



The utility of whole exome sequencing for identification of the molecular etiology in autosomal recessive developmental and epileptic encephalopathies

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Abstract

Aim Developmental and epileptic encephalopathies (DEEs) are a group of devastating disorders caused by epileptic activity, resulting in deterioration in developmental, cognitive, and motor functions. The number of genes identified as being responsible for DEEs has been increasing rapidly. However, despite a comprehensive molecular analysis, a molecular diagnosis can only be established in 50% of cases. The aim of this project is to use whole exome sequencing (WES) to determine the molecular etiology of DEEs in undiagnosed patients with a pedigree suggestive of an autosomal recessive single gene disease.

Methods Three DEE families, having either consanguineous parents of an affected individual and/or having more than one affected offspring, were enrolled in the project. Prior to this project, the families had been evaluated using a next-generation sequencing panel including 16 DEE genes in a previous study; however, no molecular diagnosis could be established. In five cases from the three selected DEEs families in our study, the genetic etiology was investigated using WES.

Results All patients in the study group had infantile onset epileptic seizures; however, semiologies varied. All patients presented with severe developmental delay. WES revealed biallelic disease causing mutations in *DENDD5A*, *GRN*, and *TBCD* genes in family 1, family 2, and family 3, respectively. In each family, the identified variants associated with the disease were segregated. Reverse phenotyping supported the molecular analysis.

Conclusion This study provided a valuable contribution to the genotype-phenotype relationship by determining rare epilepsy syndromes in undiagnosed patients previously. WES is a useful diagnostic alternative, particularly in consanguineous families.

Keywords Developmental and epileptic encephalopathies · Genetics · Infantile spasms · Mutation · Neonatal seizures

Introduction

Developmental and epileptic encephalopathies (DEEs) are a group of devastating disorders caused by epileptic activities, resulting in deterioration in developmental, cognitive, and motor functions [1, 2]. Early-onset epileptic encephalopathies appear during neonatal and infantile period. DEEs are a

heterogeneous group of disorders resulted by congenital or acquired cerebral damage. The number of genes which been identified as being responsible for DEEs has been increasing rapidly, and over 50 genes have been identified during the last 3 years [3–5]. The most common genetic anomalies causing DEEs are single nucleotide variations responsible for 30–40% of genetic etiology. Copy number variations are seen rarely and responsible for 5–10% of cases [5]. Molecular diagnosis of DEEs is not only important for confirming the clinical diagnosis but also for the selection of appropriate treatment options, giving an accurate prognosis as well as the provision of appropriate genetic counseling. There are several molecular analysis methods including gene panels, whole exome sequencing, or genome sequencing able to detect molecular pathology underlying DEEs. These methods have some advantages and disadvantages compared with each other. Hebbard et al. [5] had recommended selecting most appropriate test considering several factors such as age at seizure onset,

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severity of disease, other associated features, and patient insurance. However, despite a comprehensive molecular analysis, a molecular diagnosis can only be established in 20–40% of cases [2, 4, 6].

In a previous study, we evaluated the molecular etiology of early-onset DEEs using a targeted next-generation sequencing (NGS) panel [7]. This panel included 16 genes responsible for DEEs: *ARX*, *CDKL5*, *CNTNAP2*, *FOLR1*, *FOXG1*, *LAMC3*, *MBD5*, *MECP2*, *NTNG1*, *PCDH19*, *PNKP*, *SCN1A*, *SCN1B*, *SCN2A*, *STXBPI*, and *KCNQ2*. In that study group, a molecular diagnosis could only be made in 40% of cases. However, the diagnostic success rate was found to be higher in patients born to non-consanguineous parents (55.5%) than in patients born to consanguineous parents (16%). In consanguineous families, due to the panel containing mainly de novo and channel-encoding genes, a sufficient diagnostic success rate could not be achieved.

In this study, we aimed to evaluate the diagnostic utility of whole exome sequencing (WES) in previously molecularly undiagnosed DEE patients with a pedigree suggestive of an autosomal recessive single gene disease.

