■ Original article

Epileptic Disord 2021; 23 (6): 843-853

Epileptic Disorders

OCLN gene variants identified in three patients with severe neurodevelopmental disorder associated with epilepsy, intellectual disability and malformation of cortical development

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Received November 23, 2020;
Accepted May 11, 2021

ABSTRACT

Objective. Homozygous *OCLN* variants have been reported in patients with band-like calcification with simplified gyration and polymicrogyria (BLC-PMG) which is characterized by microcephaly, intracranial calcification and severe developmental delay. The *OCLN* gene encodes the integral membrane protein, occludin.

Methods. Herein, we report three additional cases with homozygous *OCLN* variants that were identified via Trio-WES in two consanguineous unrelated families.

Results. We detected a previously reported frameshift variant in two cases in Family 1 and a novel missense variant in a case in Family 2. The potential pathogenicity of both variants in the index cases was investigated using *in silico* tools, and both variants were determined to be rare and predicted to be pathogenic. All of the presented cases exhibited clinical features in common with earlier reported patients, such as severe intellectual disability, microcephaly, polymicrogyria, epilepsy, hypotonia and severe developmental delay. On the other hand, in addition to the common phenotypic features, our two cases in Family 1 showed features similar to those previously reported in cases from two Turkish families carrying the same frameshift variant, such as renal failure.

Significance. We herein expand the spectrum of *OCLN* gene variants with a description of an additional novel homozygous missense variant. The frameshift variant in Turkish cases may thus be a phenotype associated with renal failure in addition to the core phenotype associated with other *OCLN* gene variants, and such variants could be important for rapid molecular diagnosis and treatment. The frameshift variant in Turkish cases might also be investigated for both a potential founder effect and mutational hot spot.

Key words: *OCLN* variants, neurodevelopmental disorders, BLC-PMG, whole-exome sequencing

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doi:10.1684/epd.2021.1349

Patients with the BLC-PMG phenotype (also referred to as the pseudo-TORCH phenotype) have bilateral symmetrical polymicrogyria and grey matter calcification, as well as microcephaly, developmental arrest and early-onset seizures. Moreover, in some of the reported patients, there was evidence of an extracranial phenotype similar to renal dysfunction [1-4].

O'Driscoll *et al.* (2010) reported that the cause of BLC-PMG (band-like calcification with simplified gyration and polymicrogyria), which is a severe autosomal recessive neurodevelopmental disorder, is derived from variants in the *OCLN* gene. Multiple *OCLN* variants have been reported in numerous patients with neurological and clinical symptoms, including progressive microcephaly, brain calcification and polymicrogyria, intellectual disability, and seizures [1-7] (*table 1*).

The *OCLN* gene is located on chromosome 5q13.2 and includes nine exons [5]. Moreover, the *OCLN* pseudogene, inverted and partly duplicated, contains a copy of exons 5-9 [8]. The *OCLN* gene encodes the ~65-kDa (522 amino acids) integral membrane protein, occludin, which is necessary for cytokine-induced regulation of tight junction barriers [6, 9]. Additionally, transcripts of the *OCLN* gene exhibit splicing diversity and variable expression of alternative splice variants [8]. Occludin is highly conserved in vertebrates and also contains two functional domains: the occludin/ELL domain and the marvel (MAL and related proteins for the vesicle traffic and membrane link) domain [6]. The occludin/ELL domain is located in the C-terminal part of the protein and is a part of the ELL family of RNA polymerase II elongation factors [8]. In contrast, the Marvel domain, which has a four-transmembrane-helix region architecture, is involved in membrane apposition, and is encoded by exon 3 and exon 4 of the *OCLN* gene [10]. Protein forms lacking part or all of the Marvel domain are expressed in the cytoplasm, whereas forms containing the entire Marvel domain are expressed on the cell membrane [8].

The present study aimed to describe three additional cases (from two unrelated consanguineous families [Family 1 and Family 2]) with homozygous pathogenic variants in exon 3 of the *OCLN* gene that were identified via whole-exome sequencing. Both of the variants (NM_001205254.1:c.173_194del, p.(Trp58Phefs*10) and NM_001205254.1:c.199A>T, p.(Ile67Phe)) identified in the two families are rare and predicted to be pathogenic. All three cases exhibited common clinical features, including severe intellectual disability, microcephaly, epilepsy, hypotonia and severe developmental delay.

Clinical report

Family 1

- ***Patient 1***

Patient 1 was the third child of healthy consanguineous Turkish parents (first cousins) (*figure 1A*). She was born at term via vaginal delivery and became cyanotic just after delivery. Anamnesis showed that a sister and a brother had epilepsy, and that both of them died at about age four years. Upon examination at age three years, she weighed 5,400 g (<3rd percentile), her height was 75 cm (<3rd percentile), and her head circumference was 45 cm (<3rd percentile). She had axial hypotonia, microcephaly, and an atypical facial appearance. She did not reach any developmental milestones. Head control was absent. Moreover, she was unable to sit up, walk, or speak. She had upper and lower extremity spasticity, hyperactive deep tendon reflexes, and lower extremity clonus. She had hypernatremia and elevated ALT-AST levels.

