequencing results of eight Saudi pediatric patients with distinctive clinical features which revealed both monoallelic and biallelic variants in the *EMC1* gene (CHR1 exon4: 19568918, NM_001271429.2, c.364G>A; p.A122T), including two previously reported siblings (CHR1 exon21: 19547328, NM_015047.3, c.2602G>A; p.G868R).

Results: The patients presented with the neurological and extra-neurological clinical spectrum that included seizures, spastic diplegia, cognitive impairment, axial and appendicular hypotonia, dysmorphic features, joint hyper-flexibility, attention deficit hyperactivity disorder, skeletal dysplasia in addition to generalized global developmental delay, failure to thrive, speech delay, intellectual disability, and visual impairments. Furthermore, brain Magnetic resonance imaging findings were consistent with variable clinical features and revealed brain atrophy, thinning of corpus callosum, semi-lobar holoprosencephaly, white matter abnormality, diffuse paucity of the myelin within the brain parenchyma, and reduction of white matter arborization in the temporal lobes.

Conclusion: In conclusion, these clinical cases highlight the importance of the *EMC1* gene in disease phenotype and add up to the expanded *EMC1*-related phenotype.

Keywords: *EMC1*, whole exome sequencing, CAVIPMR, brain MRI, consanguinity.

Introduction

Endoplasmic reticulum membrane protein complex 1 (*EMC1*) [endoplasmic reticulum (ER) membrane protein complex subunit 1, OMIM#616846] gene is located in chromosome 1p36.13 and encodes a 993-amino acid single-pass type I transmembrane protein, a subunit of the endoplasmic reticulum (ER) membrane protein complex. *EMC1* is expressed ubiquitously in all human tissues and exists in different isoforms with multiple alternatively spliced transcript variants (www.gtexportal.org; www.proteomicsdb.org). Studies in Yeast, *C. elegans*, and *Drosophila* implicated *EMC1* gene to play a role in protein folding by possibly modulating ER-associated protein degradation, ER-mitochondria tethering, and stable expression of other transmembrane proteins (1,4). Studies in human patients suggested a possible association of *EMC1* in neurodegeneration and other variable clinical phenotypes such as Alzheimer's, Parkinson's disease, amyotropic lateral sclerosis (ALS), and Charcot-Marie-Tooth (CMT) disease among others (5,7).

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At the molecular genetic level, both monoallelic and biallelic variants in *EMC1* gene were first reported in retinitis pigmentosa (8) and then in four different families with cerebellar atrophy, visual impairment, and psychomotor retardation, called (CAVIPMR) (9,10). Specifically, the *EMC1* gene variant c.2602G>A; p.G868R was reported only in one family (two affected Saudi siblings) with CAVIPMR (OMIM#616875). Other novel truncating and missense variants were recently reported in patients with autism spectrum disorder, severe global development delay, and visual impairment, thereby extending the spectrum of *EMC1*-related phenotypes (11).

In the current study, we report of eight pediatric patients presenting with neurological and extra-neurological manifestations carrying heterozygous and homozygous variants of the *EMC1* gene (c.364G>A; p.A122T), including two previously reported siblings with a homozygous *EMC1* gene variant (c.2602G>A; p.G868R). This is only one of the few studies with *EMC1*-related neurological and extra-neurological phenotypes reported thus far and, therefore, is a valuable addition to the *EMC1*-related clinical phenotypes.

Results

Clinical report

EMC1 variant in association with neurological phenotypes

Case#1: 14-year-old girl, known case of global developmental delay (GDD), microcephaly, failure to thrive (FTT), spastic diplegia, cognitive impairment, and attention deficit hyperactivity disorder (ADHD). The patient had no clinical seizures; however, her electroencephalography revealed focal onset epilepsy. Hearing was normal but she had vision impairment (bilateral cataract). Parents are consanguineous with no positive family history (Figure 1A). Magnetic resonance imaging (MRI) of the brain and spine was unremarkable (Figure 2A and B). Whole Exome Sequencing (WES) revealed a missense homozygous variant within the *EMC1* gene (c.364G>A; p.A122T). A summary of the clinical features in our patients and the published results are shown for comparison in Table 1.

