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Novel Insights from Clinical Practice

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Two New Cases of Primary Microcephaly with Neuronal Migration Defect Caused by Truncating Mutations in the *ASPM* Gene

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Established Facts

- Autosomal recessive primary microcephaly is a rare disorder due to congenital deficiency in the development of the cerebral cortex, characterized by a head circumference below 2 SD.
- Neuronal migration defects are very rare findings in patients with *ASPM* gene mutations.
- Until now, polymicrogyria has been found in 4 cases with *ASPM* gene mutations and pachygyria in 2 cases as reported in the relevant literature.

Novel Insights

- Brain MRI of patient 1 showed polymicrogyria located in the right frontotemporal region, and patient 2 had pachygyria.
- Patient 1 with polymicrogyria is the fifth case and patient 2 with pachygyria is the third case described in the literature.

Keywords
ASPM · Polymicrogyria · Pachygyria · Whole-exome sequencing · Novel variant

Abstract
Autosomal recessive primary microcephaly (MCPH) is a uncommon disorder due to congenital deficiency in the development of the cerebral cortex, characterized by a head circumference below 2 SD. MCPH is a group of diseases with genetic heterogeneity and has been reported by the Online Mendelian Inheritance In Man® (OMIM) database and associated with 25 different genes. It is known that MCPH cases are most frequently associated with abnormal spindle-like, microcephaly-associated (*ASPM*) gene mutations. The ASPM protein consists of an N-terminal 81 IQ (isoleucine-glutamine) domain, a calponin-homology domain, and a C-terminal domain. It interacts with calmodulin and calmodulin-related proteins via the IQ domain and acts as a part in mitotic spindle function. The basic characteristics of cases with *ASPM* gene mutations are microcephaly (below −3 SD) present before 1 year of age, intellectual disability, and the absence of other congenital anomalies. Macroscopic organization of the brain is preserved in cases with *ASPM* mutation, and a decrease in brain volume, particularly gray matter volume loss and a simplified gyral pattern are observed. Cortical

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migration defects are a very rare finding in patients with *ASPM* mutations. In the present study, we aimed to discuss the clinical and genetic findings in 2 cases with cortical dysplasia in which truncated variants in the *ASPM* gene were detected, particularly in terms of genotype-phenotype correlation in comparison with the literature.

Introduction

Microcephaly is defined as a head circumference below 2 SD in the ethnic, age, and sex compatible population. Intellectual disability and seizures may be present in cases of microcephaly, depending on the influence on brain development. It is basically divided into primary and secondary microcephaly. Primary microcephaly, a prenatal developmental neurogenic disease, has an intrauterine onset and is detected at birth. Secondary microcephaly is a type of microcephaly in which the head circumference is normal at birth, and syndromic findings may develope due to progressive neurodegenerative disease [Naveed et al., 2018]. Autosomal recessive primary microcephaly (MCPH) is a rare disease occurring due to congenital deficiency in the development of the cerebral cortex, characterized by a head circumference below 2 SD [Mochida and Walsh, 2001]. Although the frequency of MCPH varies between populations, it occurs as 1/30,000–1/250,000 live births [Van Den Bosch, 1959; Zaqout et al., 2017]. It is more common in Asian and Arab societies where consanguineous marriage is a common practice [Zhou et al., 2013]. MCPH is a genetically heterogeneous group of diseases that has been reported in the OMIM database (http://omim.org/) associated with 25 different genes. The gene responsible for MCPH in the MCPH5 locus was the *ASPM* gene. It is known that MCPH cases are most frequently associated with *ASPM* gene mutations. This is frequently followed by mutations in the *WDR62* and *MCPH1* genes [Woods et al., 2005; Sajid Hussain et al., 2013; Jayaraman et al., 2018; Naveed et al., 2018]. The fact that no pathogenic variation in 25 different OMIM genes known to be associated with the disease was detected in some families with MCPH suggests that there may be genes that are not yet identified related to the phenotype [Mahmood et al., 2011].

