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Identification of Mosaic Variants in Neurodevelopmental Disorders

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Identification of Mosaic Variants in Neurodevelopmental Disorders

Directed by Professor Hoon-Chul Kang

Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Medical Science

Se Hee Kim

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ABSTRACT

Identification of Mosaic Variants in Neurodevelopmental Disorders

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Neurodevelopmental disorders (NDDs) refer to a group of disorders that affect cognitive and social communicative development. Growing evidence indicates that mosaicism of genes can cause NDDs, but detection of low level mosaic variants with an allele frequency < 5% remains a challenge. This study aimed to identify, quantify, and characterize NDD-associated mosaic variants by analyzing the DNA obtained from blood. We retrospectively reviewed patients with NDDs who underwent conventional next-generation sequencing (NGS) panel testing at Severance Children's Hospital between 2016 and 2021, and prospectively reviewed selected patients by using a specifically designed mosaic NGS panel. DNA was obtained from blood, and brain specimens, when available, were additionally tested. Furthermore, patients with combined malformations of cortical development (MCDs), neurocutaneous syndrome, or infantile epileptic spasm syndrome (IESS) were analyzed via various approaches. Magnetic resonance imaging (MRI) did not reveal any lesion in the patients with IESS. In the selected patients, previous genetic test results were all negative. Overall, 2,162 NDDs patients underwent conventional target-gene NGS panel testing. The diagnostic yield was 31.7% (686/2,162), and 670 (31%) and 16 (0.7%) patients had germline and mosaic variants, respectively. Among the 1476

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patients with negative genetic test results, 44 underwent the specifically designed mosaic NGS panel testing, whereby three more patients with mosaic variants of the *NF1*, *TSC2*, and *AKT3* genes were identified using blood samples. The mean read depth was 50,170 (3,615–130,792), and the variant allele frequency of the detected variants ranged from 1.7% to 10.7%. A variant was detected in both the blood and brain of a patient. The diagnostic yield was high in the patients with neurocutaneous syndrome (2/7, 28.6%) but low in the patients with IESS who exhibited no lesion on MRI (0/17, 0%). The diagnostic yield was 5% (1/20) in the patients with MCDs. Mosaic variants were identified in both the blood and brain of 0.9% (19/2,162) of the patients with NDDs via the conventional NGS and specifically designed mosaicism NGS panels. These findings will enhance our understanding of NDDs and improve patient care and management.

Keywords: neurodevelopmental disorders, mosaic, somatic, genetic, next-generation sequencing, delayed development, epilepsy

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Identification of Mosaic Variants in Neurodevelopmental Disorders

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I. INTRODUCTION

The term neurodevelopmental disorders (NDDs) applies to a group of disorders that affect cognitive and social communicative development. According to the International Classification of Diseases 11th Revision, disorders of intellectual development, autism spectrum disorders, attention deficit hyperactivity disorder, developmental motor coordination disorder, speech or language disorders, learning disorders, and stereotyped movement disorders are classified as NDDs. Neurological conditions, including epilepsy and malformations of cortical development (MCDs), are common comorbidities.

Recent studies have emphasized the role of *de novo* variants in NDDs. Haploinsufficiency of genes involved in conserved pathways can disrupt protein synthesis, synaptic signaling, and transcriptional or epigenetic regulation. Even though *de novo* variants are the most commonly identified cause of NDDs, detection rate for *de novo* variants associated with NDDs has been approximately 30% in large-size studies.[1-3]

Until recently, most identified variants were thought to be *de novo* germline variants. However, growing evidence has indicated that somatic variants from the brain can also cause NDDs.[4,5] A recent study on intellectual disability has reported that 6.5% of 107

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variants that have been presumed to be *de novo* and germline are in fact somatic.[6] Similarly, a further review of 893 patients with pathogenic variants in one of 9 common epilepsy-causing genes has revealed that 3.5% of such patients have somatic variants.[7] Somatic mosaicism has been reported in various NDDs and related disorders, including MCDs, epileptic encephalopathies, and intellectual disability.[8-11]

With the recent development of sensitive tools, including droplet digital polymerase chain reaction (ddPCR), and an increasing depth of coverage, additional somatic variants associated with NDDs are expected to be identified,[12] but data regarding the frequencies of mosaic variants in NDDs remain limited.

Here, mosaicism related to NDDs was investigated in detail. Cases with a high variant allele frequency (VAF) (10–<30%) were identified using a conventional next-generation sequencing (NGS) panel, and those with a low VAF (< 10%) were further investigated using a mosaicism panel we specifically designed. We hypothesized that mosaic variants with a low VAF in blood can be detected if a targeted NGS panel with high sensitivity is used.