She had been hospitalized several times due to epilepsy and diabetes insipidus. Phenobarbital (5 mg/kg/day) was used to control her seizures. Unfortunately, EEG was not available for this patient. Abdominal USG and DMSA (dimercaptosuccinic acid) scintigraphy showed that the right kidney was larger than the left kidney. She also had hepatomegaly. MRI at age three years showed cerebral and cerebellar atrophy and polymicrogyria (*figure 2A-C*). The corpus callosum was thinner than normal. As CT could not be performed, calcification findings were not evaluated.

- ***Patient 2***

Patient 2 is the sister of Patient 1 and the sixth child of the family (*figure 1A*). She was also born at term via vaginal delivery, like Patient 1. She had birth asphyxia and her birth weight was 2,000 g. The parents presented her to hospital for the first time when she was aged three months because of generalized tonic seizures in the form of contraction of the arms. On physical examination, she had microcephaly, axial hypotonia, and severe developmental delay. She could not sit up, walk, or speak. She also exhibited spastic tetraparesis. She had upper and lower extremity spasticity and hyperactive deep tendon reflexes. She was diagnosed with diabetes insipidus including hypernatremia by the endocrinology department and desmopressin treatment was initiated. She was subsequently hospitalized several times due to refractory epilepsy. Her seizures were controlled with the antiepileptic drug

▼ **Table 1.** Genetic and clinical features of previously reported patients and the presented patients.

Case report (Family)	Exon	Nucleotide alteration	Amino acid alteration	Clinical features														
				Gender	Feeding difficulty	Vision impairment	Hearing impairment	Developmental delay	Hypotonia	Hyperreflexia/spasticity	Renal involvement	Other organ involvement	Seizure	Microcephaly	CC	PMG	BLC	Dilated lateral V
O'Driscoll *et al.* [6] (F085)	3 and 4?	arr5q13.2(68,839,890 × 2,68,840,602-68,844,536 × 0,68,852,777 × 2)	p.Lys18_Glu243? Deletion of transmembrane domains	F	+	+	+	-	+	-	-	-	+	+	NA	+	+	NA
				M	-	+	NA	-	+	+	-	PDA, PFO, cleft lip	+	+	NA	+	+	NA
O'Driscoll *et al.* [6] (F275)	3 and 4?	arr5q13.2(68,839,890 × 2,68,840,602-68,844,536 × 0,68,852,777 × 2)	p.Lys18_Glu243? Deletion of transmembrane domains	M	+	NA	NA	+	-	+	NA	-	+	+	H	+	+	+
				M	NA	NA	NA	+	-	+	+ Renal failure	-	+	+	NA	+	+	Dysplastic V
O'Driscoll *et al.* [6] (F386)	3	c.51-?_730-?del	p.Lys18_Glu243? Deletion of transmembrane domains	F	-	+	NA	+	-	+	+ DI, mild renal dysplasia	PDA	+	+	NA	+	+	NA
				M	+	+	NA	+	-	+	-	Umbilical hernia	+	+	NA	+	+	NA
O'Driscoll *et al.* [6] (F312)	3	c.512dupA	p.Tyr171*	M	+	+	+	-	+	+	NA	-	+	+	Thin	+	+	Large V
	3	c.656T>C	p.Phe219Ser	F	+	+	NA	-	-	+	NA	NA	+	+	NA	+	+	NA
O'Driscoll *et al.* [6] (F351)	Intron 5-6	c.1037+5G>A	Alteration of donor splice site	F	-	+	NA	NA	+	+	-	-	+	+	NA	+	+	NA
O'Driscoll *et al.* [6] (F375)	3	c.171_193delATGGACC-TCTCCTCCAGGAGTG- (=NM_001205254.1: c.173_194del)	p.Lys57Asnfs*37 (=NM_001205254.1: p.Trp58Phefs*10)	M	+	NA	NA	NA	-	+	NA, hypernatremia episodes	NA	+	+	Small	+	+	NA
				F	NA	+	NA	-	-	+	NA	NA	+	+	NA	+	+	NA
Aggarwal *et al.* [2]	3	c.252delC	p.Ser85Profs*14	M	+	-	-	NA	-	+	+ Renal failure	NA	+	+	NA	-	+	NA
				F	+	-	-	NA	-	+	+ Acute on CRF	-	+	+	NA	+	+	NA
LeBlanc *et al.* [4]	9	Novel translocation	-	F	+	+	NA	+	NA	NA	+ CRF	-	+	+	NA	-	+ Mild	NA
				F	+	+	NA	+	NA	NA	+ ARF	-	+	+	NA	+	+	Enlarged V

