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Novel truncating and missense variants extending the spectrum of *EMC1*-related phenotypes, causing autism spectrum disorder, severe global development delay and visual impairment

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Abstract

The *EMC1* gene, located on 1p36.13, encodes the subunit 1 of the endoplasmic reticulum-membrane protein complex, a highly conserved and ubiquitous multiprotein transmembrane complex. Pathogenic monoallelic and biallelic variants in *EMC1* in humans have been reported only in six families, causing isolated visual impairment or in association with psychomotor retardation and cerebellar atrophy. We report a ten-year-old boy, born to unrelated parents, with early-onset severe global development delay due to novel *EMC1* biallelic pathogenic variants. A truncating variant, p.(Tyr378*) and a missense variant, p.(Phe953Ser), located in exon 11 and 23 of *EMC1* gene respectively, have been found by reanalysis of exome sequencing data. The proband's phenotype included several signs that overlap with the phenotype of previously reported patients, associating severe global developmental delay, abnormal ophthalmological examination, and postnatal slow-down of the head circumference growth. Some distinguishing clinical signs were observed in comparison to patients from literature, such as autism spectrum disorder, absence of seizures, scoliosis or facial dysmorphic features, thus extending the spectrum of *EMC1*-related phenotypes. Similarly, brain MRI, performed at 2 years, showed normal cerebellar volume and structure, whereas cerebellar atrophy was described in literature. Moreover, difficulties of clinical differential diagnosis between *EMC1*-associated disease and other etiologies of global development delay support the importance of large-scale genetic investigations. Our diagnostic approach, through reanalysis of exome sequencing data, highlights the importance of reconsidering initial negative results for patients with a strong suspicion of genetic disease, and to update analytic pipelines in order to improve the diagnostic yield of exome sequencing.

Keywords: global development delay; cerebellar atrophy; visual impairment; autism spectrum disorder; endoplasmic reticulum membrane protein complex, subunit 1; *EMC1*

Introduction

Global development delay is a clinically and etiologically heterogeneous group of disorders that affects up to 3% of children (Shevell et al., 2003). It can be related to exogenous, metabolic or non-metabolic genetic diseases with inconstant structural brain abnormalities (Majnemer and Shevell, 1995). Making the precise diagnosis is all the more important as the prognosis and management differ between the different etiologies. Clinical history, detailed examination, and imaging are crucial to define the phenotype. During the last decade, large-scale genetic investigations, especially exome sequencing, have significantly increased the number of Mendelian causes of global development delay providing a growing list of involved genes (Hunt et al., 2014; Millan et al., 2016; Zhao et al., 2018). Pathogenic monoallelic and biallelic variants in *EMC1* gene (OMIM #616846) have only been reported in three publications since 2013, in eleven individuals with isolated ophthalmological signs, or in association with psychomotor retardation and cerebellar atrophy (OMIM #616875) (Abu-Safieh et al., 2013; Harel et al., 2016; Geetha et al., 2018). The *EMC1* gene, located on 1p36.13, contains 23 exons and encodes the subunit 1 of the endoplasmic reticulum-membrane protein complex (EMC1, GenBank #AAH34589.1), a highly conserved multiprotein transmembrane complex (Nagase et al., 1995; Satoh et al., 2015). The endoplasmic reticulum-membrane protein complex (EMC) consists of chaperone proteins located in the endoplasmic reticulum (ER) where transmembrane proteins mature and acquire their correct three-dimensional shape (Jonikas et al., 2009; Satoh et al., 2015). The EMC is crucial for proper folding of multi-pass transmembrane proteins by modulating ER-associated degradation of specific proteins, thus avoiding the accumulation of misfolded proteins (Jonikas et al., 2009; Xie and Ng, 2010; Christianson et al., 2011; Smith et al., 2011; Brodsky, 2012; Satoh et al., 2015; Ninagawa et al., 2015). The EMC could also facilitate phospholipid transfer from ER to mitochondria and contacts between these organelles, perhaps by modulating the folding of proteins involved in such