ASPM , located in chromosome 1q31.3, is composed of 28 exons and encodes 3,477 amino acids [Saunders et al., 1997; Ponting 2006]. During neurogenesis, the ASPM protein is expressed in different parts of the cerebral cortex and creates transcripts of different sizes that are thought to have different functions according to the size of the IQ (isoleucine-glutamine) domain present in it. The N-terminal region and C-terminal region of the ASPM protein are located in the spindle poles of mitotic cells and the midbody, respectively. Proliferative symmetrical cell divisions have been observed to be decreased in neocortex cells that develop in *Aspm* knockdown mouse models [Fish et al., 2006]. It has been shown that the ASPM protein forms a complex with ATPase katanin and regulates the mitosis process in the progenitor cells of the nervous system [Jiang et al., 2017]. In addition, the presence of *ASPM* overexpression in brain tumors has been demonstrated; it has been suggested that it might be associated with malignant progression and could be targeted therapeutically [Bikeye et al., 2010].

In the present study, we aimed to discuss the clinical and genetic findings, particularly in terms of genotype-phenotype correlation, in 2 cases with cortical dysplasia in which truncated variants in the *ASPM* gene were detected in comparison with the available literature.

Materials and Methods

To investigate the molecular etiology of the diagnosis of primary microcephaly, genomic DNA was isolated from peripheral blood of the patients using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. All the coding regions in the human genome of the patient were sequenced using whole-exome sequencing analysis via the Illumina NovaSeq Platform using the Agilent SureSelect V5 kit (Agilent, Santa Clara, CA, USA). Raw data were analyzed using the Sophia DDM® data analysis platform. The following filtering steps were applied to detect the pathogenic variants responsible for the clinical features of the case: (1) all missense, nonsense, frameshift, splice site, indel, in-frame and synonymous variants, and (2) variants with minor allele frequency <1.0% in population studies [1000 Genome (1000G), ESP, ExAC, and Genome Aggregation Database (gnomAD)]. Integrative Genome Viewer was used for sequence data visualization. The novel variant was checked in HGMD® and ClinVar (http://ncbi.nlm.nih.gov/clinvar) databases. The pathogenicity of the novel variants was interpreted using in silico analysis tools [Mutation Taster, CADD (Combined Annotation Dependent Depletion)], probability of being loss-of-function intolerant (pLI) score, and American College of Medical Genetics and Genomics (ACMG) criteria [Richards et al., 2015].

Case Reports and Results

Two patients from 2 different families with a head circumference below 2 SD in the first 6 months after birth were included in the study. Both cases were evaluated