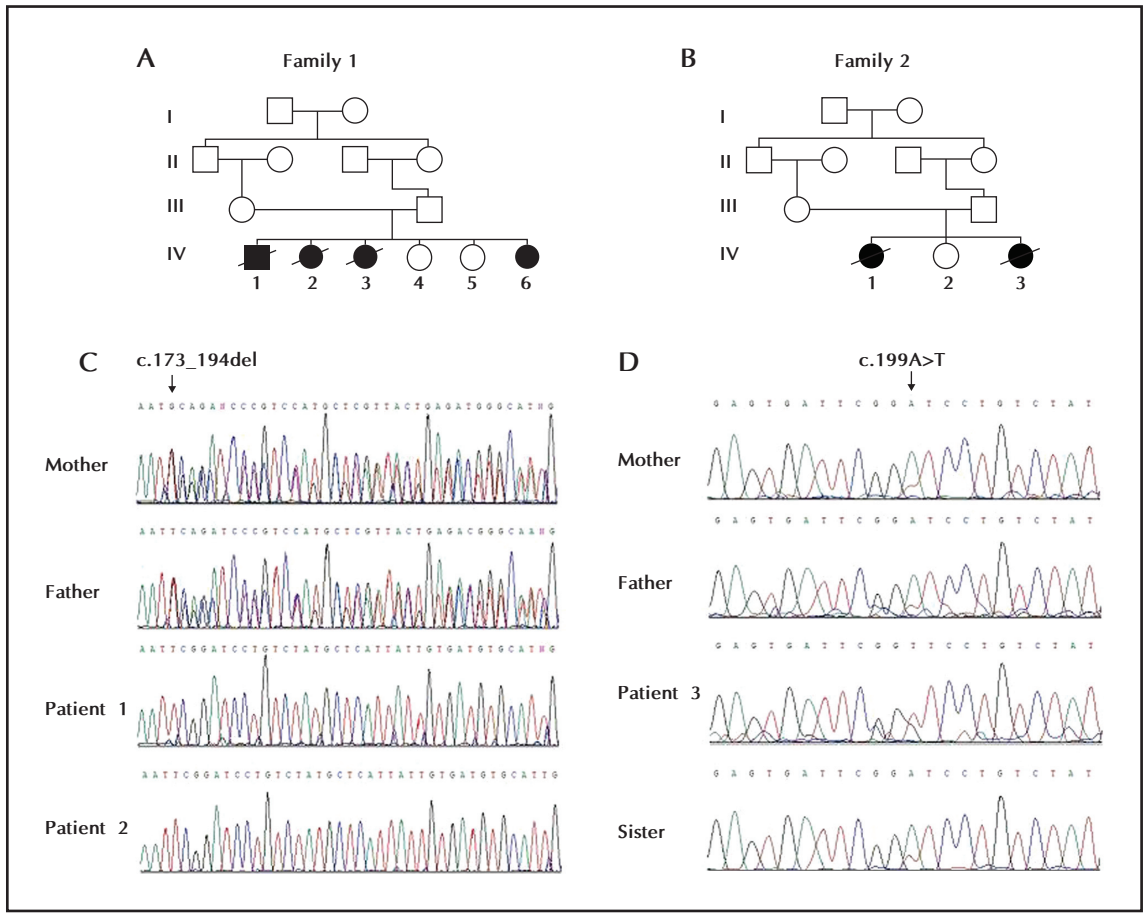
▼ **Table 1.** Genetic and clinical features of previously reported patients and the presented patients (*continued*).

Case report (Family)	Exon	Nucleotide alteration	Amino acid alteration	Clinical features														
				Gender	Feeding difficulty	Vision impairment	Hearing impairment	Developmental delay	Hypotonia	Hyperreflexia/spasticity	Renal involvement	Other organ involvement	Seizure	Microcephaly	CC	PMG	BLC	Dilated lateral V
Elsaid *et al.* [3]	5	c.981delC	p.Asn328Metfs*4	F	NA	+	+	NA	+	+	+ CRF, DI, hypernatremia	Liver calcification, GER	+	NA	NA	+	+	Enlarged lateral V
				F	NA	+	+	NA	+	+	+ Microscopic hematuria	-	+	NA	NA	+	+	Secondary ventricular dilatation
Jenkinson *et al.* [5] (F131)	6	c.1218C>T	p.Ser407Asnfs*66 Cryptic splice site	F	+	NA	NA	+	NA	+	NA	-	+	+	NA	+	+	NA
Jenkinson *et al.* [5] (F468)	7	c.1320_1336del	p.Gln441Thrfs*5	F	NA	NA	NA	+	+	NA	VUR	-	+	+	Thin	+	+	NA
Jenkinson *et al.* [5] (F523)	7	c.1284_1287del	p.Gln428Hisfs*56	M	NA	NA	NA	+	NA	NA	DI	NA	+	+	NA	-	+	NA
Jenkinson *et al.* [5] (F557)	3	c.711_714delins-ATCCCCAGTA	p.Cys237*	F	NA	NA	NA	+	+	+	-	NA	+	+	NA	+	+	NA
Abdel-Hamid *et al.* [7] (F1)	4	c.809delA	p.Lys270Argfs*62	M	+	+	-	+	+	+	-	NA	+	+	Thin	+	+	+
Abdel-Hamid *et al.* [7] (F2)	Intron 5-6	c.1037+5G>C	Splice site IVS5+5G>C	M	+	-	-	+	+	+	-	NA	+	+	Thin	+	+	+ Asymmetric
Abdel-Hamid *et al.* [7] (F3)	3	c.51-?_730-?del	p.Lys18_Glu243? Deletion of transmembrane domains	M	+	+	-	+	+	+	-	NA	+	+	Thin	+	+	+ Mild
Abdel-Hamid *et al.* [7] (F4)	3	c.51-?_730-?del	p.Lys18_Glu243? Deletion of transmembrane domains	M	+	+	+	+	+	+	-	NA	+	+	Thin	+	+	+
Abdel-Hamid *et al.* [7] (F5)	6	c.1180delG	p.Glu394Serfs*91	M	+	+	-	+	+	+	-	NA	+	+	Thin	+	+	+

▼ **Table 1.** Genetic and clinical features of previously reported patients and the presented patients (*continued*).