tethering (Lahiri et al., 2014). EMC1 is a ubiquitous protein of 993 aminoacids that contains two conserved domains: a N-terminal quinoprotein alcohol dehydrogenase-like domain (PQQ_2) ranging from residue 28 to 242, and an uncharacterized domain of unknown function 1620 (DUF1620) ranging from residue 786 to 992 (Harel et al., 2016). Here we report a ten-year-old boy, born to unrelated parents, with early-onset severe global development delay with absent speech, poor comprehension and non-verbal communication, autism spectrum disorder and strabismus due to novel *EMC1* biallelic pathogenic variants.

Clinical report

Clinical data

A ten-year-old boy, born to unrelated healthy parents, was first referred to genetics department at the age of 2 for severe global developmental delay. He was the second child of the couple and his thirteen-year-old sister was healthy (Fig. 1A). The parents were 27 and 33 years old (mother and father respectively) at time of birth.

The proband was born at full term after an uneventful pregnancy and the Apgar score was 10-10. At birth, head circumference (36.5 cm, +1 standard deviation), height (52 cm, +0.5 standard deviation) and weight (3750 g, +0.5 standard deviation) were in the normal range.

Psychomotor delay was noticed during the first months of life with severe hypotonia and lack of ocular fixation. He started to sit at 21 months of age, and to walk at 4 years of age. Speech was absent with severe intellectual disability, poor comprehension and non-verbal communication. Toilet training was not acquired at 10 years of age. He never had cerebellar syndrome, pyramidal syndrome, or seizure.

Autism spectrum disorder had been diagnosed in early childhood according to clinical

diagnostic criteria of DSM-5 (the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders) with avoidance of eye contact and manual stereotypies, supplemented by intolerance to frustration, and self- and hetero-aggressive behavior at 3 years of age. His behavioral disturbances were partially appeased by long-term medication using risperidone and cyamemazine.

Ophthalmological examination revealed an intermittent unilateral divergent strabismus and a possible amblyopia. Audiometry was normal.

Body height-weight growth was normal but a slow-down of the head circumference growth was noticed at 6 months of age. Despite the loss of 2 standard deviations, the head circumference remained within normal ranges at 10 years of age. Upon recent examination, height, weight and head circumference were proportionate, respectively 133 cm (-0.5 standard deviation), 29 kg (-0.5 standard deviation) and 51.5 cm (-1 standard deviation). No vertebral static disorder was noted. Except plagiocephaly and bifid uvula, no dysmorphic features were noted.

Routine blood counts and biochemistry (blood electrolytes, creatine, ferritin, creatine phosphokinase) were within reference limits. Blood metabolites (thyroxine, thyroid stimulating hormone, liver enzymes, chromatography of plasma amino acids, very long chain fatty acids) and urine metabolites (chromatography of urinary organic acids, creatine, urinary succinylaminoimidazole-carboxamide riboside, urinary glycosaminoglycans) were normal. Congenital disorders of glycosylation and glycogen storage disease were ruled out by targeted analysis. Cerebrospinal fluid analysis, including neurotransmitters testing, showed no significant abnormality.

Appropriate parental consent for this report has been obtained.

Imaging data

Brain MRI (1.5 tesla, 5 mm slices) with spectroscopy, performed at 2 years of age, was normal (Fig. 1B).

Genetic investigations

Karyotype analysis by G and R-banding and array-CGH were normal. Targeted analyses for Angelman, Prader-Willi, and fragile-X syndromes were negative.