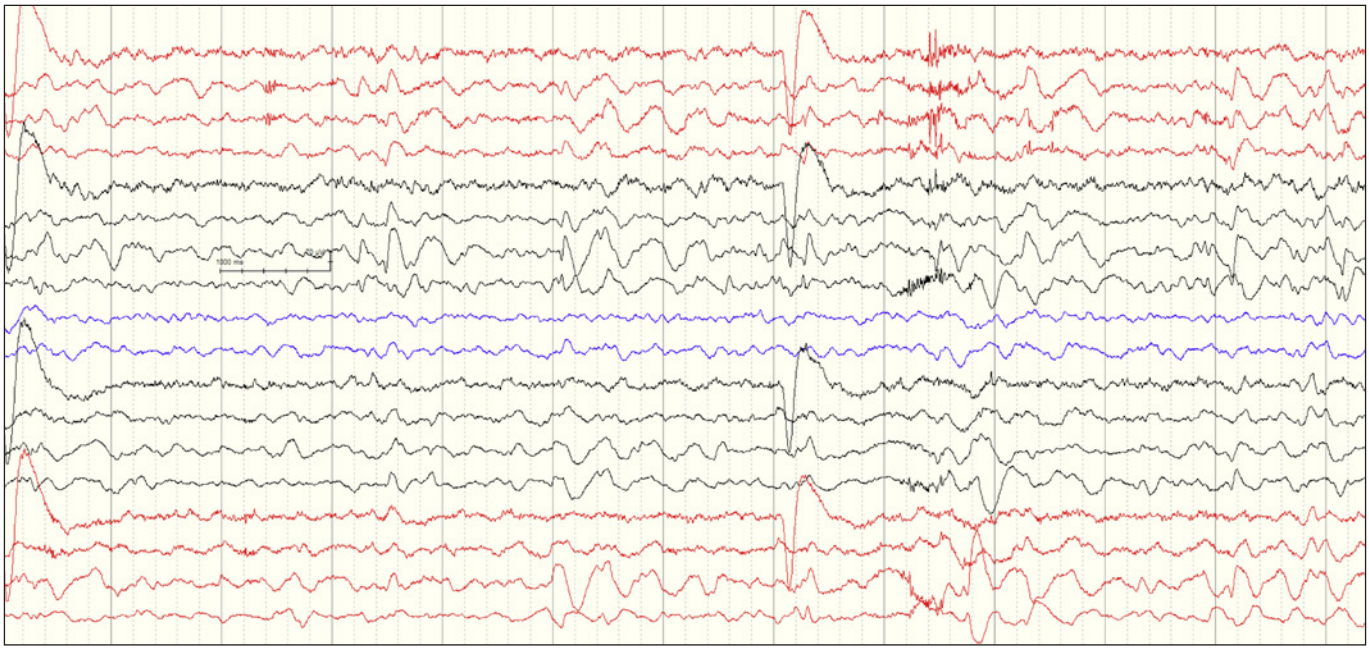

Fig. 1 Interictal EEG on patient 1 showing high amplitute slow wave spikes emerging predominantly from centroparietal regions in the left hemisphere and normal background rhythm (Sensitivity: 10 μV/mm; Bandpass 0.5–70 Hz).

with anthropometric and family history evaluation, dysmorphology examination, hearing and visual examination, echocardiography, EEG, and brain MRI.

Family 1

Patient 1, a 9-year-old female, is the first child of healthy parents who are first cousins. The patient, without any anomaly in prenatal history, was born by normal spontaneous vaginal delivery, with a birth weight of 2,500 g. The head circumference was measured as 32 cm at birth. The patient gained head control at 2 months, sat without support at 10 months, and started walking at 18 months of age. The head circumference of the patient was 43 cm (−6.07 SD) at the age of 8, and during her examination at age 9, the height, body weight, and head circumference measurements were 123 cm (−1.57 SD), 25 kg (−0.83 SD), and 43.5 cm (−6.01 SD), respectively. Her dysmorphology examination result revealed a narrow and sloping forehead. She started having secondarily generalized seizures at the age of 7, which stopped at the age of 8 with carbamazepin and valproate therapy. Each EEG revealed focal epileptiform discharges located in the left hemisphere (Fig. 1). She had mild-to-moderate intellectual disability. She could only speak 2 words: "mother" and "father."

Family 2

Patient 2, a 10-year-old male, is the first child of healthy parents who are second cousins. Microcephaly was discovered prenatally at week 32 of gestation. He was born by normal spontaneous vaginal delivery, with a birth weight of 2,900 g. It was reported that the patient, whose head circumference was 31 cm at birth, gained head control at 3 months, sat without support at 11 months, and started walking at 20 months of age. During the examination at age 10, his height, body weight, and head circumference were 131 cm (−1.12 SD), 31 kg (−0.21 SD), and 45 cm (−5.88 SD), respectively. His dysmorphology examination revealed synophrys and a narrow, sloping forehead. He started having focal motor seizures at the age of 5 which stopped at the age of 7 under valproate therapy. His EEG revealed focal epileptiform discharges located in the left hemisphere. He had severe intellectual disability.

The results of both the patients' ophthalmologic and audiological examinations, as well as metabolic screening tests and their karyotype analyses were all normal.

The craniofacial and neurological features of the patients are summarized in Table 1.

The radiological findings of the patients are summarized in Table 1 and illustrated in Figure 2. Patient 1 had symmetrical mild ventriculomegaly, simplified gyral pat-

Table 1. Clinical and genetic findings of the previously reported and present cases with polymicrogyria and pachygyria