Case report (Family)	Exon	Nucleotide alteration	Amino acid alteration	Clinical features														
				Gender	Feeding difficulty	Vision impairment	Hearing impairment	Developmental delay	Hypotonia	Hyperreflexia/spasticity	Renal involvement	Other organ involvement	Seizure	Microcephaly	CC	PMG	BLC	Dilated lateral V
Abdel-Hamid *et al.* [7] (F6)	4	c.858_861delTTAT	p.Ile286Metfs*45	F	+	-	-	+	+	+	-	NA	+	+	Thin	+	+	+
				F	+	-	-	+	+	+	-	NA	+	+	Thin	+	+	+
				M	+	+	-	+	+	+	-	NA	+	+	Thin	+	+	+ Mild
Abdel-Hamid *et al.* [7] (F7)	6	c.1169C>G	p.Ser390*	F	+	+	-	+	+	+	-	NA	+	+	Thin	+	+	+
				M	+	-	NA	+	+	+	-	NA	+	+	Thin	+	+	-
Abdel-Hamid *et al.* [7] (F8)	3	c.51-?_730-?del	p.Lys18_Glu243? Deletion of transmembrane domains	M	+	-	Mild	+	+	+	-	NA	+	+	Thin	+	+	+ Mild
Abdel-Hamid *et al.* [7] (F9)	3	c.51-?_730-?del	p.Lys18_Glu243? Deletion of transmembrane domains	M	+	-	-	+	+	+	-	NA	+	+	Thin	+	+	+
Abdel-Hamid *et al.* [7] (F10)	3	c.51-?_730-?del	p.Lys18_Glu243? Deletion of transmembrane domains	F	+	-	-	+	+	+	-	NA	+	+	Thin	+	+	+
Ekinci *et al.* [1]	3	c.173_194del	p.Trp58Phefs*10	F	+	NA	NA	+	NA	+	+ DI, hypernatremia	GER	+	+	NA	+	+	+
Our patients (P1 and P2)	3	c.173_194del	p.Trp58Phefs*10	F	+	-	-	+	+	+	+ DI, hypernatremia	NA	+	+	Thin	+	NA	NA
				F	+	+	-	+	+	+	+ DI, hypernatremia	NA	+	+	Thin	+	NA	NA
Our patient (P3)	3	c.199A>T	p.Ile67Phe	F	+	+	-	+	+	+	-	-	+	+	Thin	+	+	+

ARF: acute renal failure; BLC: band-like calcification with simplified gyration; CC: corpus callosum; CRF: chronic renal failure; DI: diabetes insipidus; F: family; GER: gastroesophageal reflux; H: hypogenesis; NA: not available; PDA: patent ductus arteriosus; P: patient; PFO: patent foramen ovale; PMG: polymicrogyria; V: ventricle; VUR: vesicoureteral reflux.



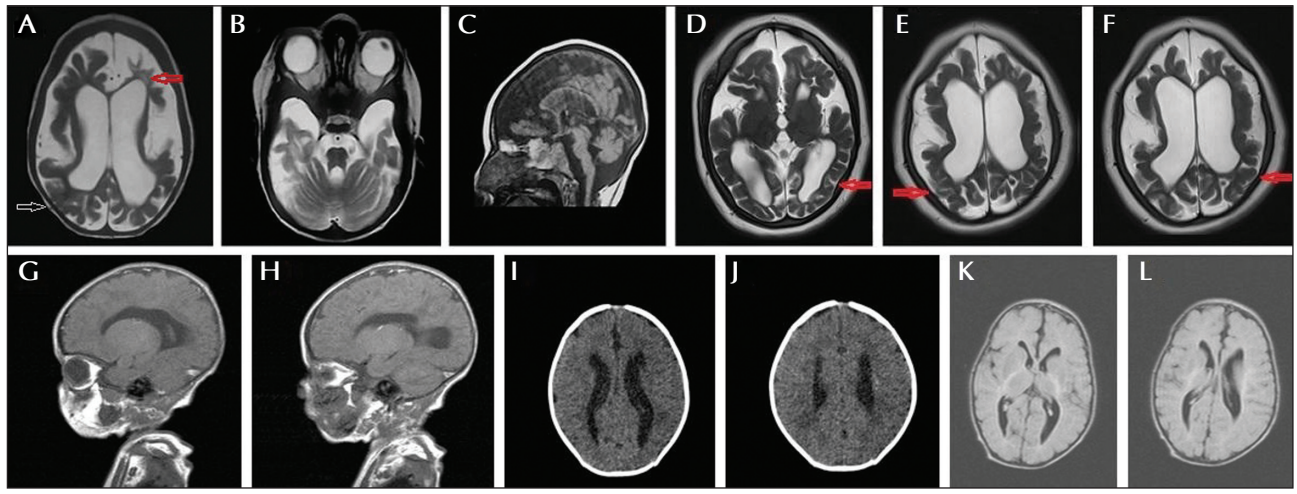
■ **Figure 1.** (A, B) Pedigrees of the two families. Squares represent males, circles represent females, and black symbols indicate clinically affected individuals. (A) Family 1. IV-3: Patient 1; IV-6: Patient 2. (B) Family 2. IV-3: Patient 3. (C, D) Partial electropherograms show the *OCLN* variants in the two families. The NM_001205254.1: c.173_194del, p.(Trp58Phefs*10) variant detected in Family 1 (C) was homozygous in Patient 1 and Patient 2, but heterozygous in their mother and father. In Family 2 (D), Patient 3 was homozygous for the *OCLN* NM_001205254.1:c.199A>T, p.(Ile67Phe) variant, whereas the father, mother, and sister were heterozygous.