Case#3: 4-year-old boy with GDD, normocephalic, axial, and appendicular hypotonia, subtle dysmorphic

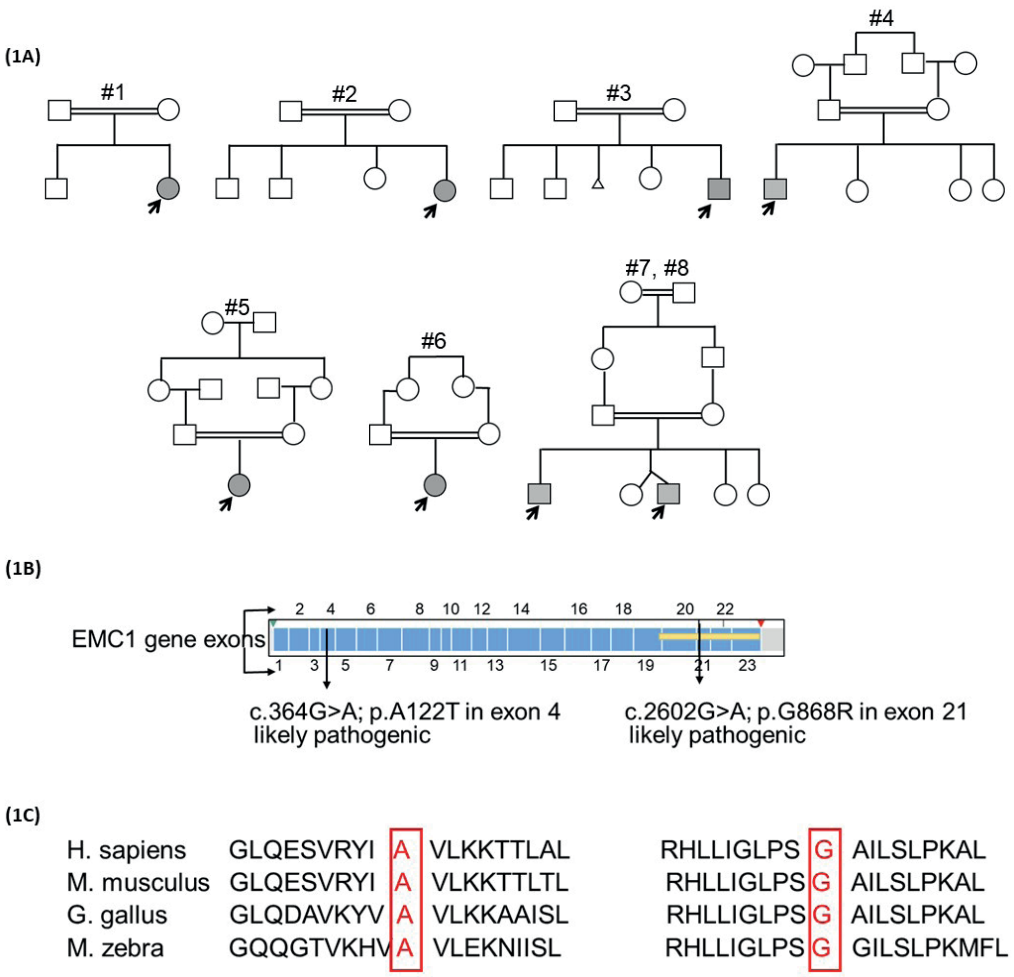

Figure 1. *Family pedigree, exon map of EMC1 gene variants, and conservation of mutated nucleotides across species. (A) Extended pedigree showing consanguineous marriage with the arrows pointing to the affected cases (solid box) including the twin gestation and other healthy siblings (open box). (B) Detailed exon map of the EMC1 gene showing all the 23 exons (numbered 1-23) including the variant 364G>A; p.A122T in exon4 and c.2602G>A; p.G868R in exon21. (C) Part of the EMC1 gene nucleotide sequence obtained from NCBI showing the conservation of the mutated nucleotide (T122 and G868) across species.*

