Official Journal of the European Paediatric Neurology Society

Original article

Novel *WWOX* deleterious variants cause early infantile epileptic encephalopathy, severe developmental delay and dysmorphism among Yemenite Jews

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https://doi.org/10.1016/j.ejpn.2019.02.003

ARTICLE INFO

Article history:
Received 26 September 2018
Received in revised form
9 February 2019
Accepted 13 February 2019

Keywords:
WW Domain Containing Oxidoreductase
Early infantile epileptic encephalopathy
Intellectual disability
3′ splice site

ABSTRACT

The human WW Domain Containing Oxidoreductase (*WWOX*) gene was originally described as a tumor suppressor gene. However, recent reports have demonstrated its cardinal role in the pathogenesis of central nervous systems disorders such as epileptic encephalopathy, intellectual disability, and spinocerebellar ataxia. We report on six patients from three unrelated families of full or partial Yemenite Jewish ancestry exhibiting early infantile epileptic encephalopathy and profound developmental delay. Importantly, four patients demonstrated facial dysmorphism. Exome sequencing revealed that four of the patients were homozygous for a novel *WWOX* c.517-2A > G splice-site variant and two were compound heterozygous for this variant and a novel c.689A > C, p.Gln230Pro missense variant. Complementary DNA sequencing demonstrated that the *WWOX* c.517-2A > G splice-site variant causes skipping of exon six. A carrier rate of 1:177 was found among Yemenite Jews. We provide the first detailed description of patients harboring a splice-site variant in the *WWOX* gene and propose that the clinical synopsis of *WWOX* related epileptic encephalopathy should be broadened to include facial dysmorphism. The increased frequency of the c.517-2A > G splice-site variant among Yemenite Jews coupled with the severity of the phenotype makes it a candidate for inclusion in expanded preconception screening programs.

1. Introduction

Compelling evidence supports the notion that WWOX (WW Domain-Containing Oxidoreductase; OMIM 605131) is a protein of multiple functions, interacting with many proteins in different pathways at different locations in the cell membrane, cytoplasm, and nucleus.[1] Initially recognized as a tumor suppressor, it is now known to play a role in tumorigeneses, neural disorders and metabolic syndrome related traits.[2] Deleterious variants in the *WWOX* gene cause a severe form of an autosomal recessive Epileptic Encephalopathy, Early Infantile, 28 (EIEE); OMIM 616211,[3,4] and Spinocerebellar ataxia, autosomal recessive 12 (SCAR12); OMIM 614322.[5,6] The salient clinical synopsis of EIEE is comprised of pharmaco-resistant epilepsy, ataxia and intellectual disability. Dysmorphism has only been reported in one patient and is not considered part of the clinical signs of WWOX encephalopathy. Interestingly, the neural disorders caused by *WWOX* pathogenic variants do not include spontaneous tumor formation among previously reported patients. *WWOX*-related EIEE cases are rare and limited to a few dozen affected families.[3–14] Although WWOX splice-site variants have been reported,[11–13] there is no clinical report describing those patients and thus far only patients with missense, nonsense, frameshift, exon deletion,[7] and gene deletion identified as part of a copy number variant[3,6–10] have been described in details. We report the genetic investigation and resolution by whole exome sequencing of six patients from three unrelated families, presenting with EIEE, profound developmental delay, peripheral spasticity, dysmorphism in four of the six patients, and infantile death in one.

2. Materials and methods

2.1. Ethical approval

DNA was available from all six patients and their family members. Genomic DNA was extracted from peripheral leukocytes following standard protocols. The molecular studies were approved by the ethical committee of Rabin Medical Center, Sheba Medical Center and the Israeli Ministry of Health. Written informed consent was obtained from all participants or their respective legal guardians. The molecular investigations of the three families was conducted independently with the investigators unaware of their parallel efforts.

2.2. Whole exome sequencing and bioinformatics

2.2.1. Family 1

Molecular work-up was completed at the Genomic Research Center, Gene By Gene (Houston, TX). Molecular investigation of the proband included homozygosity mapping completed on both probands and Whole Exome Sequencing (WES) of patient II-2 (Fig. 1A). Homozygosity mapping was completed using the Illumina HumanOmniExpress BeadChip array composed of ~730K SNPs (Illumina, San Diego, CA). Genotyping was performed following the manufacturer's protocol. Data was evaluated and analyzed using Illumina's GenomeStudio v2011.1 and the Nexus CN 7.0 Standard Edition. The twenty largest regions of Loss of Heterozygosity (LOH) with an apparent normal copy number that were shared between the two sisters were identified and systematically screened for genes with a potential role in neurological phenotypes. WES was completed using the Nextera Rapid Capture Enrichment