combination of lacosamide, clobazam, and levetiracetam. She could not sit up, walk, or speak. EEG findings showed generalized epileptic activity originating from the right hemisphere. VEP (visual evoked potential) showed bilateral mildly delayed potentials. MRI findings showed that she had diffuse cerebral and cerebellar atrophy, bilaterally advanced enlargement of the lateral ventricular, corpus callosum atrophy, and bilaterally pathological bright lesions in the white matter of both cerebral hemispheres, similar to Patient 1. Polymicrogyria findings were not noted on her first MRI because of low-resolution image quality. When this patient subsequently underwent 3 Tesla Cranial MRI visualization at age 11 years, polymicrogyria was observed (*figure 2D-F*).

Family 2

• *Patient 3*

This nine-month old female was born at 38 weeks of gestation to an 18-year-old mother (*figure 1B*). During the prenatal period, foetal movements were normal and there were no abnormalities detected. The patient's delivery was normal and problem-free. Birth weight was 3,000 g, length was 50 cm, and head circumference was 35 cm. In the hours following birth, she became lethargic, exhibited poor feeding, and generalized hypotonia was clinically observed. The patient was intubated due to an apnoeic respiratory pattern. She was monitored in the NICU (neonatal intensive care unit) for 40 days, during which time she remained intubated and received total



■ **Figure 2.** Brain images of the three patients. (A-C) Patient 1. Axial T2-weighted MRI shows cerebral atrophy, occipital polymicrogyria (white arrow) and periventricular white matter hyperintensity (red arrow) (A), and slight cerebellar atrophy (B). Note the thin corpus callosum with mild vermis hypoplasia/atrophy and polymicrogyria on the T1-weighted image (C). (D-F) Patient 2. Polymigrogyria findings are indicated by the arrows on axial T2 sequences (3 Tesla cranial MRI visualization). (G-L) Patient 3. Sagittal T1A-weighted image shows a thin corpus callosum as well as small hyperintense and hypointense areas in the periventricular region; there is a small periventricular hyperintense area consistent with calcification (G, H). Brain CT shows that the ventricles are mildly dilated and a subacute-phase haemorrhage that appears mildly hyperintense in periventricular white matter areas (I, J). Axial T-FLAIR shows a moderately wide view of the lateral ventricles within physiological limits (K, L).

parenteral nutrition, intravenous antibiotics, and phenobarbital. At age 1.5 months, she presented to the paediatric neurology clinic with hypotonia. Anamnesis showed that she was born to consanguineous parents (first cousins) and that a sibling had the same symptoms and died two years ago at age 13 days.

Physical examination showed microcephaly, generalized hypotonia, absence of deep tendon reflexes, and growth and motor retardation. Laboratory findings showed that glycaemia, counter blood cells, serum ammonia, serum pyruvic acid, serum lactate, biotinidase, urine amino acid, urine organic acid, and plasma amino acid were normal. MRI performed using a 1.5 Tesla scanner (1.5T) (Gyroscan Intero, Philips, Best, the Netherlands) showed a thin corpus callosum and dilatation of the lateral ventricles, polymicrogyria and band-like calcification at two months old (*figure 2G-L*). A CSF (cerebrospinal fluid) study indicated normal glucose and normal protein levels, but elevated glycine levels were noted via plasma and urine analysis. The ratio of CSF glycine to plasma glycine was 0.27 (normal: 0.08). Sodium benzoate (100 mg/kg/day) and dextromethorphan (6 mg/kg/day) were initiated. During clinical follow-up, generalized tonic-clonic and clonic seizures developed, and clonazepam was added to her phenobarbital treatment, followed by the addition of vigabatrin. EEG showed multifocal spikes and sharp waves. The patient also developed drug-resistant epilepsy.

The clinical features of the patients are presented in *table 1*.

Materials and methods

Editorial policies and ethical considerations

The study protocol was approved by the Dr. Behçet Uz Children's Hospital and Dokuz Eylul University Ethics Committee, and the parents of the patients provided written informed consent.