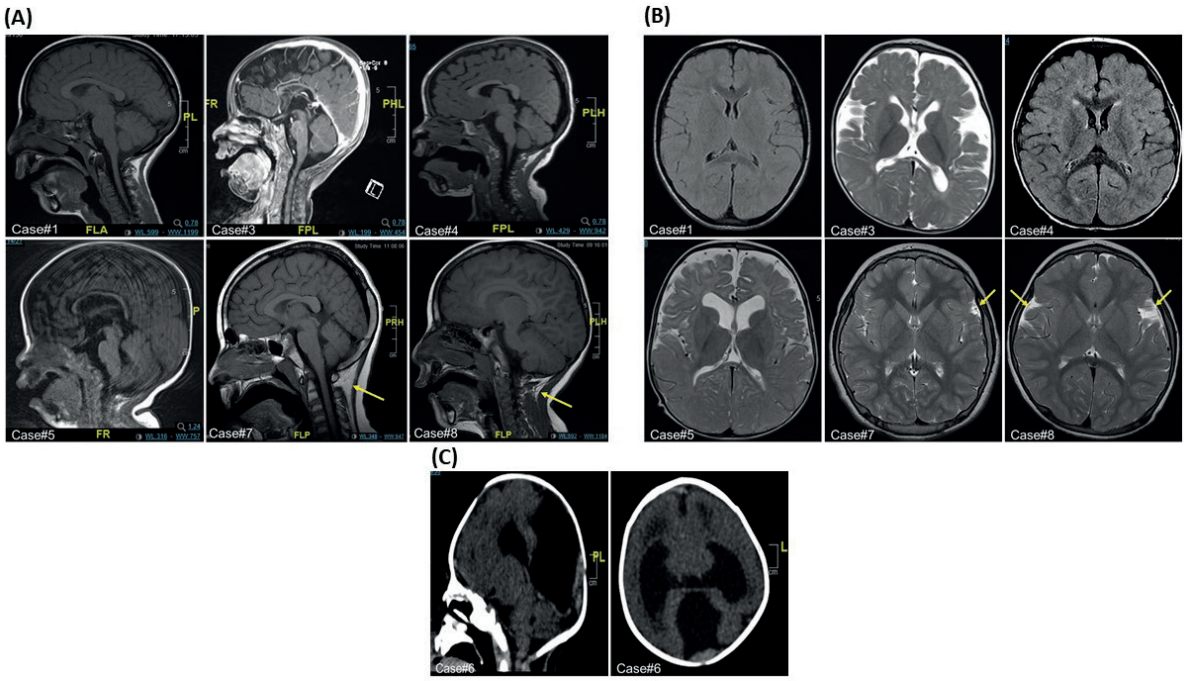

Figure 2. *Radiological images from MRI (A and B) and CT (C) showing brain abnormalities in patients with EMC1 gene variants. (A) Sagittal T1-weighted brain MRI images and (B) fast recovery fast spin-echo (FRFSE) T1/T2-weighted images of affected cases showing various brain abnormalities (see description with individual cases in the text) characteristic of the reported EMC1 gene variants. For comparison, arrows in case#7 and #8 (reported cases previously) show normal cerebellar volume and structure and hyperintense signal abnormality and white matter changes in frontal lobe, respectively. (C) CT brain showing semi-lobar/alobar holoprosencephaly for case#6 (no MRI available).*

feature, joint hyper-flexibility, and normal vision. He is a product of a full-term uncomplicated pregnancy from consanguineous parents with no previous medical history or hospital admission and has other healthy siblings (Figure 1A). MRI of the brain exhibited generalized atrophy with thinning of the corpus callosum (Figure 2A and B). WES revealed a missense heterozygous variant within the *EMC1* gene (c.364G>A; p.A122T).

Case#7 and #8: These are two siblings (15 and 17-year-old boys) from first cousin consanguineous parents (Figure 1A) who were previously reported in 2016 with a missense homozygous variant within the *EMC1* gene (c.2602G>A, p.G868R) by a group in Baylor College of Medicine (9). Using our in-house bioinformatics pipeline at King Fahad Medical City (KFMC), we also detected the same *EMC1* gene variant (c.2602G>A, p.G868R) in the two siblings. The two siblings had GDD, intellectual disability, and hypotonia. Brain MRI, carried out at 6-years in one (Case#7) and at 3 years (Case#8) in the other, showed very mild abnormality and normal cerebellar volume and structure (Figure 2A and B, Case#7, see arrows), and mild brain abnormalities right temporal arachnoid cyst and some diffuse paucity of myelin within brain parenchyma and reduction of white matter arborization in the temporal lobe (Figure 2A and B, Case#8, see arrows), respectively.