Material and methods

Study group

Five patients from 3 unrelated families having early-onset refractory seizures with global developmental delay and cognitive dysfunction were enrolled into the study. Prior to this project, we evaluated the molecular etiology of early-onset DEEs using a targeted NGS panel in 30 patients [7]. NGS panel which included 16 DEE genes (*ARX*, *CDKL5*, *CNTNAP2*, *FOLR1*, *FOXG1*, *LAMC3*, *MBD5*, *MECP2*, *NTNG1*, *PCDH19*, *PNKP*, *SCN1A*, *SCN1B*, *SCN2A*, *STXBPI*, *KCNQ2*) failed to identify a molecular etiology in 60% of these families. Among these patients who could not be molecularly diagnosed, patients whose pedigrees suggestive of an autosomal recessive inheritance (having either consanguineous parents and/or more than one affected sibling) were evaluated. Demographic data, family history, and laboratory and imaging test results were all obtained from hospital records. Clinical, laboratory, and electrophysiological findings of the patients were evaluated by experienced pediatric neurologist and geneticist, and three families with no specific clinical diagnosis were selected.

The study was approved by the Ethical Committee of the Ege University Medical Faculty (Date: March 9, 2015; number: 15-9/49) and financially supported by Ege University Scientific Research Projects Coordination (Grant Number 17-TIP-006). Samples from the patients were collected in accordance with the Helsinki Declarations. Written informed

consent for genetic testing was obtained from all cases or their parents/guardians.

Whole exome sequencing

Whole exome sequencing was performed on affected siblings in families 1 and 3 and affected offspring and healthy parents in family 2. Genomic DNA samples were extracted from 1 ml of peripheral blood leukocytes obtained from the parents and probands using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). DNA quality and quantity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Approximately 2 µg of high-quality genomic DNA from each sample was prepared as the starting material for generating the sequencing library using the SeqCap EZ Human Exome Library v3.0 (F. Hoffmann-La Roche Ltd., Basel, Switzerland), in accordance with the manufacturer's instructions.

SeqCap EZ Exome v3.0 Kit (F. Hoffmann-La Roche Ltd., Basel, Switzerland) was used for target enrichment. Paired-end sequencing was performed in all samples using the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA).

Burrows–Wheeler Alignment (BWA) tool was used for mapping to the reference genome (hg19) and alignment [8]. Variant calling was performed using SAMtools and GATK best practices pipeline [9, 10].

Data analysis

Sequencing data was analyzed using Illumina VariantStudio 3.0 software and Integrative Genomics Viewer (IGV). Variants with a frequency of less than 0.5% were selected based on NCBI dbSNP build141 (<http://www.ncbi.nlm.nih.gov/SNP/>), 1000 Genomes Project (<http://www.1000genomes.org/>), Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>), and Genome Aggregation Database (gnomAD) (<http://gnomad.broadinstitute.org/>). Any variants which had a read depth below 10X were excluded. The impact of the variants on the protein structure was identified using several in silico prediction tools such as MutationTaster, SIFT, REVEL, DANN, dbSNV, and CADD [11–16]. Conservation of residues across species was evaluated using PhyloP algorithm and GERP [17, 18]. Variant pathogenicity was classified in accordance with American College of Medical Genetics (ACMG) recommendations [19].

Confirmation and segregation analysis

The most likely disease-causing variants, identified by data analysis, were then confirmed using direct Sanger sequencing on ABI PRISM 3130 DNA analyzer (Applied Biosystems). Following this segregation analysis was performed.

Results

Study group

Family 1

In case 1, a 14-year-old boy was presented with a focal seizure at the age of 2.5 months. He was born at the 39th week of gestation via spontaneous vaginal delivery following an uneventful pregnancy. He had intellectual disability and motor retardation. There was no consanguinity between the parents; however, both were coming from the same small village.

On physical examination, weight, height, and head circumference were measured as 32 kg (< 3 p, - 2.9 SD), 141 cm (< 3 p, - 3.1 SD), and 48.5 cm (< 3 p, - 4.9 SD), respectively. Intellectual disability was considered severe. Poor eye contact and repetitive stereotypic movements were observed. He also had microcephaly, wide nasal tip, short philtrum, open mouth, thick and everted lips, and spasticity on lower extremities (Fig. 1).

His biochemical and metabolic screening tests and hormonal profile, hearing, and ophthalmologic examination were all normal. Cranial MRI revealed periventricular nodular heterotopia, and EEG findings were compatible with multifocal epileptic activity.