A *solo* exome sequencing was performed when he was 7 years old. Covaris shearing of DNA was used for library preparation (End Repair, A-Tailing, Adaptors ligation, PCR amplification) and captured using the SeqCap EZ MedExome kit (Roche, Madison, WI, USA). The captured library was sequenced by 2X150-bp paired-end reads protocol using the NextSeq 500 (Illumina, San Diego, CA, USA). Ninety seven percent exons were covered by at least 10X. Initially negative (no pathogenic variant was found in 2016), exome analysis was recently reconsidered with the latest data from literature. Sequence alignment to the human reference genome (hg19) and variants call and annotation were performed using an updated in-house bioinformatic pipeline. Synonymous variants, strand bias, mosaic <10%, depth <10 reads, or variants with frequency >1% in public database including the 1000 Genome Project and the 6500 Exome Sequencing Project were filtered out. Only exonic and splicing variants were considered. Out of a total of 111,304 detected variants, 626 passed the first filtering. No relevant result was found for a monoallelic or X-linked hypothesis considering all variants in genes that are expressed in brain tissues, involved in neurodevelopment and OMIM-reported genes. Analysis was then widened by considering the biallelic variants (details available in Supplementary data). Only two variants were retained after interpretation of each variant according to the corresponding *in silico* predictions if missense, gnomAD data, and available data from the literature (about

the involved gene, the function of the encoded protein, and its involvement in human pathology). Both variants were located in the *EMC1* gene, respectively in exons 11 and 23, and consisted of a nonsense variant chr1(hg19):g.19,564,589G>T (RefSeq accession number NM_015047.2), c.1134C>A, p.(Tyr378*), and a missense variant chr1(hg19):g.19,545,921A>G, c.2858T>C, p.(Phe953Ser). Each parent was heterozygous for one of these variants. Variants were verified by Sanger sequencing (Fig. 2A, 2B). The combination of these two variants had never been reported in control individuals: each variant was present only once in the gnomAD database, separately in two heterozygous control individuals (allele frequency: $4 \cdot 10^{-6}$) and absent from other control databases. The nonsense variant p.(Tyr378*) leads to an early truncated protein and the missense variant p.(Phe953Ser) is located in the functional domain DUF1620 (Fig. 2C). Both are expected to cause disease with the highest probability of 1 according to MutationTaster. The missense variant was also predicted as deleterious by SIFT and PROVEAN and as probably damaging by PolyPhen. Therefore, these two variants in *EMC1* were considered likely pathogenic with an autosomal-recessive mode of inheritance. These data were submitted to the ClinVar public database (SCV63969822 and SCV6396845).

Discussion

We report a ten-year-old boy, born to unrelated parents, with early-onset severe global development delay with autism spectrum disorder, absent speech, and strabismus, without cerebellar atrophy, due to novel *EMC1* biallelic variants found by exome sequencing: a truncating variant, p.(Tyr378*), and a missense variant, p.(Phe953Ser). These two variants were considered as likely pathogenic based on their very low frequency in control databases, the concordant familial segregation for autosomal recessive hypothesis, the *in silico* predictions from MutationTaster, SIFT, PROVEAN, and PolyPhen (details available

in Supplementary data), and the literature data. Initially negative, the proband's exome analysis was reconsidered three years later allowing genetic diagnosis. Initial report, carried out in 2016, did not retain any pathogenic variant because of poor literature on the involvement of *EMC1* in human pathology at that time and because of the low specificity of the proband's phenotype. Indeed, the initial report was done only few weeks before the first publication describing *EMC1* as a causative gene of global development delay (Harel et al., 2016). Reanalysis was based on updated annotation and filtering bioinformatics tools and on the last data from literature about novel genes involved in human diseases. This case highlights the importance of re-analyzing exome data to include recent findings especially for patients with a strong suspicion of genetic disease, and to update analytic pipelines. According to Li et al., the suitable time points for reanalysis might be 6-12 months after initial report (Li et al., 2019).

Pathogenic nucleotide variants in *EMC1* have been reported in human pathology only in three publications, first in 2013, as a candidate gene in retinal dystrophy (Abu-Safieh et al., 2013). By exome sequencing of individuals from 149 families with retinal dystrophy, Abu-Safieh et al. found the missense variant c.430G>A, p.(Ala144Thr) in *EMC1* in two homozygous individuals. More recently in two publications, *EMC1* have been involved in nine children from five unrelated families with a broader phenotype associating global development delay with visual impairment and cerebellar atrophy (OMIM#616875) (Harel et al., 2016; Geetha et al., 2018). They were all found to have biallelic pathogenic variants (truncating, missense and intronic splice variants) except one 12-year-old child, with a *de novo* monoallelic missense variant and a similar phenotype, suggesting either an additional unidentified deep intronic variant on the second allele (copy number variations and variants in the putative promoter region of *EMC1* having been ruled out) or that *EMC1* belongs to the genes for which both autosomal-dominant and autosomal-recessive inheritance exist (Harel et al., 2016; Eldomery et al., 2017).