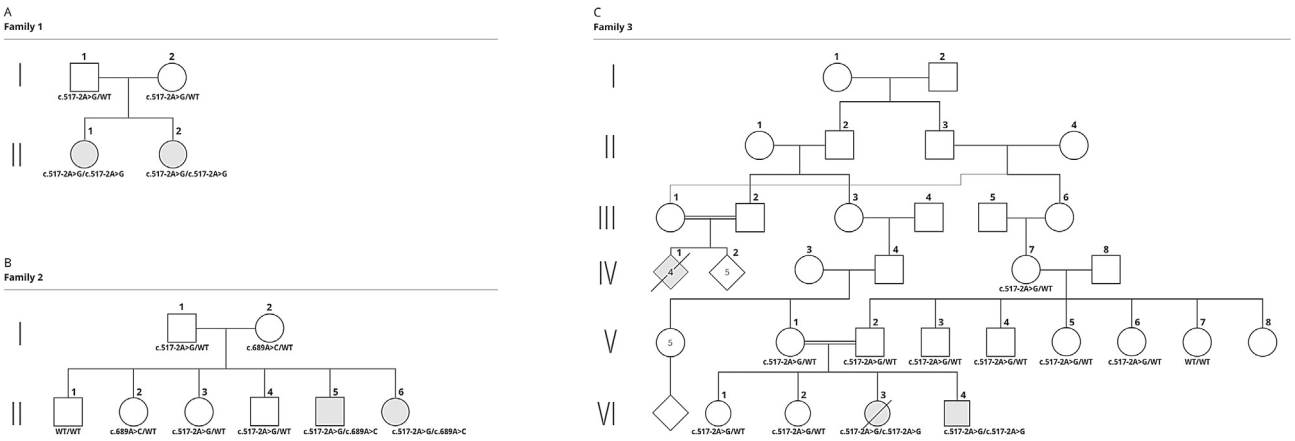

Fig. 1 – Pedigrees of the three studied families showing the affected individuals and the segregation of the deleterious *WWOX* c.517-2A > G splice-site variant and c.689A > C, p.Gln230Pro missense variant. Wild-type *WWOX* allele are noted as WT. Filled symbols indicate affected individuals. Diagonal lines across symbols indicate deceased individuals.

kit (Illumina, cat# FC-140-1000) and the Illumina HiSeq2500 platform. A standard analytic pipeline was used to map the obtained fragments to the human reference genome (hg19 assembly, UCSC Genome Browser), and generate the final list of candidate pathogenic variants. Private and rare (minor allele frequency < 0.01), presumptively damaging variants that intersected with the results from the homozygosity mapping were highlighted.

2.2.2. *Family 2*

WES was performed on DNA of the two affected siblings, Patients II-5 and II-6 (Fig. 1B). Exome enrichment was performed by Macrogen (Seoul, South Korea) using Agilent SureSelect v5+UTR kit (Santa Clara, CA). Sequencing was performed using Illumina HiSeq 4000 (San Diego, CA). The bioinformatics platform "Genomic Intelligence®" available at Variantyx Inc. (Framingham, MA) was used to analyze the data. Variants were filtered to generate a final list of rare functional variants.[15]

2.2.3. *Family 3*

WES was performed on DNA of patient VI-3. Genomic DNA was captured using the SureSelect Human All Exon v4 Kit (51 Mb; Agilent Technologies, Santa Clara, CA) and sequenced on the Illumina HiSeq2000 (Illumina, San Diego, CA). Exome data processing, variant calling, and variant annotation were performed as previously described.[16]

2.3. *Validation, segregation and carrier rate determination*

In all cases, validation and segregation studies of the *WWOX* variants detected by WES were performed by Sanger sequencing. Following purification, the obtained fragments were sequenced bi-directionally to determine the noted variants state. Sequencing was performed on various DNA Analyzers (Applied Biosystems) available at the different facilities, and the resulting sequences were analyzed with the Sequencher software (Gene Codes Corporation). Variants were scored relative to the reference sequences deposited in the National Center for Biotechnology Information [*WWOX*: (NM_016373)].

To calculate the carrier frequency of the novel *WWOX* c.517-2A > G splice-site variant, three different sets of individuals of Yemenite Jewish origin cumulatively available to the authors were screened. A total of 353 (706 chromosomes) samples were either sequenced as described above or were subject to investigation by Restriction Fragment Length Polymorphism (RFLP).