EMC1 variant in association with extra-neurological phenotypes

Case#2: 6-year-old girl, known case of GDD, and congenital muscular dystrophy, Ullrich type. She had difficulty in motor skills. She is a product of full-term pregnancy from consanguineous parents with three healthy siblings (Figure 1A). MRI of the brain was not available. WES revealed a missense heterozygous variant within the *EMC1* gene (c.364G>A; p.A122T).

Case#4: 6-year-old boy, known case of GDD, doparesponsive dystonia, alternative leg dystonia with flexion at knee with multiple falls, congenital jaundice, motor repression, hyperactive, dysarthric speech, and no dysmorphism. He is a product of a full-term pregnancy from consanguineous parents with no family history of neurological diseases or similar condition (Figure 1A). MRI of the brain suggested mild volume loss and focal hypoplasia of the corpus callosum in the posterior aspect of the body (Figure 2A and B). There was also a mild fluid-attenuated inversion recovery hyperintense signal abnormality around the frontal horns and the occipital lobe white matter. WES revealed a missense heterozygous variant within the *EMC1* gene (c.364G>A; p.A122T).

Case#5: 3-year-old girl, known case of primordial dwarfism and hypotonia. She has GDD, FTT, asthma, bilateral club feet, exaggerated thoracic kyphotic posture, apparent leg length discrepancy, hearing loss, and normal vision. She is a product of a full-term normal spontaneous vaginal delivery (NSVD) pregnancy from consanguineous parents with no family history (Figure 1A). She was admitted to the Neonatal Intensive Care Unit due to feeding difficulty and oxygenation but no intubation. MRI of the brain showed thin corpus callosum, mild dilatation and wavy in contour ventricles, and no evidence of cortical malformation (Figure 2A

Table 1. *Summary of EMC1 pathogenic variant characteristics and clinical and imaging observations in current patients and patients reported in the literature.*

Case	Age (years) sex (M/F)	Clinical feature(s)	Brain MRI/CT	EMC1 variant
EMC1 variants in association with neurologic phenotype				
Case#1	14, F	GDD, dysmorphism, FTT, microcephaly, cerebral palsy, spastic diplegia, vision impairment (bilateral cataract)	unremarkable	c.364G>A; p.A122T (homozygous)
Case#3	4, M	GDD, subtle dysmorphic feature, axial and appendicular hypotonia, joint hyper-flexibility, normal vision	Cerebral atrophy, thinning of corpus callosum	c.364G>A; p.A122T (heterozygous)
Case#7 and #8, Harel et al. [9]	3–13, M/F	GDD and mild and severe ID, dysmorphism, P/M retardation, Truncal/axial hypotonia, scoliosis, dystonic posturing, speech delay, vision impairment	Cerebral/cerebellar atrophy/ atrophic corpus callosum	c.2602G>A; p.G868R (homozygous), c.2619_2622delTCCT; p.P874Rfs*21 (homozygous), c.245C > T; p.T82M (homozygous), c.1411G > C; p.G471R (heterozygous, de novo)
EMC1 variants in association with extra-neurologic phenotype				
Case#2	6, F	GDD, no dysmorphism, Diagnosed to have Aicardi goutieres syndrome in association with homozygous pathogenic variant in RNASEH2B gene	NR	c.364G>A; p.A122T (heterozygous)
Case#4	6, M	GDD, no dysmorphism, dopa-responsive dystonia, hyperactive, dysarthric speech He has homozygous VUS in SPR gene	Mild volume loss, corpus callosum hypoplasia, mild white matter abnormality	
Case#5	3, F	GDD, dysmorphism, FTT, primordial dwarfism, hypotonia, asthma, hearing loss, normal vision	Thin corpus callosum, brain atrophy	
Case#6	6, F	GDD with ID, dysmorphism, primary microcephaly, hypertelorism, cortical malformation, holoprosencephaly	Semi-lobar/alobar holoprosencephaly	
Geetha et al. [10]	5, M/F	GDD/ID severe, central hypotonia scoliosis, seizures, scaphocephaly, poor swallowing, speech delay, dystonia, muscle wasting, disuse atrophy, visual impairment	Cerebral atrophy, brainstem atrophy	c.1212+1G > A (splicing)
Cabet et al [11]	10, M/F	GDD/severe ID, global hypotonia, P/M retardation, ASD, bifid uvula, strabismus	No cerebral atrophy	c.2858T > C; p.F953S and c.1134C > A; p.Y378* (compound heterozygous)
Abu-Safieh et al. [8]		Retinitis pigmentosa	No cerebral atrophy	c.430G>A; p.A144T (homozygous)