Several clinical signs observed in the proband reported here overlap with the phenotype of the recent cases from literature, associating both severe global developmental delay with absent speech, and abnormal ophthalmological examination limited to strabismus. In the literature, ophthalmological disorders appear heterogeneous, including cortical visual impairment, retinopathy, optic neuropathy, refractive disorders and strabismus. The postnatal slow-down of the head circumference growth reinforces a unifying molecular pathogenesis between the proband and the cases reported by Harel et al. and Geetha et al. among which four presented a progressive microcephaly (Family 2 from Harel et al., individual IV.1 from Geetha et al.).

However, some distinguishing clinical signs in comparison to previously reported patients were observed in our proband, thus extending the spectrum of *EMC1*-related phenotypes. Notably, he presented with severe autism spectrum disorder that had never been reported. On the contrary, several additional clinical features reported in literature cases were not observed in our proband such as scoliosis, diminished deep tendon reflexes, limb dystonia, or early infantile onset seizures (Harel et al., 2016; Geetha et al., 2018). In the same way, no facial dysmorphic features were retained in our proband, unlike in the individuals from the literature who all displayed mild dysmorphic features such as deep-set eyes, short philtrum, retromicrognathia, or scaphocephaly.

Varying degrees of parenchymal cerebral atrophy were described in literature, primarily affecting the cerebellum (before the age of 2 in three children). Our proband benefited from a brain MRI at 2 years of age which showed normal cerebellar, brainstem and supratentorial trophicity. We considered the more pronounced foliation of the culmen as a variant within the normal range (Fig. 1B). Reviewing individual III-2 of family 3 in Harel et al., the cerebellar atrophy predominating on the upper part of the vermis they retained at 3 years old, seems debatable according to our opinion and the available images. A neurodegenerative process was suggested by Harel et al. face to a progressive cerebellar

atrophy in two children but, unfortunately, no subsequent MRI control was available to judge the evolution in the proband. Notably, regular neurological follow-up of the proband showed no evidence of cerebellar syndrome, which was also not preponderant in literature. This supports the fact that radiologists should attentively analyze cerebellum trophicity in case of global development delay, even in the absence of clinical cerebellar syndrome; especially since brain MRI showed a higher abnormality rate in global development delay with additional clinical signs, as in *EMC1*-associated disease, than in isolated development delay (Griffiths et al., 2011).

The clinical findings, *EMC1* pathogenic variant characteristics and imaging features of the present patient and patients reported in literature are summarized in Table 1.

Our case confirms the variability in phenotypic features that seems independent of the variant type or its location on the protein (Fig. 2C), based on, however, few reported cases. Given this variability and the role of the EMC in the stable expression of multi-pass transmembrane proteins such as rhodopsin (Sato et al., 2015), it seems plausible to hypothesize that the missense variant identified by Abu-Safieh et al. might represent a mild allele with an isolated ophthalmological phenotype (Abu-Safieh et al., 2013). This variability may be due to the effect of the pathogenic variant on the multiple functions of the EMC including protein folding and organelle crosstalk that have already been implicated in neurodegenerative processes such as Alzheimer disease, amyotrophic lateral sclerosis, Charcot-Marie-Tooth disease, or Parkinson's disease (Schon and Area-Gomez, 2013; Xia et al., 2014; Credle et al., 2015; Guardia-Laguarta et al., 2015; Bernard-Marissal et al., 2019). Additional functional studies are required to delineate the pathogenic mechanism and to precise the genotype-phenotype correlations.