2.4. *Complementary DNA analysis*

Total RNA was isolated from blood using Trisol reagent (Ambion). cDNA was formed using random primers, Reverse-iT 1 st Strand synthesis kit (ABgene, Surrey, UK). cDNA amplification was carried out with Red load Taq Master*5 (LAEOVA), followed by gel electrophoresis and sequencing by ABI Prism 3100 Genetic Analyzer (Perkin Elmer). cDNA segment containing exon six was amplified with the primers 5′ TGGTTGTGGTCACTGGAGCTA 3′ and 5′ AGGATGCACTGCGTTCGAC 3'.

2.5. *Quantitative real-time polymerase chain reactions*

Total RNA from fresh blood was isolated using Trizol reagent (Ambion). cDNA was synthesized using random primers (qScript cDNA Synthesis Kit, Quanta). The qPCR was performed using the power SYBR Green PCR master MIX (Applied Biosystems) and run on the StepOnePlus™ (Applied Biosystems). The amplification was done with the following primers which amplified ex8-9: 5′ CTTTCACCAAGTCCATGCAA 3′and 5′CGTCTCTTCGCTCTGAGCTT3'.

A total of 12 controls were compared to patient VI-4 from family 3 in four different runs (4 different controls to each run). Every sample was analyzed in triplicate and gene expression was standardized against the *GAPDH* mRNA.

The relative quantification was calculated with the following equation.

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta CP_{\text{target}}(control-sample)}}{(E_{\text{ref}})^{\Delta CP_{\text{ref}}(control-sample)}}$$

P-value was calculated with an independent sample t-test (two tails).

3. Results

3.1. Case descriptions

3.1.1. Family 1

Family 1 comprised of four family members including two unrelated healthy parents and two affected sisters (Fig. 1A). The mother was of Yemenite Jewish ancestry and the father was of mixed Yemenite and Ashkenazi Jewish ancestries.

The proband (Fig. 1A, II-2) was born at term following an uneventful pregnancy at 39 weeks. Birth weight was 3200 g (34% percentile), and increased tone in her lower limbs was noted at birth. At the age of three months, she presented with failure to thrive, recurrent vomiting, and recurrent episodes of convulsions. At this age she was reported to achieve smiling and midline hand position. However, significant developmental delay and severe hypertonicity followed. Physical examination at the age of 30 months showed abnormal gaze, no ocular pursuit, severely increased tone in four limbs, flexed toes in both feet, adducted thumbs, extensor planter reflex, and normal patellar reflexes. Dysmorphic features included microcephaly (head circumference (HC) < 3%), frontotemporal narrowing, long eye lashes, uplifted ear lobes, short and wide neck, mild pectus carinatum, and tapering fingers. Growth parameters demonstrated short stature with length at -3SD and weight at 3rd percentile. Magnetic resonance imaging (MRI) of the brain at 18 months showed delayed myelination, thin corpus callosum (CC), moderate dilatation of the lateral ventricles and mild dilatation of the extra-ventricular CSF (cerebrospinal fluid) spaces (Fig. 2A).

The elder sister (Fig. 1A, II-1) was born at term after an uneventful pregnancy and birth growth parameters were normal (50% for age). At the age of three weeks she developed episodic vomiting and rhythmic movements suggesting seizures. Developmental delay followed. Extensive metabolic workup including muscle biopsy yielded no diagnostic findings. Electroencephalogram (EEG) at seven months of age showed asymmetric bilateral temporal spikes and sharp waves with background slowing. She underwent two surgeries due to complications of para-esophageal hernia. Brain MRI at the age of 33 months (Fig. 2B) demonstrated a thin CC, moderate dilatation of the lateral ventricles and the extra-ventricular CSF spaces representing cortical atrophy. Delayed myelination for age and mega cisterna magna were noted. Physical examination revealed similar dysmorphic features as her sister.

3.1.2. Family 2

Family 2 comprised of eight family members including two parents, two affected children and four unaffected siblings (Fig. 1B). The father was of Yemenite and Moroccan Jewish origin and the mother was of Kurdish and Moroccan Jewish origin.