Genetic analysis

- ***DNA Extraction***

Genomic DNA was extracted from peripheral blood using a NucleoSpin® Blood L Kit (Macherey-Nagel, Duren, Germany), according to the manufacturer's instructions.

• ***Whole-exome sequencing (WES) and analysis***

Exome sequencing was performed on samples from two unrelated, not previously reported families. The NimbleGen SeqCap EZ Human Exome Kit v2.0 was used for exome capture. Samples were sequenced using paired-end 50-bp reads and an Illumina HiSeq2000 sequencing platform. Exome variants were called by the UW-CMG GATK (Genome Analyzer Toolkit) pipeline (version date Dec 2015) (https://uwcmg.org/) and annotated using VEP v.83 (Variant Effect Predictor). Then, the data were analysed using GEMINI v.0.19.1 and suspected variants were screened in the following databases: dbSNP (https://www.ncbi.nlm.nih.gov/snp); ESP6500 (v2) (http://evs.gs.washington.edu/EVS/); 1000 Genomes (phase 3) (http://www.1000genomes.org/); and UK10K (February, 2016) (https://www.uk10k.org).

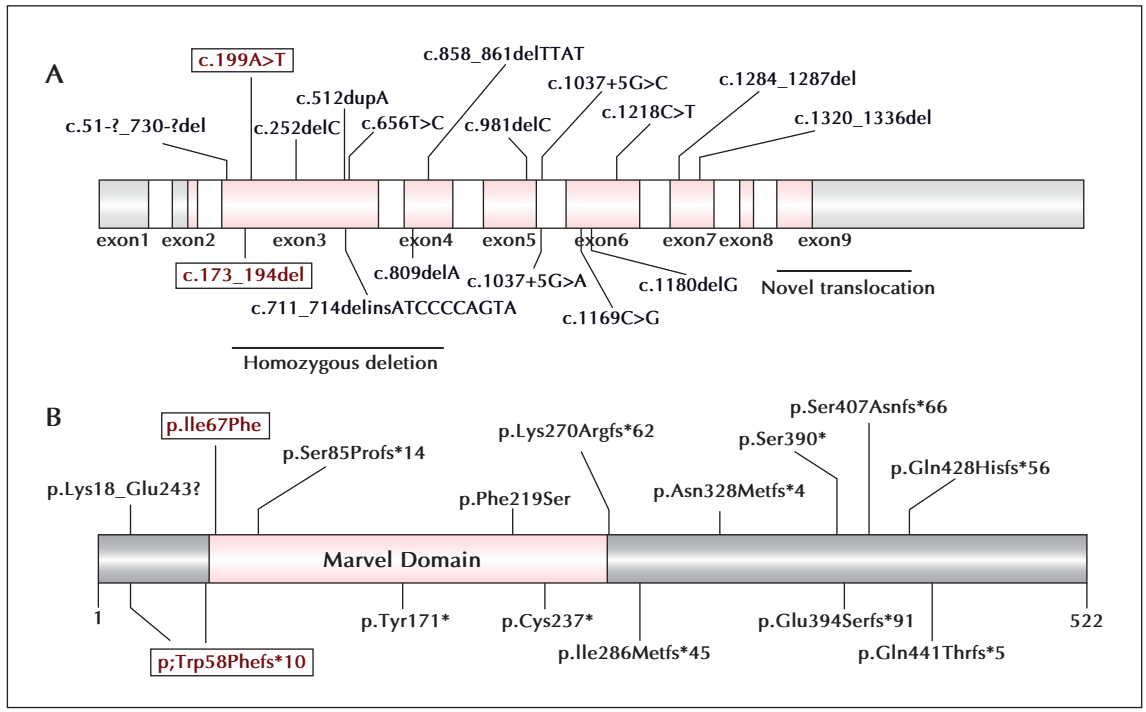
After the variants in the two families were filtered, the disease candidate variants were chosen according to the autosomal recessive segregation model. The parents were heterozygous, and affected cases were homozygous. Rare variants were the focus (low minor allele frequency in the gnomAD variome) (http://gnomad.broadinstitute.org). Potentially damaging variants were prioritized using the American College of Medical Genetics Organization criteria [11].

Following application of a segregation filter, two exonic non-synonymous variants in the methylcrotonoyl-CoA carboxylase 2 (*MCCC2*) gene and WD repeat domain 36 (*WDR36*) gene, and an exonic frameshift variant in the occludin (*OCLN*) gene were investigated in greater detail with *in silico* tools in Family 1. Four exonic non-synonymous variants in the ankyrin repeat domain 28 (*ANKRD28*) gene and dynein cytoplasmic 2 heavy chain 1 (*DYNC2H1*) gene, and three autosomal recessive variants in the kinesin family member 15 (*KIF15*) gene, keratin associated protein 5-10 (*KRTAP5-10*) gene and PX domain-containing protein kinase-like protein (*PXK*) gene, and one exonic missense variant in the *OCLN* gene were investigated in greater detail with *in silico* tools in Family 2. The *OCLN* gene (OMIM 602876) was prioritized as a candidate gene according to the clinical characteristics of the patients, as well as gene function, protein expression, and assessment based on *in silico* prediction tools (*supplementary table 1*).