In case 2, a 9-year-old boy is the second affected offspring of this family. Firstly, he displayed a generalized tonic-clonic seizure at the age of 3 months. He displayed refractory focal and generalized motor seizures in spite of antiepileptic treatment. In conjunction with his older sibling, intellectual disability, stereotypic movements, microcephaly, and dysmorphic facial features were also present.

Family 2

In case 3, a 10-year-old girl had a normal neurocognitive development until the age of 7 months. Loss of cognitive and motor functions was observed following this period. At the age of 12 months, she had a seizure in the form of infantile spasm for the first time. These seizures repeated 10–12 times a day and were resistant to antiepileptic drugs. She was born to consanguineous parents at the 38th week via cesarean section. Family history revealed that an older sister with neurodegeneration, severe developmental delay, and refractory epilepsy died due to pneumonia at the age of 7 years. She was a 10-year-old girl, and her parents described that she had normal development until 7 months of age. She exhibited severe loss of cognitive and motor functions within time. At the age of 12 months, she presented with epileptic spasms refractory to multiple antiepileptic drugs.

On physical examination, weight, height, and head circumference were 41 kg (10–25 p, 0.87 SD), 120 cm (< 3 p, - 3.07

SD), and 56.5 cm (> 97 p, 2.26 SD), respectively. She had severe developmental delay. Generalized hypotonia, macrocephaly, proptosis, and strabismus were observed (Fig. 1). Deep tendon reflexes were hypoactive.

Biochemical and metabolic screening tests were found to be normal. A cranial MRI revealed severe diffuse cerebral, cerebellar, and brain stem atrophy.

Family 3

In case 4, an 18-year-old boy was presented with focal motor seizures at the age of 12 months. Despite multidrug regimen, his seizures had not been fully controlled. He was born at the 39th week of gestation via a spontaneous vaginal delivery. There was no consanguinity between the parents; however, both were coming from the same small village.

On physical examination, weight, height, and head circumference were 47 kg (10–25 p, - 1.08 SD), 156 cm (10–25 p, - 0.81 SD), and 53 cm (- 1.72 SD), respectively. He had poor eye contact, and he was not able to speak. Severe global developmental delay was noted. Deep tendon reflexes were diminished.

His biochemical and metabolic screening tests and hormonal profile, auditory, and ophthalmologic examinations were all normal. EMG revealed sensorimotor axonal polyneuropathy. Cranial MRI demonstrated bilateral periventricular leukomalacia and thin corpus callosum.

In case 5, the second affected child of family 3 had refractory epileptic seizures since 7-months-old. She had also severe developmental delay, periventricular leukomalacia, and sensorimotor axonal polyneuropathy.

Clinical features, biochemical analysis, and electrophysiological and imaging test results of all patients are given in Table 1.

WES and segregation analysis

Sequencing depth for each sample was more than 95.16% of target regions having at least a 10-fold coverage. Detailed coverage metric values for each sample within the target region are given in Table 2.

Family-based variant filtering was performed (two affected siblings in families 1 and 3, proband and parents in family 2). After selection variants with a minor allele frequency of < 0.5% in public databases, a read depth > 10X, and having a predicted deleterious effect (missense, nonsense, frameshift, and splice site variants), 47, 140, and 152 variants remained in families 1, 2, and 3, respectively. To evaluate their putative pathogenic impact, a series of prediction tools were used and a comprehensive literature search was performed for each variant.

In family 1, a homozygous c.110-3T>G variant in *DENND5A* gene was identified in both affected siblings.