In summary, our case, with novel pathogenic variants of *EMC1* expands the spectrum of *EMC1*-associated phenotypes. Our diagnostic approach, through reanalysis of exome

sequencing data, highlights the importance of reconsidering initially negative results for patients with a strong suspicion of genetic disease, and to update analytic pipelines in order to improve the diagnostic yield of exome sequencing.

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Figures

-Figure 1:

(A) Genealogy tree of the proband.

(B) Brain MRI (1.5 tesla, 5 mm slices).

Mid sagittal T2-weighted image performed at 2 years of age showed normal trophicity of the vermis.

-Figure 2:

(A, B) Sanger sequencing of the variants c.1134C>A and c.2858T>C of *EMC1* gene

(NM_015047.2) in the proband and his parents.

The variants were confirmed in the compound heterozygous proband (individual II.2), each inherited from one of the heterozygous parents (individuals I.1 and I.2 are the father and the mother respectively).

(C) Structure of *EMC1* gene.

Scheme of *EMC1* gene (NM_015047.2), with location of pathogenic variants identified in the proband, p.(Tyr378*) in exon 11 and p.(Phe953Ser) in exon 23 (upper part of the scheme), and six previously published variants (lower part of the scheme - Abu-Safieh et al., 2013; Harel et al., 2016; Geetha et al., 2018). Rectangles schematize the 23 exons and horizontal bars the two functional domains of EMC1.

Table

-Table 1: Summary of *EMC1* pathogenic variant characteristics, clinical and imaging observations in the present patient and patients reported in literature.

Supplementary data

-Table 1s: Interpretation of biallelic variants in the proband.

A *solo* exome sequencing was performed. Synonymous variant, strand bias, mosaic <10%, depth <10 reads, or frequent variants were filtered out. Out of a total of 111,304 detected variants, 626 passed the first filtering. No relevant result was found for a monoallelic or X-linked hypothesis considering all variants in genes that are expressed in brain tissues, involved in neurodevelopment or associated a phenotype in OMIM. Analysis was then widened by considering all the biallelic variants. According to variant frequency in several

control databases and *in silico* predictions, variants in *EMC1* gene were considered as likely pathogenic and familial segregation was verified by Sanger sequencing.

For readability, known polymorphisms and benign variants (matching all considered softwares of in silico prediction) had not been reported in the table.