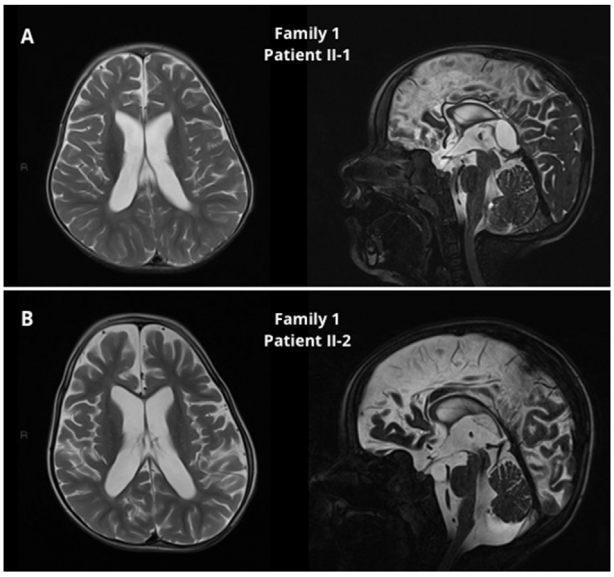

Fig. 2 – Brain MRIs. (A) Family 1, patient II-1. An axial and midsagital T2 weighted images at the age of 18 months demonstrating a thin corpus callosum, moderate dilatation of the lateral ventricles and mild dilatation of the extra-ventricular CSF spaces representing cerebral atrophy. Delayed myelination for age is evident. Posterior fossa elements are preserved. Cavum septum verga is noted as midline cyst. (B) Family 1, patient II-2. An axial and midsagital T2 weighted images at the age of 33 months demonstrating a thin corpus callosum, moderate dilatation of the lateral ventricles and moderate dilatation of the extra-ventricular CSF spaces representing cerebral atrophy. Delayed myelination for age is evident. Posterior fossa mega cysterna magna is noted.

The proband (Fig. 1B, II-5) was born at term following an uneventful pregnancy. His weight and HC at birth were 4,150 g (97%) and 35.5 cm (79%), respectively. At the age of two weeks he started exhibiting tonic contractions with head and eye deviation. EEG showed right fronto-central epileptic focal discharges. Extensive metabolic investigation was normal and brain MRI at the age of seven months demonstrated only thin CC. Various antiepileptic drugs (AED) failed to control the seizures. Physical examination at five months of age demonstrated truncal hypotonia with significant head lag, peripheral hypertonicity and normal deep tendon reflex (DTR). No developmental milestones were achieved. At the age of four years, he did not acquire any developmental milestones, established no eye contact, and truncal hypotonia with peripheral hypertonicity and normal DTR were documented. He required gastrostomy and tracheostomy due to eating difficulty and repeated aspirations. His examination revealed short stature and microcephaly with head circumference in the 1st percentile. Dysmorphic features included low anterior hairline, bushy eyebrows, long eyelashes, broad nasal bridge, short neck, brachydactyly and tapering fingers (Fig. 3A–C).

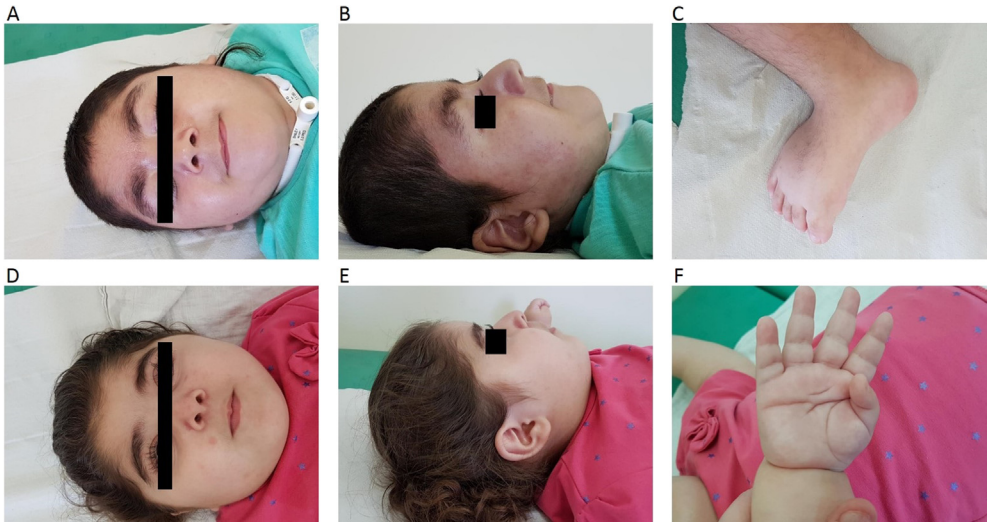

Fig. 3 – Dysmorphism of patients II-5 (A–C) and II-6 (D–F) from family 2. Panels A, B, D,E- Demonstrate short neck, low anterior hairline, bushy eyebrows, long eye lashes and broad nasal bridge in both patients. Panels C, F- Demonstrate brachydactyly and tapering fingers accordingly.