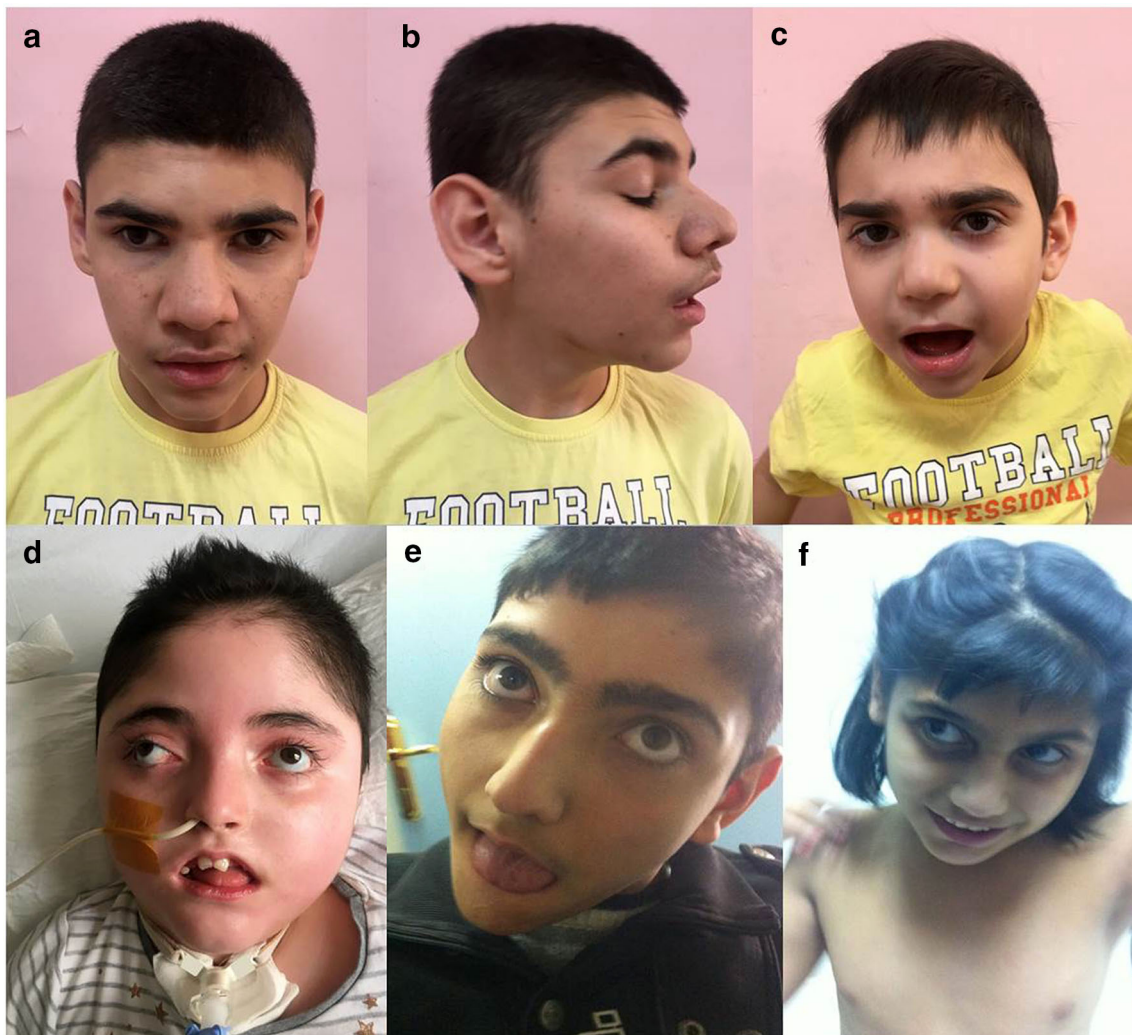


Fig. 1 Dysmorphic facial features of the study group: case 1 (a–b), case 2 (c), case 3 (d), case 4 (e), and case 5 (f)

This variant had not been reported in any database to date. It was predicted as being deleterious based on its DANN, GERP, and dbSNV (v1.1) scores. The variant classified as “variant of unknown significance (VUS)” in accordance with ACMG recommendations. Via segregation analysis, both parents were found to be heterozygous carriers. The mutation analysis of a healthy brother was negative.

In family 2, a homozygous missense variant c.480G>A (p.Asp144Asn) was detected in *GRN* gene. This variant (rs200591137) had been reported in gnomAD exomes database with a minor allele frequency of 1/125.729. It was considered to be deleterious based on prediction tools and classified as “VUS” in accordance with ACMG recommendations. Both parents were found to be heterozygous carriers for the same variant.

In family 3, two heterozygous variants in *TBCD* gene were identified in both affected siblings. The variant c.202C>T (p.Gln68Ter) had not previously been reported in the databases and was classified as “pathogenic.” The second variant

c.880C>T (p.Arg294Trp) (rs200591137) had been reported in gnomAD genomes and gnomAD exomes databases with a minor allele frequency of 1/31.182 and 1/123.543, respectively. It was classified as “VUS” in accordance with ACMG recommendations. The amino acid Arg294 is highly conserved across species, and additionally, a different missense variant affecting the same amino acid has been registered on ClinVar as “likely pathogenic.” Segregation analysis revealed that the mother was a heterozygous carrier for c.202C>T variant, with the father a heterozygous carrier for c.880C>T variant.