Abbreviations : NA, not applicable

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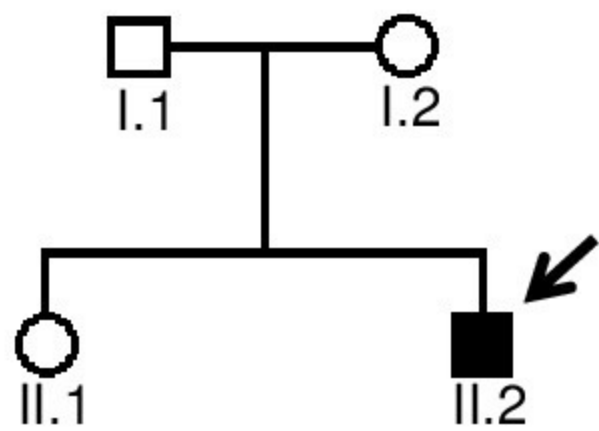
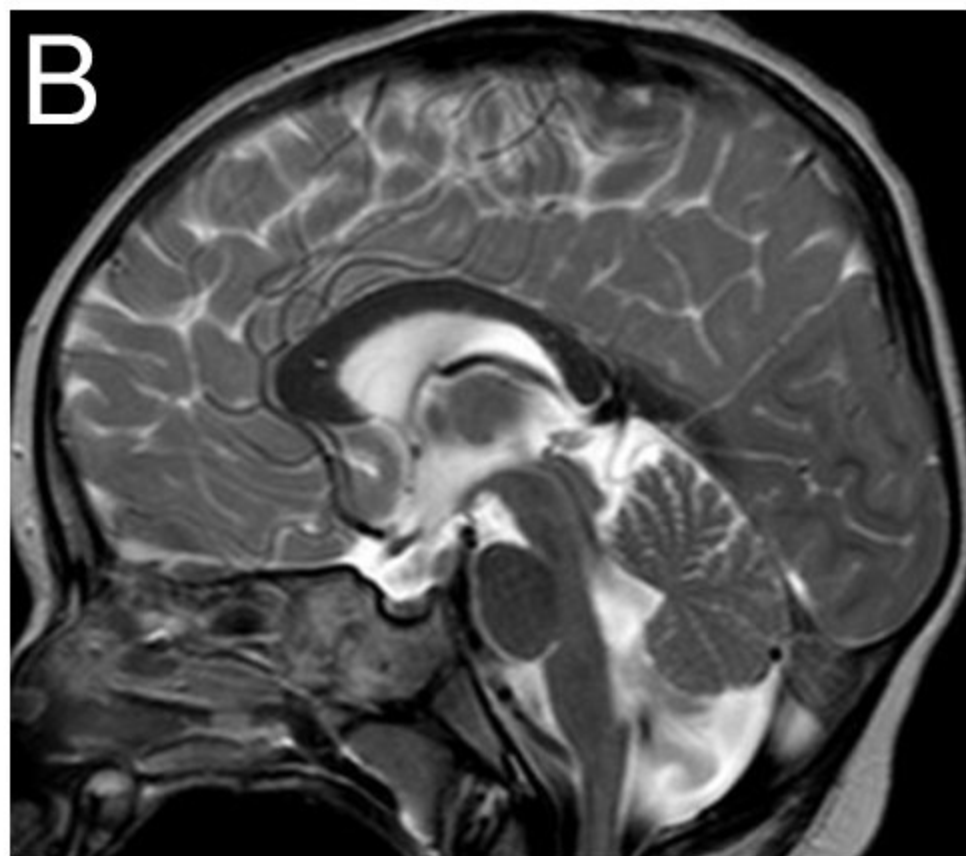
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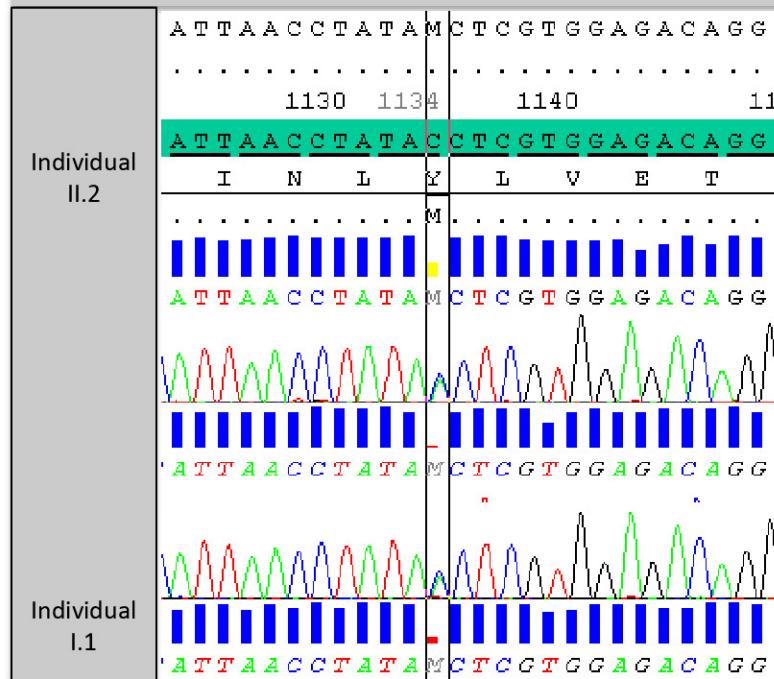
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A**B**