	Patient 1	Patient 2	Passemard et al. [2009]	Ariani et al. [2012]	Hu et al. [2014]	Nakamura et al. [2015]	Letard et al. [2018]	Letard et al. [2018]
Sex	F	M	M	M	NA	M	M	M
Age, years	9	10	20	29	23	8	4	7
HC at birth, cm	32	31	NA	NA	NA	29.5	NA	NA
HC at examination age, SD	−6.01	−5.88	−5	−7.8	NA	−7.6	−10.8	−5.6
Intellecual disability	Moderate	Severe	Mild	Moderate-severe	Moderate	Severe	NA	NA
Age of epilepsy onset, years	7	5	14	–	4	4	–	6
Type of epilepsy	Secondary generalized tonic-clonic seizures	Focal motor epilepsy	Generalized tonic-clonic seizures	–	Focal and generalized epilepsy	Tonic seizures	–	–
Additional neurological findings	–	–	–	–	Fine motor problems and required surgery for strabismus	Increased muscle tone and mild spasticity in the arms and severe spasticity in the legs	Spastic tetraplegia	–
Dysmorphic features	Narrow, sloping forehead	Synophrys Narrow, sloping forehead	Narrow bitemporal distance, sloping forehead, oxycephaly	Sloping forehead, long face, thick lips	NA	–	NA	NA
MRI findings	Symmetrical ventriculomegaly, thinning of the corpus callosum, simplified gyral pattern located in the left frontotemporal region, and polymicrogyria located in the right frontotemporal region	Symmetrical ventriculomegaly, simplified gyral pattern located in both hemispheres and pachygyria	Extensive unilateral perisylvian polymicrogyria from the frontal pole to the occipital pole; contralateral simplified frontal and occipital gyral pattern	Global reduction in brain size, thin brain stem, normal corpus callosum,temporal pachygyria	Reduced gyration, parietal polymicrogyria and a myelination defect	Frontal-dominant pachygyria with enlarged lateral ventricles and a slightly thickened corpus callosum	Thick frontal gyri, gyral simplification, thick corpus callosum, extensive bilateral posterior polymicrogyria	Gyral simplification, polymicrogyria in frontoinsular region
ASPM mutation	Homozygous c.5219_5225delGAGGATA (p.Arg1740Thrfs*7)	Homozygous c.7792C>T (p.Gln2598*)	Homozygous c.9507delG (p.Ile3170Leufs*9)	Compound heterozygous c.3796 G>T (p.Glu1266*); c.7815_7816del (p.Glu2605Aspfs*31)	Compound heterozygous c.8227C> T (p. Arg2743*); c.7772_7775delAAAA (p.Lys2591Argfs*24)	Compound heterozygous c.3055C>T (p.Arg1019*); c.6750delT (p.Phe2250Leufs*10)	Homozygous c.8702delA (p.His2901Leufs*37)	Homozygous c.7744delA (p.Ile2582Serfs*34)
Mutation location	Exon 18	Exon 18	Exon 23	Exon 16 and 18	Exon 18 and 18	Exon 11 and 18	Exon 18	Exon 18

MRI, magnetic resonance imaging; HC, head circumference; NA, not available; SD, standard deviation.