Confirmation and segregation analysis results of all families are given in Fig. 2.

Reverse phenotyping

Following WES results, some additional tests were performed in families to evaluate genotype-phenotype correlation. In family 1, a cranial computed tomography was performed to

Table 1 Clinical features and neurophysiological and imaging test findings of the study group

Family no.	Case no.	Sex	Age (year)	The onset age of seizures (month)	Seizure semiology	DD/ID	Head circumference (SDS)	EEG	Cranial MRI	Others
1	1	M	14	2.5	Focal motor	Severe	−4.96	Focal epileptic activity	Mega cisterna magna	Severe language delay, autistic behavior, and stereotypic movements
	2	M	9	3	Infantile spasms, focal/generalized motor seizures	Severe	−4.2	Multifocal epileptic activity	Periventricular nodular heterotopia	Severe language delay, autistic behavior, and stereotypic movements
2	3	F	10	12	Infantile spasms	Severe	2.26	Hypsarrhythmia/multifocal epileptic activity	Cerebral and cerebellar atrophy	Hypotonia
3	4	M	18	12	Focal motor	Severe	−1.72	Multifocal epileptic activity	Periventricular leukomalacia and thin corpus callosum	Spasticity, sensorimotor axonal polyneuropathy
	5	F	13	7	Focal motor	Severe	−0.9	Focal epileptic activity	Periventricular leukomalacia and thin corpus callosum	Spasticity, sensorimotor axonal polyneuropathy

investigate intracranial calcifications reported in biallelic *DENND5A* mutation carriers. Intracranial calcifications on deep white matter, basal ganglia, and thalamus were detected in both siblings. In family 2, a detailed family history revealed that the grandmother of the father showed clinical features of dementia and cognitive impairment at the age of 60 years. On peripheral blood smear using light microscopy, vacuolated lymphocytes were observed (Fig. 3). However, further electron microscopic evaluation for lipopigment accumulation or

any biochemical tests to determine blood progranulin dosage were not available at the time of study. An ophthalmological examination revealed no signs for retinal degeneration. In family 3, a cranial MRI was considered necessary for the confirmation of cerebral atrophy. However, due to adverse behavioral characteristics of both siblings, it was an impossibility.

Discussion

In this study, the diagnostic success of WES in three DEE families with pedigrees suggestive of autosomal recessive single gene disorders was evaluated. Prior to WES, the families had been analyzed using a next-generation sequencing panel which included 16 known DEE genes; unfortunately, no molecular diagnosis could be established [7]. WES was able to provide a diagnosis of rare Mendelian disorders in each family.

DENND5A, located at 11p15.4, plays a role in the regulation of membrane traffic between Golgi and endosomal complex. In 2016, Han et al. showed that biallelic *DENND5A* mutations were responsible for DEEs [20]. They reported

Table 2 Whole exome sequencing coverage metric values for each sample within the target region

Patient no.	1x (%)	10x (%)	20x (%)	30x (%)	Mean coverage
1	99.61	95.77	87.62	73.87	55.9X
2	99.86	97.33	92.66	83.57	65.5X
3	99.75	95.16	83.2	64.86	46.2X
4	99.89	97.48	93.05	84.59	70.7X
5	99.77	97.82	95.01	89.94	81.3X
6	99.72	97.26	93.33	85.79	73.8X
7	99.9	97.72	94.03	87.1	78X