His sister (Fig. 1B, II-6) was born at term after an uneventful pregnancy with normal growth parameters. At the age of three weeks she started exhibiting tonic contractions accompanied with head and eye deviation that appeared in clusters. EEG showed multifocal bilateral epileptic activity, which transformed at the age of four months to a pattern of modified hypsarrhythmia. Partial metabolic investigation was normal, and a head MRI was not performed due to parents' refusal. On examination at the age of four months she displayed HC of 39 cm (10th percentile) and dysmorphic features similar to those of her brother (Fig. 3D–F). Eye contact was lacking and truncal hypotonia with peripheral hypertonicity and normal DTR were noted. At the age of three years, no developmental milestones were reached.

3.1.3. *Family 3*

Family 3 comprised of six family members including two parents, two affected children and two unaffected siblings. The parents are 3rd degree cousins of Yemenite Jewish origin. There was a family history of epileptic encephalopathy with infantile death in four of nine children of the aunt of a paternal grandmother who was married to the uncle of the maternal grandmother (Fig. 1C). The proband (Fig. 1C, VI-3), was born at term after an uneventful pregnancy with Apgar scores of 7/9 at one and 5 min. Her birth weight was 2040 g (−3.11 SD) and HC was 36 cm (89%). At the age of one month she started exhibiting abnormal movements of focal tonic contractions with eye staring lasting for seconds-minutes. Examination at three months of age demonstrated poor growth with weight at -2SD and microcephaly (HC in the 2nd percentile), lethargy, lack of eye contact, truncal hypotonia with head lag and peripheral hypertonicity with normal DTR. EEG showed bilateral multi focal spikes and sharp waves. Extensive metabolic investigation was within normal limits. Brain MRI exhibited thin CC and MRS was non-diagnostic. Abdominal sonography and heart echocardiography were normal. Brainstem Evoked Response Audiometry demonstrated bilateral hearing loss. Chromosomal Microarray Analysis (CMA) did not find any deletions or duplications. Various AED failed to control the seizures. At the age of nine months she passed away due to an aspiration episode. No developmental milestones had been achieved.

Her brother (Fig. 1C, VI-4), was born at term after a normal delivery and an uneventful pregnancy. Birth weight and HC were 3700 g (76%) and 33 cm (12%), respectively. He was noted to have increased peripheral tone with normal DTR already on the second day of life. Head sonography was normal. At six weeks of age he started exhibiting clusters of flexor spasms accompanied with grunting and irritability. On examination he presented with lack of eye contact, truncal hypotonia with poor head control, peripheral hypertonicity and normal DTR. EEG showed right centro-temporal epileptic focal discharges. A comprehensive metabolic investigation was within normal limits. Brain MRI at seven weeks of age showed no abnormality, however a repeated MRI at the age of four months showed cortical atrophy. Heart echocardiography showed mild pulmonic stenosis and mild atrial-septal defect (ASD) and abdominal sonography showed left hydro-nephrosis. BERA was normal. Various AED failed to control the seizures. On examination at the age of six months HC was at the 3rd percentile and weight at the 10th percentile for age. No developmental milestones were achieved.

3.2. *Whole exome sequencing results*

3.2.1. *Family 1*

The homozygosity mapping suggested a total of three genes that were previously associated with neuro-developmental phenotypes (*FA2H*, *KCTD7* and *WWOX*). WES followed and demonstrated overall good quality of the recorded variants with a mean coverage depth of 73x. Sequencing of the *FA2H and KCTD7* yielded no variants suspected to be pathogenic or likely pathogenic. The novel homozygote chr16:g.78420755A > G (GRCh37); c.517-

2A > G (NM_016373) variant was identified in the *WWOX* gene of the proband (Fig. 3A). This variant was highlighted as the *WWOX* gene was previously associated with EIEE. In addition, this gene mapped to the largest shared region of homozygosity identified for the two probands. This region was approximately 4.9 Mb long, included a total of 1657 markers and spanned chromosome 16:74085326–78980401. This variant is extremely rare and its prevalence in the general population according to gnomAD database was 0 (out of 245,866 alleles).

3.2.2. *Family 2*

WES of the two affected siblings yielded shared significant variants in five genes of which three were unrelated to the clinical phenotype of the patients and one is not currently described with respect to any human disorder. They included heterozygous variants in the *DNAH5* gene related to primary ciliary dyskinesia, *TGIF1* which is associated with Holoprosencephaly 4, *RYR1* which is associated with central core disease and other neuromuscular disorders, and two homozygous variants in the *SARM1* gene. In addition, a compound heterozygote state in the *WWOX* gene was identified and flagged due to its clinical relevance. The identified variants included the splice-site c.517-2A > G variant and the novel missense variant c.689A > C, p.Gln230Pro (Fig. 2B). The latter variant was found to be rare according to gnomAD, with an allele count of three out of a total of 246,218 alleles in the general population and was assigned an "Aggregated Predicted Severity Score" of 0.75 out of 1, representing the fraction of prediction tools (13 tools in this case) that consider the variant severe or the MetaLR score.[17]

3.2.3. *Family 3*

The homozygous *WWOX* splice-site c.517-2A > G variant described above was the only clinically relevant significant variant flagged in the exome of patient VI-3 (Fig. 1C).