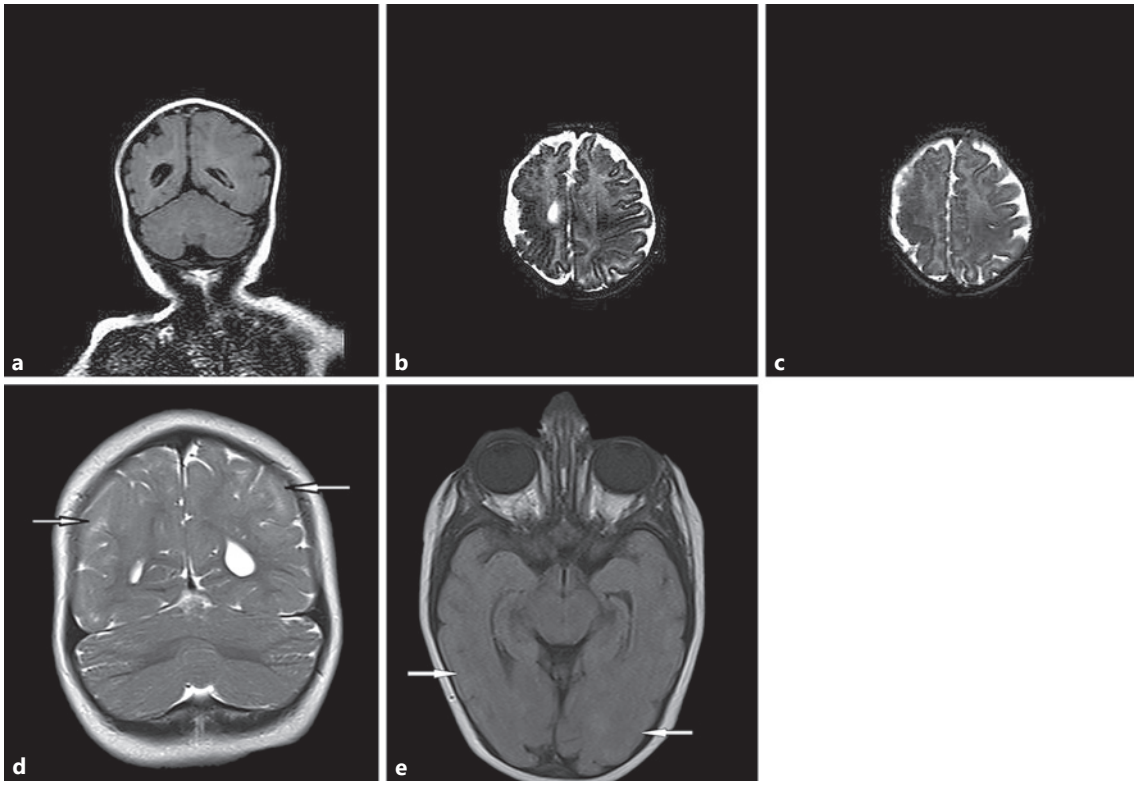

Fig. 2. Radiological findings. Patient 1: **a** Symmetrical mild ventriculomegaly, simplified gyral pattern, and cortical dysplasia located in the right temporaparietal lobe on T2 FLAIR coronal image. **b**, **c** T2-weighted axial images of brain. Patient 2: **d** Pachgyria pattern of cortical thickness on coronal T2-weighted image. **e** Axial FLAIR image.

tern, and cortical dysplasia located in the right temporoparietal lobe on T2 FLAIR coronal image and T2-weighted axial images of brain (Fig. 2a–c). Patient 2 had pachgyria patern of cortical thickness on coronal T2 weighted (Fig. 2d) and axial FLAIR images (Fig. 2e).

In whole-exome sequencing analysis, patient 1 was found to have a homozygous frameshift variant (NM_018136: c.5219_5225delGAGGATA, p.Arg1740Thrfs*7) in exon 18 of *ASPM* . Patient 2 had a novel homozygous nonsense variant (NM_018136: c.7792C>T, p. Gln2598*) in exon 18 of *ASPM* (Fig. 3). Due to the restrictions imposed because of the Covid-19 outbreak, segregation analysis could not be performed in the parents and other family members.

Discussion

The ASPM protein consists of an N-terminal 81 IQ (isoleucine-glutamine) domain, a calponin-homology domain, and a C-terminal domain. It interacts with calmodulin and calmodulin-related proteins through the IQ domain and plays a role in mitotic spindle function. Together, these observations suggest that mutations in the human *ASPM* gene may cause microcephaly due to the dysregulation of mitotic spindle activity in neuronal progenitor cells [Naveed et al., 2018]. Since the ASPM protein has a role in centriol duplication, it has been stated that primary microcephaly is a "centriolopathy" [Jayaraman et al., 2016]. However, *ASPM* functions are still not clearly known. Till now, 230 different variants (98 nonsense/missense, 20 splice site, 90 small and 2 gross deletions, 17 small insertions, 2 small indels, and 1 complex rearrangement) have been reported in the *ASPM* gene registered in Human Gene Mutation Database® (HGMD®) Professional (https://portal.biobase-international.com/hgmd/pro/all.php).

Verloes et al. [2020] described the clinical features of *ASPM*-associated MCPH phenotype in patients [Verloes et al., 2020]. These criteria are (i) microcephaly (below −3 SD) present at birth and present before the age of 1 year, and (ii) no other congenital anomalies [Nicholas et al.,