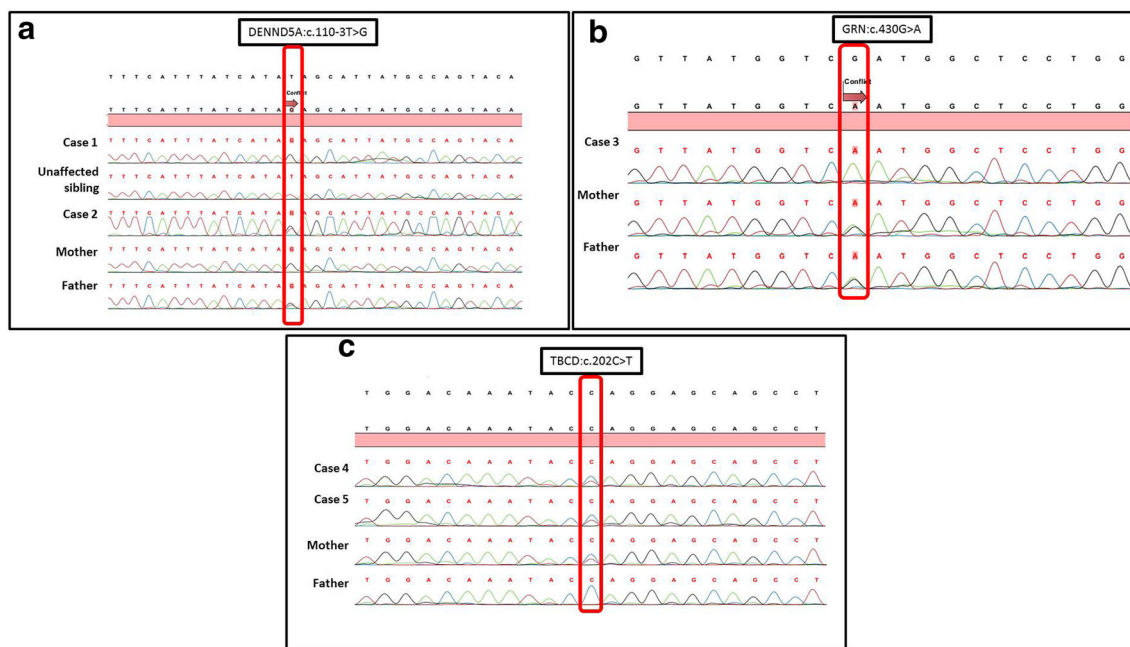


Fig. 2 Sequencing electropherograms of the mutations identified in family 1 (a), family 2 (b), and family 3 (c)

two different homozygous null variants in three epileptic encephalopathy cases from two unrelated families. Dysmorphic facial features, microcephaly, and global growth retardation were described in all three cases. Seizures started in the newborn period, with tonic, myoclonic, or generalized tonic-clonic seizures all been observed. Intracranial calcifications had been noted in all three cases. They also showed the loss of function of *DENND5A* alternating neurite and dendrite outgrowth during neuronal differentiation via functional studies. In 2017, Anazi et al. also reported homozygous variants in the *DENND5A* gene in 2 different cases presenting with epilepsy, global developmental delay, and microcephaly [21]. Detailed clinical features of biallelic *DENND5A* mutations are given in Table 3. In our study, we identified a novel splice site *DENND5A* variant in family 1. The clinical findings of both siblings were consistent with *DENND5A*-related epileptic

encephalopathy. As reported in the literature, seizures are early onset and have been observed in different semiologies. Dysmorphic facial features of our patients (triangular face, thick eyebrows, wide nasal tip, prominent nostrils, short and deep philtrum, open mouth, and thick lower and upper vermillion) have been found to be similar to the patients described in the literature.

GRN gene is located at 17q21.31 and encodes the progranulin glycoprotein. It is known that monoallelic loss of function *GRN* mutations are responsible for frontotemporal lobar degeneration (FTLD) [22]. FTLD is the most common cause of dementia after Alzheimer's disease. In 2012, Smith et al. described a homozygous mutation in the *GRN* gene in two siblings presenting with adult onset progressive vision loss, retinal dystrophy, recurrent seizures, ataxia, and cerebellar atrophy [23]. Supporting molecular findings, they also