As the *OCLN* gene is associated with BLC-PMG (OMIM 251290), which is characterized by early-onset seizures, progressive microcephaly, severe developmental delay, and cortical brain malformations, two different recessive variants in the *OCLN* gene (NM_001205254.1) were identified and further investigated in the two unrelated families. The two detected variants in the *OCLN* gene were validated via Sanger sequencing. The oligonucleotide primers 5′-CATTTCAGCAAACCGAATCA-3′ and 5′-CCAAAGCCACTTCCTCCATA-3′ were used for amplification of the variant in Family 1, whereas primers 5′-ATGCTCTCTCAGCCAGCCTA-3′ and 5′-TTGCTGCTCTTGGGTCTGTA-3′ were used for amplification of the variant in Family 2. The affected siblings (Patient 1 and Patient 2) in Family 1 had a rare homozygous frameshift deletion variant, NM_001205254.1:c.173_194del, p.(Trp58Phefs*10) (maxAAF:0.0019 no CADD score) (*figure 1C*). This frameshift variant has previously been reported by O'Driscoll *et al*. (2010) and Ekinci *et al*. (2020). In addition, the affected patient (Patient 3) in Family 2 had a rare homozygous missense variant, NM_001205254.1:c.199A>T, p.(Ile67Phe); rs766112658 (maxAAF:0.0005; CADD score:27.2) (*figure 1D*). This rare missense variant is not documented in homozygous state in GnomAD. Based on Mutation Taster, SIFT, PROVEAN and SNAP2, the homozygous missense variant is predicted to have a damaging/disease-causing effect on *OCLN* (*supplementary table 1*).

Discussion

Whole-exome sequencing is a powerful method to identify genetic causes of neurodevelopmental disorders [12]. The present study describes two recessive variants in the *OCLN* gene (NM_001205254.1) based on whole-exome sequencing in three cases in two unrelated families whose children have neurodevelopmental disorders. To date, 16 distinct *OCLN* variants have been described in 35 cases from 24 families, associated with the development of neurological and clinical symptoms [1-7] (*table 1, figure 3*). Both of the variants described in the present study are rare, predicted to be pathogenic, and located in the third exon (*figure 3*). All three of the presented cases with variants exhibited clinical features in common with earlier reported patients, such as severe intellectual disability, microcephaly, epilepsy, hypotonia, and severe developmental delay. Polymicrogyria associated with *OCLN* variants was observed in all our patients. On the other hand, a calcific area was observed only in Patient 3, as brain CT could not be performed in the other patients. To the best of our knowledge, the missense variant is novel, whereas the frameshift variant has been reported in three cases from two unrelated Turkish families [1, 6] who had diabetes insipidus in addition to other common phenotypic features, as well as other *OCLN* variants. Cases with the same frameshift variant, previously reported by O'Driscoll *et al*. (2010) and Ekinci *et al*. (2020), presented with polymicrogyria, brain calcification, hypernatremia, renal dysfunction, microcephaly, intellectual disability, and seizures, similar to the presented cases in Family 1. As such, we believe that c.173_194del, p.Trp58Phefs*10 could be a relatively



■ **Figure 3.** Schematic representation of the *OCLN* gene (A) and the occludin protein (B), showing the variants reported to date. The two variants described in this study are indicated in red in boxes.

common frameshift variant with a probable founder effect in the Turkish population. *Supplementary table 2* shows the possible homozygous region surrounding the *OCLN* gene and the estimated haplotypes of individuals carrying the frameshift variant according to the segregation of parental alleles based on SNP positions.

The frameshift variant in Turkish cases should be investigated further to elucidate whether the variant is derived from a haplotype exerting a potential founder effect or from diverse haplotypes associated with a mutational hot spot. The first founder variant, p.Lys18_Glu243, in *OCLN* was reported in five Egyptian families [7]. The present study describes the second most frequent variant (p.Trp58Phefs*10) in the same exon (exon 3) in the *OCLN* gene. *Table 1* summarizes the genetic and clinical features of the previously reported patients and the presented patients. All patients in *table 1* have common features, including epilepsy, microcephaly, spasticity and severe developmental delay. The two siblings in Family 1 had a thin corpus callosum, polymicrogyria, cerebral and cerebellar atrophy, renal failure (diabetes insipidus/hypernatremia), and bilateral optic atrophy. Band-like calcification was not evaluated because of the absence of CT data. Polymicrogyria was detected in more recent 1.5 Tesla MRI images obtained for Patient 2, who was recalled for 3 Tesla Cranial MRI, as in previous cases. In Patient 1, 1.5 Tesla MRI images revealed polymicrogyria although the level of resolution of the MRI images was low. Because this patient died, high-resolution cranial MRI could not be performed. This experience shows that the combination of high-resolution MRI and next-generation sequencing are important for clinical evaluation. Another important phenotypic feature in Patient 1 and Patient 2, but not in Patient 3, was central diabetes insipidus/hypernatremia due to renal failure. Hypernatremia is a common phenotypic feature in patients with multiple variants that have been previously reported [2-4]. Hypernatremia was observed in the two affected siblings in the present study, carrying the same homozygous indel variant (c.173_194del), as reported by O'Driscoll *et al.* (Family F375) and Ekinci *et al* [1, 6].