A

Variant c.1134C>A

**B**

Variant c.2858T>C

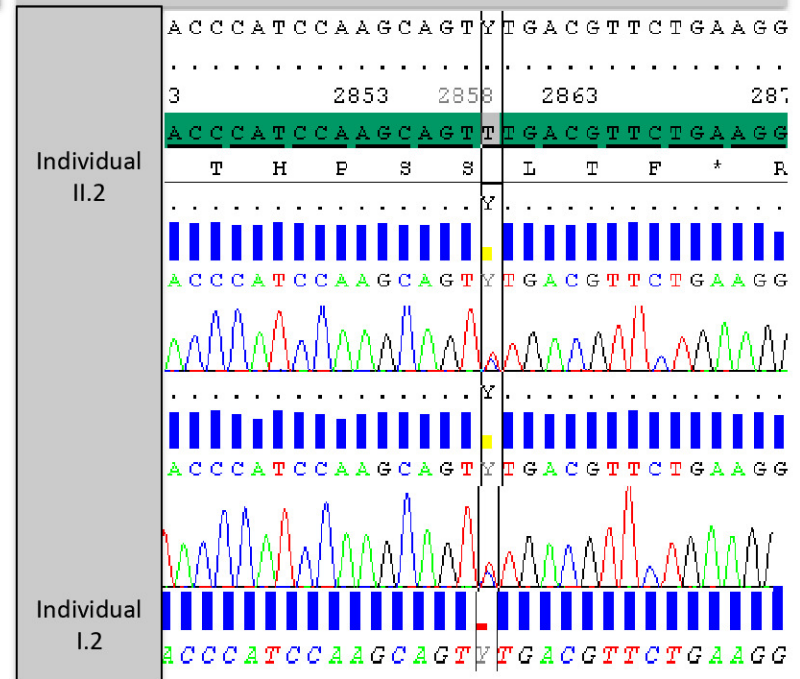
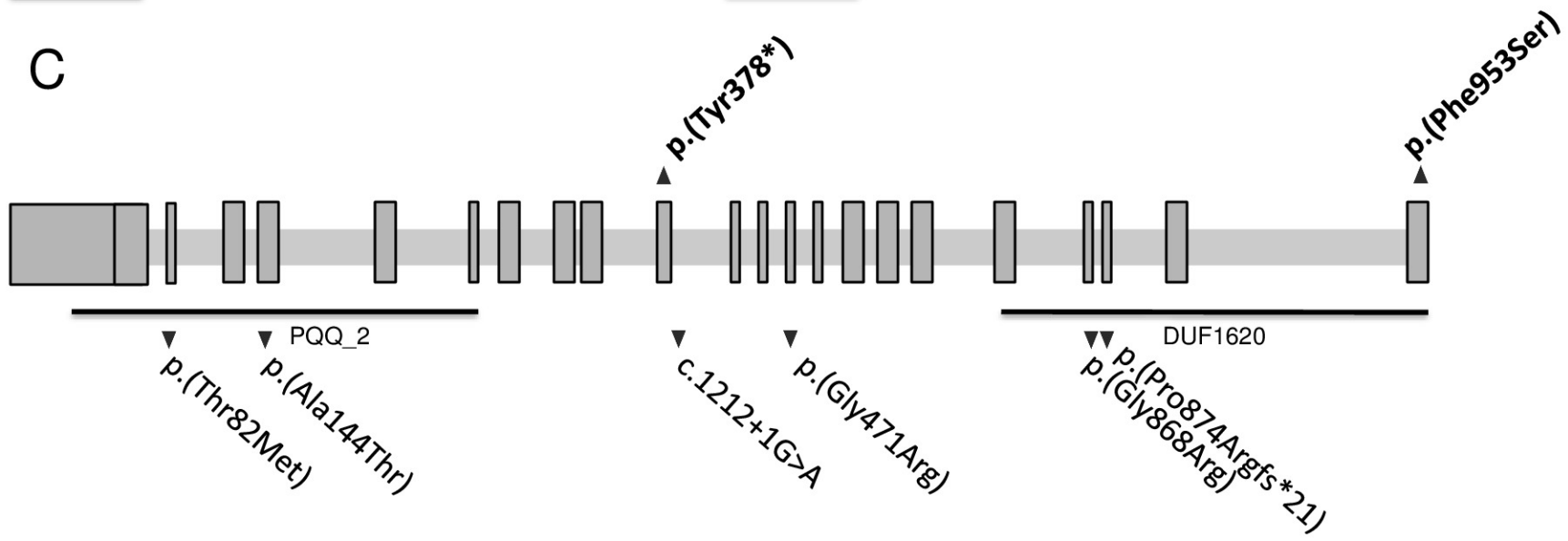
**C**