Fig. 3 Peripheral blood smear of case 3 revealing vacuolated lymphocytes

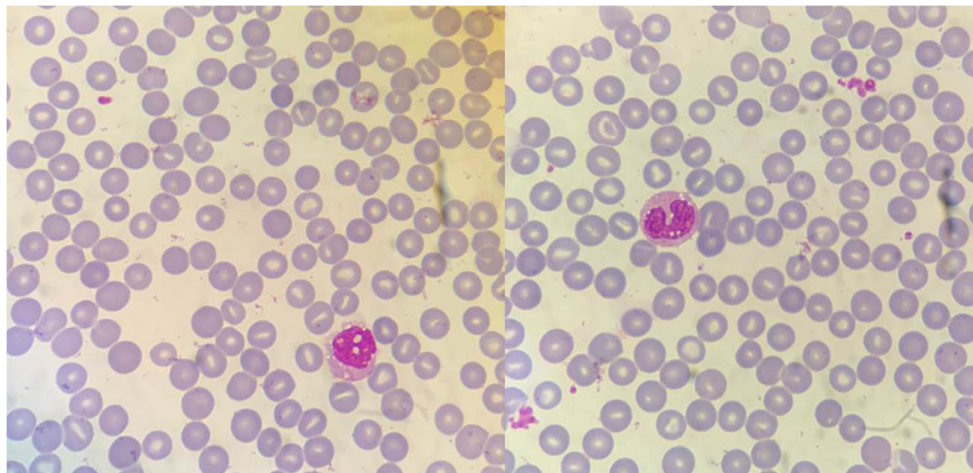


Table 3 Clinical features and molecular findings of the cases carrying biallelic *DENND5A* mutations

Publication	Han, 2016		Anazi, 2017		Present study
Gender	Female	Female	Female	Female	Male
Dysmorphic features	Microcephaly Coarse face Frontal bossing Broad eyebrows Long eyelashes Large ears Prominent nose Large nostrils Open mouth Tented upper vermilion Thick everted lower lip Short philtrum	Microcephaly Coarse face Frontal bossing Broad eyebrows Long eyelashes Large ears Prominent nose Large nostrils Open mouth Tented upper vermilion Thick everted lower lip Short philtrum	Microcephaly Hypotelorism Full cheeks	Microcephaly Hirsutism	Microcephaly Broad eyebrows Long eyelashes Large ears Prominent nose Large nostrils Open mouth Tented upper vermilion Thick everted lower lip Short philtrum
Developmental delay	Severe	Severe	Severe	Severe	Severe
The onset of seizure	Neonatal period	?	Neonatal period	Neonatal period	3 months
Seizure semiology	Tonic, myoclonic, generalized tonic-clonic	Startle, tonic, myoclonic, generalized tonic-clonic	Tonic-clonic	Focal, generalized	Infantile spasm, focal/generalized motor
Neurological findings	Spastic tetraplegia	?	Axial hypotonia Peripheral hypertonia Hyperreflexia	Axial hypotonia Peripheral hypertonia Hyperreflexia Clonus Positive Babinski reflex	Spasticity of lower extremities autistic behavior stereotypic movements Severe language disorder
Cranial MRI	Dandy-Walker malformation Corpus callosum dysgenesis Arachnoid cyst Hypoplastic vermis Diffuse hydrocephaly Basal ganglion calcification	Ventriculomegaly Corpus callosum dysgenesis	Hydrocephaly Septum pellucidum agenesis Holoprosencephaly	Thin corpus callosum Incomplete hippocampal inversion Mega cisterna magna	Thin corpus callosum Nodular heterotopia
Additional findings	Basal ganglion calcification	Basal ganglion calcification	Periventricular white matter calcification	Bilateral optic atrophy	Intracranial calcifications on deep white matter, basal ganglions, and thalamus
<i>DENND5A</i> gene mutations	c.[517_518delGA]; [517_518delGA] p.[Asp173Profs*8]; [Asp173Profs*8]	c.[517_518delGA]; [517_518delGA] p.[Asp173Profs*8]; [Asp173Profs*8]	c.[3811del]; [3811del] p.[Gln1271Argfs*67]; [Gln1271Argfs*67]	c.[1622A4G]; [1622A4G] p.[Asp541Gly]; [Asp541Gly]	c.[110-3T>G]; [110-3T>G]

Table 4 Clinical features and molecular findings of the cases carrying biallelic *GRN* mutations