3.3. *Validation, segregation and carrier rate determination*

Both variants were validated by Sanger sequencing and fully segregated with the disease in all 3 families consistent with autosomal recessive inheritance (Fig. 1A–C and 4A, B).

The prevalence of the c.517-2A > G splice-site variant among the general population according to the gnomAD database is 0 out of 245,866. However, this variant was detected in two out of the 353 (706 chromosomes) Yemenite Jewish control samples that were tested, establishing a carrier rate of 1:177 for this variant indicating a 95% confidence interval of 0.0016–0.0204 for carrier frequency in this population, using the binomial exact confidence interval approach.

3.4. *Complementary DNA analysis in families 2 and 3*

cDNA analysis of the compound heterozygote patients II-5 and II-6 of family 2 yielded two PCR products sized 593 bp and 504 bp. In contrast, the homozygote patient VI-4 of family 3 yielded only the 504 bp band (Fig. 4C). This is consistent with the prediction that the mutated splice-site variant abolishes the acceptor splice-site between exons five and six resulting in an 89 bp deletion of exon six in the mutant allele.

3.5. *Quantitative real time polymerase chain reactions*

Quantitative real time polymerase chain reaction (qPCR) was conducted to assess *WWOX* mRNA levels in patient IV-4, family 3, and controls. qPCR analyses showed low level of *WWOX* mRNA expression in the patient compared to 12 controls (Fig. 4D). The results were analyzed by two tails T test and found to be highly significant (P = 0.0003).

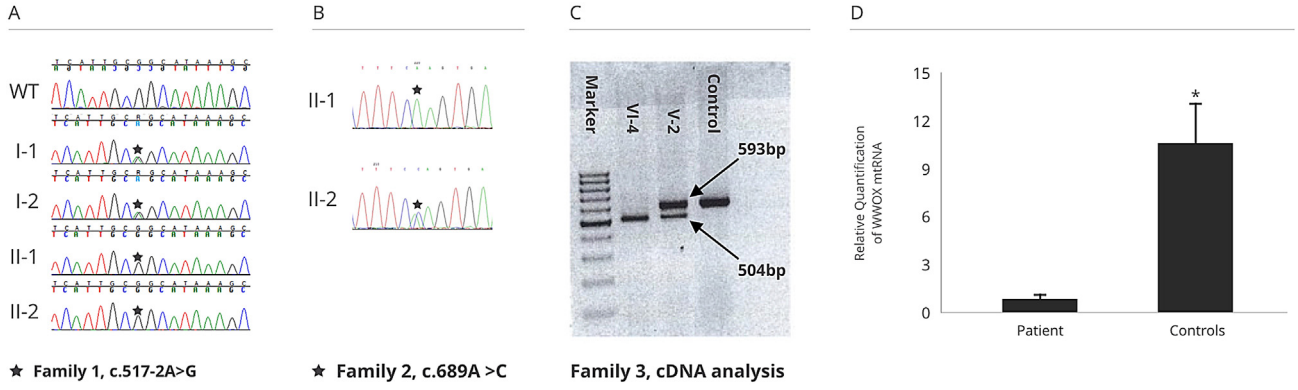

Fig. 4 – Variant analysis. (A) Sanger sequences of all individuals comprising family 1 are shown. Both parents demonstrate a heterozygote state. Both sisters demonstrate a homozygote affected state. One homozygote WT control sample is shown. The c.517-2A > G splice-site variant is marked by an asterisk. (B) Sanger sequences of family 2 individuals II-1 and II-2 represent one healthy homozygote WT non-carrier sibling, and one healthy heterozygote carrier state for the c.689A > C, p.Gln230Pro missense variant, respectively. (C) cDNA analysis of the *WWOX* allele harboring the c.517-2A > G splice-site variant in family 3. A control sample yielded one PCR product sized 593 bp. The compound heterozygote father V-2, yielded two PCR products sized 593 bp and 504 bp. The homozygote patient VI-4 yielded only the mutant 504 bp band. (D) *WWOX* expression studies. The *WWOX* expression in patient IV-4, family 3, measured with quantitative real-time PCR and standardized against *GAPDH*. The graph shows the normalized relative quantities values (Axis Y- ratio) of *WWOX* mRNA expression vs. a cohort of 12 controls. The bars indicate the average ratio of each group, asterisks denote a significant difference, as p = 0.0003 between the patient (Mean 1.08 ± 0.2) and the controls (Mean10.7 ± .1.8).