Table 1. Summary of *EMC1* pathogenic variant characteristics, clinical and imaging observations in the present study and in literature

Reference	AR forms						AD form
	Abu-Safieh et al., 2013	Harel et al., 2016			Geetha et al., 2018	Present study	Harel et al., 2016
patient	2 individuals	F1 II.1	F2 III.1, III.2, III.3, III.4	F3 III.1, III.2	IV.1	II.2	F4 II.6
age at examination	no data	4 y	3, 5 and 13 y	10 and 12 y	4.8 y	10 y	12 y
<i>EMC1</i> pathogenic variant characteristics							
nucleotide	c.430G>A	c.2619_2622delTCCT	c.245C>T	c.2602G>A	c.1212+1G>A	c.2858T>C and c.1134C>A	c.1411G>C
protein (if exon variant)	p.Ala144Thr	p.Pro874Argfs*21	p.Thr82Met	p.Gly868Arg		p.Phe953Ser and p.Tyr378*	p.Gly471Arg
allelic status	homozygous	homozygous	homozygous	homozygous	homozygous	compound heterozygous	heterozygous, <i>de novo</i>
Clinical data							
head circumference (SD) / age	no data	-1.6 SD / 2.75 y	0 SD / birth from -3.3 to -4 SD / 3, 5 and 13 y	0 SD / 10 and 12 y	-2 SD / 1.5 y	loss of 2 SD at 0.5 y -1 SD / 10 y	-0.34 SD / 12 y
facial dysmorphic features	no data	short upper lip mild hypertelorism retrognathia	deep-set eyes gingival hyperplasia shortiltrum retrognathia	deep-set eyes retrognathia	deep-set eyes scaphocephaly	low anterior hairline	low anterior hairline dysplastic ears gingival hyperplasia micrognathia
development delay	no data	yes	yes	yes	yes	yes	yes
intellectual disability	no data	severe	severe	mild	severe	severe	severe
autism spectrum disorder	no data	no	no	no	no	yes	no
ophthalmological signs	retinal dystrophy	cortical visual impairment	abnormal visual evoked potentials abnormal electroretinogram	esotropia hyperopia astigmatism	cortical visual impairment	strabismus	myopia optic atrophy
seizures	no data	yes	no	no	yes	no	no
additional features	no data	scoliosis truncal hypotonia diminished deep tendon reflexes dystonic posturing increased tone in the extremities	scoliosis truncal hypotonia diminished deep tendon reflexes dystonic posturing increased tone in the extremities	truncal hypotonia	truncal hypotonia diminished deep tendon reflexes dystonic left upper limb muscle wasting and disuse atrophy poor swallowing coordination hypoplastic nipples	global hypotonia bifid uvula	scoliosis truncal hypotonia diminished deep tendon reflexes
Brain MRI							
cerebellar atrophy / age	no data	yes / 0.5 y	yes / 1, 2.8, 3 and 5 y	yes / 3 y on the superior cerebellar vermis	yes	no / 2 y	yes / 1.3 y
additional features	no data	atrophic foreshortened corpus callosum cerebral atrophy small hippocampi	thin foreshortened corpus callosum cerebral atrophy	foreshortened corpus callosum	cerebral atrophy brainstem atrophy	no	foreshortened corpus callosum cerebral atrophy

Table 1 summarizes clinical signs for our proband and literature cases. The following abbreviations are used: AD, autosomal-recessive; AR, autosomal-recessive; y, year(s).