This table summarizes the clinical signs for our patients and previously reported cases, and a comparison is made thereby to show the expanded *EMC1*-related phenotype.

The following abbreviations are used: M/F = male /female; P/M = psychomotor; GDD = global developmental delay; ID = intellectual disability; FTT = failure to thrive; ASD = autism spectrum disorder; NR = not reported; ADHD = attention deficit hyperactivity.

and B). WES revealed a missense heterozygous variant within the *EMC1* gene (c.364G>A; p.A122T).

Case#6: 6-year-old girl with primary microcephaly, dysmorphism, GDD with intellectual disability, hypertelorism, cortical malformation, and holoprosencephaly. She is a product of a full-term NSVD pregnancy from consanguineous parents with no family history (Figure 1A). No MRI was carried out but computed tomography (CT) of the brain showed semilobar/alobar holoprosencephaly (Figure 2A and B). WES revealed a missense heterozygous variant within the *EMC1* gene (c.364G>A; p.A122T).

Discussion

Through the use of WES, the *EMC1* gene mutations in the form of homozygous and *de novo* heterozygous variants, truncating mutations, and deletions have been reported in the literature (8,11), albeit with only a few publications. We report here a series of six new patients with variable clinical presentation in which WES revealed a homozygous *EMC1* gene variant (c.364G>A; p.A122T) in one patient, and a heterozygous variant (c.364G>A; p.A122T) in five patients. A homozygous *EMC1* gene variant (c.2602G>A, p.G868R) reported previously in two Saudi siblings (9) was confirmed by our laboratory using the in-house pipeline.

The patients presented with similar clinical features such as GDD, hypotonia, and psychomotor retardation, but inter-patient variability such as vision impairment in some and epilepsy in others were also evident. Four of the six patients showed some form of brain abnormality in MRI and/or CT and other similar features and characteristics of CAVIPMR (9), while two patients did not show any remarkable brain MRI. All the six patients were from consanguineous marriages without any significant family history. In the absence of any family history, it is conceivable that heterozygous the *EMC1* gene variant (c.364G>A; p.A122T) was detected in five of the six patients. One patient with a homozygous *EMC1* gene variant (c.364G>A; p.A122T) presented with a relatively severe disease including epilepsy. The other two siblings confirmed by our laboratory to have a homozygous *EMC1* gene variant (c.2602G>A, p.G868R) came with a strong family history and disease. Thus, it is apparent that different *EMC1* gene variants cause similar clinical phenotypes (8,11) and the zygosity of these *EMC1* gene variants is linked to the severity of the disease. The dichotomy of heterozygous and homozygous *EMC1* gene variants in this expanded clinical phenotype also raises a possibility of other contributing genetic variations implicated in disease pathogenesis. In fact, two of our patients were also reported with mutations in *RNASEH2B* and *SPR* genes. However, this is an open question that warrants investigation.