Table 1 – Demographic and Clinical characteristics of patients with *WWOX* gene deleterious variants.

	This study	Elsaadany et al. 2016[9]	Tabarki et al. 2015[8]	Valduga et al. 2015[10]	Ben-Salem et al. 2015[7]	Mignot et al. 2015[4]	Abdel-Salam et al. 2014[3]	Mallaret et al. 2014[6], Gribaa et al.2007[5]
No of Families	3	1	2	1	1	4	1	2
No of pts	6	2	5	2	1	5	1	6
Gender	4F,2M	2F	3F,2M	1F,1M	M	4F,1M	F	4F,2M
Consanguinity	+/–	+	+	+	+	–	+	+
Ethnicity	Yemenite Jews	Qatari	Saudi Arabia	Turkish	Emirati	NA	Egyptian	Saudi-Arabia Israeli Palestinian
Acquired microcephaly	+	–	+	+	+	2/5	+	NA
Neonatal hypotonia	+/–	–	–	–	+	+	+	NA
Psychomotor delay	+	+	+	+	+	+	+	+
Cerebellar ataxia	–	–	–	–	–	–	–	+
Spasticity	+	+	+	+	+	3/5 (–), 2/5 (+)	+	4/6 (–), 2/6 (+)
DTR	Normal	Exaggerated	Exaggerated	Exaggerated	Exaggerated	3/5 Normal, 2/5 exaggerated	Exaggerated	4/6- Diminished, 2/6-NA
Ophthalmological involvement	+/–	+	+ 2-abnormal ERG	+	+	3+, 2-2-abnormal ERG	+ abnormal ERG	4+, 2 NA
Age of onset of epilepsy	3w-2m	2m	2m	3m	2w	2m - 5m	2m	9–24m
Seizure type	Generalized (tonic clonic, myoclonic and IS), focal and multifocal	Myoclonic seizure	Multifocal, IS, LGS	IS	Multifocal/IS	Focal tonic/generalized/ multifocal/IS	Focal and generalized tonic clonic and myoclonic	Generalized tonic-clonic
Response to AED	Partial	Partial	Partial	Partial	Partial	Partial	Partial	Yes (2/6), partial (2/6), 2/6 NA
Brain MRI	Thin CC Cerebral atrophy, delayed myelination – 2/6 small vermis – 1/6 Moderate dilatation of lateral ventricles 2/6	Brain atrophy	Brain atrophy	Brain atrophy, thin CC, enlargement of subarachnoid spaces, opercula malformation, polymicrogyria 1/2, bil. Frontal pseudocysts 1/2	Brain atrophy polymicrogyria - frontoparietal region	Brain atrophy Thin CC 2 Normal	Brain atrophy	Mild cerebellar vermis atrophy-2/6, abnormal signal intensity at terminal zone of myelination 1/6
Premature death	1/6 (9m)	–	1/5 (<3y)	2/2 (pregnancy, 22m)	–	2/5 (16m,38m)	1 (16m)	–
Dysmorphism	+/–	–	NA	NA	–	NA	+	NA

Mutations	F1/F3: homozygous c.517-2A > G F2: comp. het. c.517-2A > G c.689A > C; p.Q230P	Homozygous: c.131G > A:p.W44[a]	Homozygous: c.606-1G > A	Homozygous del: Chr16:78,084,187 –78,424,234	Homozygous del: Chr16:78,180,603– 78,208,482	F1: comp.het. Chr16q23.1 (78,090,499–78,221,499)del Chr16q23.1 (78,376,499–78,458,499)del F2: comp.het. Chr16q23.1 (78,260,521–78,417,344) del c.1005G > A; p.W335[a] F3: comp.het. c.45_48delGGAC; pD.16Sfs63 c.140C > G; pP47R F4: comp.het. Chr16q23.1 (77,697,750–80,507,621)del c.889A > T; p.K297[a]	c.160G > T; p.R54[a]	F1: c139C > A; p.P47T F2: c.1114G > C; p.G372R

F - female, M - male, No - number, pts - patients, DTR - deep tendon reflexes, NA - not applicable, m - months, w – weeks, IS - infantile spasms, LGS – Lennox- Gastaut syndrome, AED – anti epileptic drugs, CC – corpus callosum, F1- family 1, comp.het. – compound heterozygous, del-deletion, bil.-bilateral, MRI- magnetic resonance imaging.
[a] - stop codon.