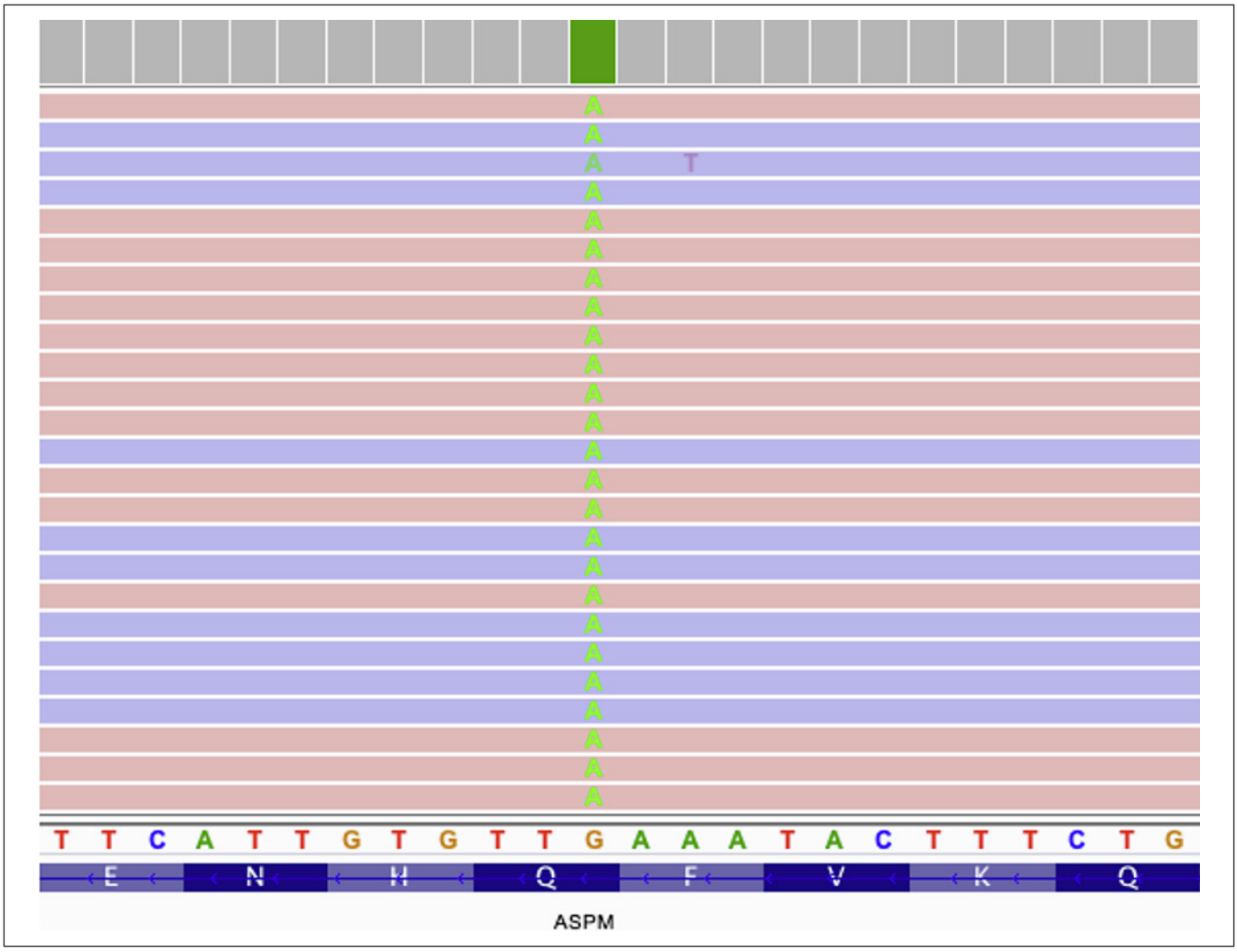

Fig. 3. A novel homozygous nonsense variant (NM_018136: c.7792C>T, p.Gln2598*) in exon 18 of the *ASPM* gene detected in patient 2.

2009]. Létard et al. [2018] classified the structural brain anomalies seen in the *ASPM*-associated MCPH phenotype. According to that study, the most common *ASPM* gene-related MCPH phenotype was characterized by gyral simplification (90%), corpus callosum abnormalities (shape and size anomalies, 52%), and mild-to-moderate cerebellar and/or pontine hypoplasia (10%). Cortical migration defect was found in a few cases [McSherry et al., 2018].