Publication	Smith, 2012	Almeida, 2016	Kamate, 2019	Present study
Gender	Male	Female	Female	Female
The onset of the symptoms	Adult onset	Adult onset	Juvenile onset	Infantile onset
Ophthalmological findings	Vision loss, recurrent episodes of optic disc, recurrent episodes of palinopsia	Vision loss, retinal dystrophy	Normal	Strabismus
The onset of the seizures	25 years	–	13 years	1 year
Seizure semiology	Generalized tonic-clonic, myoclonic	–	Generalized clonic	Infantile spasm
Neurological findings	Mild ataxia	Cerebellar ataxia with asynergia, dysarthria	Mild intellectual disability, poor short-term memory	Progressive loss of acquired developmental milestones, hypotonia, spasticity, decreased DTR
EEG	8 Hz background activity and rare paroxysms of slow waves, often preceded by small spikes	Normal	Generalized epileptic activity with normal background activity	Hypsarhythmia/multifocal epileptic activity
Cranial MRI	Severe cerebellar atrophy	Severe global cerebellar atrophy	Diffuse vermian and cerebellar atrophy	Severe bilateral cerebral, cerebellar, and brain stem atrophy
Other	Numerous fingerprint profiles in membrane-bound structures in eccrine secretory cells and in endothelium	Fingerprint profiles on peripheral blood leukocytes	–	–
<i>GRN</i> gene mutations	c.[813_816del]; [813_816del] p.[Thr272SerfsTer10]; [Thr272SerfsTer10]	c.[813_816del]; [813_816del] p.[Thr272SerfsTer10]; [Thr272SerfsTer10]	c.[912G>A]; [912G>A] p.[Trp304Ter]; [Ser301CysfsTer61] [Trp304Ter]	c.[430G>A]; [430G>A] p.[Asp144Asn]; [Asp144Asn]

showed that plasma progranulin levels were low in heterozygous carriers and undetectable in homozygous in related family. The authors considered that biallelic *GRN* mutations were responsible for neuronal ceroid lipofuscinosis (NCL) type 11. In a further study conducted by the same group, fingerprint storage and the absence of progranulin protein were observed in the skin biopsy and peripheral blood leukocytes of those two siblings [24]. Following this, three further NCL cases carrying homozygous *GRN* mutations were reported (Table 4) [25, 26].

Neuronal ceroid lipofuscinosis is clinically and genetically heterogeneous neurodegenerative disease [27, 28]. To date, 14 different types have been identified. The disease is characterized by progressive loss of cognitive and motor functions, retinal degeneration, cerebellar atrophy, and seizures. It is classified as infantile, late-infantile, juvenile, or adult onset based on the age of onset of clinical features. The clinical features of the patient in family 2 including infantile onset progressive neurodegeneration, epilepsy, and cerebellar atrophy were considered to be compatible with NCL. Vacuolated lymphocytes shown in peripheral blood smear of case 3 supported the diagnosis. To our knowledge, our patient carrying biallelic *GRN* gene variant is the first described infantile onset NCL type 11 case in the literature. However, while visual loss and retinal degeneration are major clinical features of NCL type 11 in adults, no retinal involvement was observed in our patient or her sister during their clinical follow-up. Kamate et al. reported two siblings whose symptoms appeared at the age of 8 and 13, respectively [26]. No retinal involvement was observed in their study either. Retinal degeneration may be specific to NCL type 11 in adulthood.

Biallelic *TBCD* gene variants are responsible for early-onset and progressive encephalopathy [29]. To date, approximately 34 cases have been described in the literature [29–33]. The disease is characterized by progressive encephalopathy which usually begins during infantile period. Epilepsy has been described in approximately 90% of cases, and seizure types vary [33]. The clinical findings of two affected siblings in family 3 were considered to be compatible with the clinical picture caused by biallelic *TBCD* gene mutations.

The majority of DEEs are resulted by de novo variants in responsible genes [4]. However, in a recent study conducted by Papuc et al. [34], autosomal recessive inheritance was found to be responsible for 38% of molecularly diagnosed cases. In a highly consanguineous study group, Nashabat et al. [35] also identified autosomal recessive etiology in 50% of 72 molecularly characterized early infantile epileptic encephalopathy cases. In our previous study, a targeted gene panel failed to succeed a high molecular diagnostic rate in consanguineous families. In three of these undiagnosed families, WES revealed molecular diagnosis of rare autosomal recessive genetic disorders. WES can be considered as a first

step diagnostic test in the DEE families with consanguineous marriage and/or two or more affected siblings.

The limitation of the present study is that we could not perform functional studies to determine the disease-causing effects of the identified variants in the patients. Therefore, the molecular diagnosis could only be supported by segregation analysis and reverse phenotyping.

To conclude, in this study, three rare syndromes associated with epilepsy were identified, contributing to genotype-phenotype relationship. It was thought that WES might provide a more appropriate diagnostic method in DEE cases with suspected autosomal recessive inheritance than a gene targeted panel.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Ethical Committee of the Ege University Medical Faculty (Date: March 9, 2015; number: 15-9/49). Written consent was obtained from all subjects.

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