4. Discussion

Epilepsy is relatively common in childhood, however EIEE represents a rare subgroup of age-related epilepsy syndromes characterized by multiple seizures and developmental delay or developmental regression.[18,19] EIEE presents with a wide range of overlapping clinical phenotypes and involves an large number of genes and environmental factors. Even the very rare EIEE phenotype induced by *WWOX* deleterious variants has a wide range of CNS manifestations and variable phenotypes.[4] A paucity of families and patients were previously reported in the literature demonstrating multiple neurological manifestations.[3–10,14] Table 1 shows the variability in clinical findings, imaging studies and EEG findings between the patients reported herein and the patients described in the literature. MRI findings in patients with the milder form of the disease - SCAR12 showed mild cerebellar atrophy and changes in the posterior white matter intensities.[5,6] Patients with EIEE demonstrated mainly cerebral atrophy which is suggested to be progressive, but also thin CC and cortical malformation such as polymicrogyria. Repeated brain MRIs in one patient of this study showed no abnormalities at the age of 7 weeks yet demonstrated brain atrophy by the age of 4 months which emphasizes the neurodegenerative process in the EIEE phenotype. The seizure semiology was non-specific and variable and included both focal as well as generalized seizures such as tonic clonic, myoclonic and infantile spasms. Similarly, the EEG abnormalities were non-specific and are common to many disorders of early onset epileptic encephalopathies. There are several factors that may contribute to the seizures observed in patients with *WWOX* mutations: WWOX is expressed in seizure susceptible brain regions, such as the hippocampus and cortical neurons. The mutations induce pathological changes in these sensitive brain areas of mutant rats and abnormal brain development in humans. WWOX has a role as signaling protein involved in different protein–protein interactions and loss of function results in disruption of neuronal pathways, neuronal differentiation and neuronal survival, which may lead to seizures.[6,8]

A genotype–phenotype correlation in *WWOX* related epilepsy was previously suggested with the milder phenotypes attributed to hypomorphic variants. Although splice-site variants in *WWOX* are registered in ClinVar, to the best of our knowledge this is the first detailed report of patients harboring such variants in this gene. The c.517-2A > G splice-site variant described herein is associated with a severe developmental delay and, accordingly, fits with the phenotypes that were previously observed in patients carrying two predicted null alleles.[4] The decreased *WWOX* mRNA levels that were demonstrated in patient VI-4 (Fig. 4D) imply this might be attributed to a nonsense-mediated mRNA decay (NMD) process affecting the mutated transcript.

Although postnatal microcephaly is commonly found in epileptic encephalopathies, dysmorphism is not considered a consistent feature of *WWOX* related encephalopathy and has been described to date in only one patient.[3] It is noteworthy however, that in many of the reports no physical description was given and the co-occurrence of dysmorphism cannot be ruled out.[4,6,10] Among the six patients described here, four

exhibited distinct dysmorphic features. The common dysmorphic features described in families 1 and 2 were: long eyelashes, short neck, tapering fingers with brachydactyly and short stature. In addition, Patients II-1,2 from family 1 exhibit mild pectus carinatum and patients II-5,6 from family 2 exhibit broad nasal bridge, low anterior hairline and bushy eyebrows (Fig. 3). While hypertrichosis could potentially be related to AED, and bi-temporal narrowing to microcephaly, long eyelashes, broad nasal bridge, brachydactyly, tapering fingers and short stature are features which are unlikely to be the byproduct of any drug treatment. We therefore suggest that the dysmorphic features presented could serve as additional clinical signs of *WWOX* related EIEE.

The phenotypic diversity, variable time of onset, and non-specific EEG patterns characterizing epileptic encephalopathies make final genetic diagnosis elusive and many times impossible based on clinical signs and symptoms *per se*. Repeatedly, and as reflected in this study, most cases are diagnosed following next generation sequencing without a prior possibility to pinpoint a specific gene involved. Interestingly, all previously reported *WWOX* related EIEE have been in patients from the Middle East. Since each report describes different pathogenic variants, this fact might be related to the higher prevalence of consanguineous marriages in this region with local founder effects. Accordingly, the identification of founder pathogenic variants prevalent in specific populations should be of utmost value to be included in preconception screening programs. The carrier rate of the splice-site variant reported herein among Yemenite Jews was 1:177, making it a potential candidate to be included in expanded preconception screening panels and in patients presenting with EIEE and dysmorphic features with the appropriate ancestral origin.

Declaration of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. DMB and CB are compensated and serve as the chief medical officer and the laboratory director of Gene by Gene, respectively.

Acknowledgments

The authors would like to acknowledge the patients and their family members for their collaboration. The study was supported by funds from the Israel Science Foundation (grant number 2023/14) and the Pinchas Borenstein Talpiot Medical Leadership Program.

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