An overview of our patients and patients with cortical dysplasia as reported in the literature suggests that the locations of migration anomalies vary. The simplified gyral pattern specified in other studies is observed in both the patients included in our study. In addition, secondary generalized tonic-clonic seizures were observed in all patients except one patient. In the available literature, the incidence of epilepsy without cortical migration defect in patients with *ASPM*-associated MCPH phenotype was defined as approximately 3–8%. This suggests the possibility that cortical dysplasia that cannot be detected by MRI images may still be present in these patients. Mochida and Walsh [2001] reported that MCPH is rarely associated with epilepsy. Mutations in *ASPM* do not seem to affect the later stages of cortical development such as neuronal migration, and this might be responsible for the low epileptogenicity of this malformation [Passemard et al., 2009]. Alternatively, the existence of the "absence of seizure" criterion in the primary microcephaly diagnosis may cause the epilepsy phenotype to be ignored. Although it falls outside the

diagnostic criteria in patients with microcephaly and a history of seizures, there is a possibility of mutation in *ASPM* or other primary microcephaly genes [Shen et al., 2005].

A review of the mutation distribution of the *ASPM*-associated MCPH cases in the literature suggests that there is no obvious hot-spot region and that the mutations are distributed throughout the gene. Exon 18, which constitutes 45% of the open reading frame of the *ASPM* gene, is the most frequently mutated exon. Mutations detected in all *ASPM*-associated MCPH cases cause loss of function. It is predicted that transcription of mutated *ASPM* may cause nonsense-mediated decay and/or truncated protein formation. A clear genotype-phenotype correlation could not be established between the position of the mutation in the gene and the size of the truncated protein formed, and the head circumference, degrees of mental involvement, and epilepsy phenotypes [Bond et al., 2003; Ariani et al., 2013]. In patient 1, we detected a frameshift variant in exon 18 that was previously reported in literature. In a previously reported case with the same mutation, the patient was a 2-year-old girl with severe microcephaly, narrow frontal area, and large ears phenotype, as well as cortical atrophy, and deep sulci detected on brain computed tomography images [Hu et al., 2014]. Patient 1 included in our study differed from the previous case in findings of polymicrogyria, ventriculomegaly, and a simplified gyral pattern in the right frontotemporal region. In patient-2, the novel homozygous nonsense variant was detected in exon 18. The CADD score of this variation, which was evaluated as likely pathogenic according to the ACMG criteria, was 36, and further, in silico analysis predicted it to be pathogenic. Consistent with the literature, we found mutations in exon 18 in both cases. Bilateral cerebral sulcus shallowness, decreased number of gyri, and pachygyria were detected in brain MRI in patient 2. Until now, polymicrogyria has been found in 4 cases with *ASPM* mutation and pachygyria in 2 cases as reported in the relevant literature [Passemard et al., 2009; Ariani et al., 2013; Hu et al., 2014; Nakamura et al., 2015; Létard et al., 2018]. Detailed clinical findings of the cases are summarized in Table 1. When all cases with polymicrogyria and pachygyria were evaluated, no correlation was found among the mutation site and truncated protein size and head circumference, severity of intellectual disability, or the presence of epilepsy.

It was emphasized by Abdel-Hamid et al. [2016] that mild-to-severe intellectual disability, speech delay, and hyperactivity disorder may be present in patients with primary microcephaly associated with the *ASPM* gene. In patients of primary microcephaly, it may be necessary to apply special education and appropriate behavior management strategies.

Primary microcephaly is a group of diseases with genetic heterogeneity, mostly inherited as autosomal recessive [Woods et al., 2005]. Determining the molecular etiology in patients with primary microcephaly provides an opportunity to calculate the risk of recurrence, to analyze the carrier status of the parents, and to present prenatal diagnosis options [Faheem et al., 2015].

In conclusion, neuronal migration defect is a rare finding in patients with *ASPM*-associated MCPH. Whether this defect detected in the MCPH cases occurs as a result of *ASPM* gene mutation cannot be clearly stated unless it is proven by in vitro/in vivo experimental studies and animal models. The mechanism of the defect should be investigated with further functional studies. The neuronal migration defect detected in these cases may also be a coincidental finding. The present study contributes to the expansion of the phenotypic spectrum based on the cases with neuronal migration defect in which a truncating mutation in the *ASPM* gene was detected.

Statement of Ethics

All experimental procedures were performed in terms of the guidelines of the local Ethics Committee and conducted in accordance with the principles of the Declaration of Helsinki, and written informed consent was obtained from patients or their guardians.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.T. performed the whole-exome sequencing analysis as well as the segregation study and drafted the manuscript. S.G.S. conducted the patient's physical examination and critically reviewed the manuscript.

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