Occludin is an integral membrane protein located at tight junctions, and may play a role in the formation and regulation of the tight junction (TJ) paracellular permeability barrier in epithelial and endothelial cells [13, 14]. Our knowledge of members of the claudin family of tight junction-associated membrane proteins is critical to our understanding of the structure and function of tight junctions in epithelia, such as in the kidney [15, 16]. Moreover, claudin-1, occludin and ZO-1 (zonula occludens-1) form the tight junctions of

glomerular parietal epithelial cells (PECs) [17]. Based on a *OCLN* knockout mouse model, abnormalities of the salivary glands, gastric epithelium, kidney, bone, testes and intracranial calcification and purkinje cells of the cerebellum were demonstrated [6, 18]. These results confirm the effect of occludin in other tissues as well as the brain, and may indicate how *OCLN* variants affect the kidney. Further study of the renal parameters of patients affected with BLC-PMG may provide further insight into the course of renal involvement, and assist in developing optimal management protocols [2].

Patient 3 from Family 2 had a rare homozygous missense variant (c.199A>T, p.Ile67Phe) in exon 3. Missense variants can affect the binding of SR proteins (serine-arginine-rich proteins) to ESEs (exonic splicing enhancers), resulting in failure of splicing and exon skipping or inclusion of intron segments in mRNA [19]. Therefore, SR protein binding motifs in wild-type and c.199A>T mutant exon 3 sequences were compared. This variant slightly increases the score for an ESE finder-predicted binding site for SC35 (threshold: 2.383), from 5.210135 (motif: GGATCCTG) to 5.651885 (motif: GGTTCCTG) (http://rulai.cshl.edu/tools/ESE2/). In addition, it may lead to a change in splicing pattern, however, *in silico* analysis should be followed by *in vivo* and *in vitro* studies to examine this further.

In the study of O'Driscoll *et al*., only a missense variant in the *OCLN* gene was reported (Family F312) [6]. Both the missense variant (c.656T>C, p.Phe219Ser) reported by O'Driscoll *et al*. (2010) and the missense variant (c.199A>T, p.Ile67Phe) detected in Patient 3 were analysed using *in silico* tools to predict pathogenicity. *In silico* analysis showed that both variants appear to be pathogenic (*supplementary table 1*). Patient 3 was diagnosed with dilatation of the lateral ventricles in addition to polymicrogyria. The patient with the missense variant reported by O'Driscoll *et al*. (2010) exhibited polymicrogyria, band-like calcification, and other common clinical features previously reported (*table 1*). The multiple phenotypes in previously reported cases and Patient 3 in Family 2 might be related to pathogenic variants in other genes and/or other factors, and overlapping phenotypes may be associated with mutational variation within the *OCLN* gene.

Based on the present findings, as well as the literature, renal failure (diabetes insipidus/hypernatremia), epilepsy, and microcephaly may therefore be considered as phenotypic features typically associated with *OCLN* gene mutation.

Conclusion

Herein, we describe a novel homozygous missense variant and a frameshift variant with a potential founder effect in the Turkish population. This additional novel homozygous missense variant therefore expands the spectrum of *OCLN* gene variants. Based on the previously reported cases and those reported here, we suggest that the frameshift variant in Turkish cases is typically associated with renal failure in addition to the core phenotype associated with other *OCLN* gene variants. The presence of the same variants reported in different cases may enhance molecular diagnosis and treatment, thus, the frameshift variant in Turkish cases should be investigated further with regards to both a potential founder effect and mutational hot spot. Identifying the genetic basis of neurodevelopmental disorders may facilitate subtyping, accurate diagnosis, and informed genetic counseling, as well as primary prevention measures. ■

Supplementary material.
Summary slides and supplementary table accompanying the manuscript are available at www.epilepticdisorders.com.

Acknowledgements and disclosures.
We are very grateful to the families that participated in this study. This work was supported in part by a grant given to the SC Scientific and Technological Research Council of Turkey (TÜBİTAK-BIDEB 2219) (International Postdoctoral Research Scholarship Programme) and a grant from the US National Institutes of Health (NIH R01 MH101221 to E.E.E). Sequencing was provided by the University of Washington Center for Mendelian Genomics (UW-CMG) and was funded by the National Human Genome Research Institute (NHGRI) and the National Heart, Lung and Blood Institute (NHLBI); grant HG006493 to Drs. DAB, MJB, and SML. This work has been supported by the Dokuz Eylul University Fund (2018.KB.SAG.116 to S.C) and the Scientific and Technological Research Council of Turkey (TÜBİTAK) (106S099 to S.C). TOO was granted a fellowship by the Scientific and Technological Research Council of Turkey (TUBITAK-2228/B). Evan E. Eichler is an investigator of the Howard Hughes Medical Institute.
The authors declare that they have no conflicts of interest.

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TEST YOURSELF

(1) What is the characteristic brain malformation associated with pathogenic biallelic pathogenic *OCLN* variants?

(2) What is the role of *OCLN* in the kidney?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com.