EMC1 gene variants (Figure 1B, CHR1 exon4; 19568918, NM_001271429.2, c.364G>A; p.A122T and CHR1 exon21; 19547328, NM_015047.3, c.2602G>A; p.G868R) had a Combined annotation-dependent depletion score of 25/29.4 and deleterious predictions in PolyPhen, Mutation Taster, and SIF prediction algorithms, including BLOSUM predicting a biochemical impact. Additionally, the *EMC1* gene variant (g.19568918/g. 19547328) occurs at an allele frequency of 0.5/0.08 in our in-house database (2564 cases) and both are conserved across species (Figure 1C) suggesting its deleterious nature and possible contribution to the phenotype in our patients. Furthermore, the American College of Medical Genetics and Genomics; Tandem-based scoring system for variant classification (specific criteria g.19568918/g. 19547328; PS4, PM2, PP3/ PM2, PP3, PP5) of the *EMC1* gene variants and the presence of the variants in HGMD as disease mutation and in ClinVar revealed similar pathogenic classifications of the *EMC1* gene variants (364G>A; p.A122T and c.2602G>A; p.G868R) (Figure 1B). It must be kept in mind that no functional studies have been carried out for any of the reported *EMC1* variants so far. However, with the increase in the number of reported cases (including ours in this study) having *EMC1*-related clinical features, it is evident that *EMC1* plays an important role in disease pathogenesis. A putative association between an *EMC1* variant and human disease was first reported by Abu-Safieh et al. (8) who identified a homozygous missense *EMC1* variant (c.430G>A; p.A144T) in a family with non-syndromic retinitis pigmentosa. Owing to the lack of functional data, the variant was classified as a variant of uncertain clinical significance. It is interesting to note that some of the common features of all the *EMC1* variants reported till date including ours in terms of clinical phenotype are GDD, hypotonia, and motor retardation (Table 1). Harel et al. (9) reported multiple monoallelic and biallelic *EMC1* variants in the form truncating, missense mutations, and deletion in patients with CAVIPMR, an autosomal recessive neurodegenerative disorder characterized by developmental delay, intellectual disability, hypotonia, scoliosis, cerebellar atrophy, and variable dysmorphic features. The *EMC1* gene variant (364G>A; p.A122T) identified in our six patients with variable features was reported in a family (c.430G>A; p.A144T) with retinitis pigmentosa (8). The *EMC1* gene variant (c.2602G>A; p.G868R) confirmed by our laboratory and other heterozygous and homozygous *EMC1* gene variants have been reported previously in patients with CAVIPMR (9). This suggests on one hand, the contribution of *EMC1* in multiple clinical phenotypes and on the other hand, multiple disease loci for *EMC1*-related phenotypes as suggested for recessive disorders previously (15). In a consanguineous Turkish family, the authors have reported a homozygous *EMC1* variant (c.245C>T: p.T82M) in patients with CAVIPMR that segregated with the disorder in the family (9). In a 4-year-old boy with CAVIPMR, born of unrelated parents of European descent, a homozygous *EMC1* gene 4-bp deletion c.2619_2622delTCCT; p.P874Rfs*21 was reported in the same study (9). In a 12-year-old boy with CAVIPMR, a *de novo* heterozygous missense *EMC1* variant (G471R) was reported (9). A novel homozygous intronic splice variant in the *EMC1* gene (c.1212 + 1G>A) was reported in a patient with cerebellar atrophy, visual impairment, psychomotor retardation, and epilepsy, suggesting aberrant *EMC1* Ribonucleic acid splicing as a potential cause of disease pathogenesis (10). Some of our patients also presented with brain abnormalities including cerebral atrophy and also with epilepsy and abnormal

movements indicating distinctive clinical features with the *EMC1* gene variants. Recently, novel truncating and missense *EMC1* variants were reported in patients with autism spectrum disorder, severe global development delay, visual impairment, absence of seizures, scoliosis, or facial dysmorphic features (11), suggesting yet unrecognized clinical phenotypes associated with *EMC1* gene variants. WES is currently an important diagnostic tool with a higher yield in patients with strong genetic etiology as reported previously by us and others (12,14).

Conclusion

In conclusion, this study highlights three important points: first, the importance of WES in revealing genetic and clinical heterogeneity, thereby helps contributing to the expanded *EMC1*-related clinical phenotypes; second, with the increase in the number of cases carrying the same *EMC1* gene variant, the role of *EMC1* in disease pathogenesis will become evident; and third, there is a need for addressing in-depth functional studies of *EMC1* gene variants and the importance of investigating the contribution of other gene variants in *EMC1* gene-related clinical phenotype.

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List of Abbreviations

CAVIPMR	Cerebellar atrophy, visual impairment, and psychomotor retardation
MRI	Magnetic resonance imaging
WES	Whole exome sequencing

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Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval
Considering the study design, formal ethical approval was declared as "exempt" by Institutional Review Board, King Fahad Medical City, via Ref # 15-204.

Consent to participate
Written informed consent was obtained from the parents/ guardians of the subjects.

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