II. MATERIALS AND METHODS

1. Subjects and Study Design

Overall, 2,162 NDD patients underwent our conventional targeted epilepsy, NDD, or MCD NGS gene panel testing between 2016 and 2021. A specific target gene panel was ordered by the primary physician based on the clinical diagnosis. We retrospectively reviewed the genetic test results of these patients and identified mosaic variants with an alternative allele frequency (AAF) < 30%. These mosaic variants had a frequency of 10–30% and were categorized into “high-grade mosaicism.”

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Then, a subset of patients with unidentified genetic etiology were selected for further mosaicism testing. Only patients who agreed to undergo further genetic testing by using already available DNA samples were included. The previous NGS test results of all the patients were negative. Patients were selected from the following three groups: infantile epileptic spasm syndrome (IESS), neurocutaneous syndrome, and MCD. For patients with MCD or neurocutaneous syndrome, those with multiple or diffuse lesions were specifically selected. Patients who had undergone epilepsy surgery were also selected because samples of their brain tissue were available. For patients with IESS, those with severely delayed development but no identified etiology were selected. Only patients with negative etiological conclusion from analyses such as MRI and with metabolic test results were included. Informed consent was obtained.

2. Genetic Analysis

A. DNA extraction, library construction, and sequencing

Genomic DNA (gDNA) was extracted from peripheral blood and brain samples by using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. To reduce the frequency of false-positive variants, gDNA was treated with uracil-DNA glycosylase (UDG; NEB, MA, USA) by following the manufacturer's protocol. Briefly, 100 ng gDNA was incubated at 37 ℃ with 1 unit UDG for 30 min in a final volume of 50 μL an then purified using AmpureXP beads (Beckman Coulter Inc., CA, USA). The purified gDNA was quantified using the Qubit BR dsDNA kit (Invitrogen, Carlsbad, CA). For the conventional target panel testing, the library preparation and target enrichment were performed as previously described.[13,14] For the mosaicism-specific target panel testing, approximately 100 ng gDNA was prepared using the Twist Library Preparation EF Kit

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(Twist Bioscience, San Francisco, USA). The fragmentation at 32 °C was performed for 20 min, followed by enzyme inactivation at 65 °C for 30 min. Target enrichment was performed using a custom-design enrichment panel following the manufacturer's instructions (Dxome, Seoul, Korea). The panel specially designed for mosaicism included 41 genes (*SCN1A, STXBP1, SCN2A, KCNQ2, CDKL5, CHD2, SLC2A1, PCDH19, SCN8A, GRIN2A, GRIN2B, GABRA1, GABRB3, IQSEC2, SLC9A6, SYNGAP1, DNM1L, KCNT1, KCNB1, GANO1, EEF1A2, DNM1, MBD5, DCX, PAFAH1B1, TUBA1A, TUBA8, TUBB2B, TUBB3, DEPDC5, TSC1, TSC2, SLC35A2, PIK3CA, AKT3, PTEN, NSD1, MTOR, PIK3R2, GNAQ*, and *NF1*). This gene panel consisted of commonly identified genes related to NDDs, with a focus on IESS, neurocutaneous syndrome, and malformation of cortical development (MCD). The enriched DNA was sequenced using a NextSeq 550Dx System or NovaSeq 6000 instrument (Illumina, San Diego, CA, USA), whereby we achieved approximately 150 million reads per sample. A 151 bp, dual-indexed, paired-end sequencing configuration was used.

B. Bioinformatic analysis, and variant interpretation

For further processing, the sequencing data were analyzed using our bioinformatic pipeline as previously described.[9,10] To accurately detect low-frequency variants, a positional indexing sequencing (PiSeq) algorithm (Dxome) was used to call single-nucleotide variants and small indels. The genomic positions of the mapped reads were determined and then used as indices to detect true rare variants, thereby improving the detection accuracy.

Variants were classified into five categories (benign, likely benign, variant of uncertain significance, likely pathogenic, and pathogenic) based on the recommendation of the American College of Medical Genetics and Genomics.[12] Population frequency data from multiple databases, namely 1000 Genomes, the Genome Aggregation Database, the Exome

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Sequencing Project, and the Korean Reference Genome Database, were used to interpret the detected variants. To annotate the variants, we searched and reviewed reports from ClinVar and the Human Gene Mutation Database. *In silico* analyses were conducted using SIFT, MutationTaster, FATHMM, and MetaSVM. Finally, the clinical impact of the variants with genotype-phenotype correlations was reviewed by pediatric neurologists and geneticists. If needed, peripheral blood samples were obtained from parents to confirm the pathogenicity of the variant.

Ideally, the AAF of a heterozygous germline variant is expected to be 50%. Mosaicism was suspected when the AAF of a variant was significantly lower than the expected allele frequency for a heterozygous germline variant. To exclude experimental and analytic effects on allele frequency, we defined a variant as mosaic when its AAF was < 30%.

C. Confirmation of the detected variants

To confirm the mosaic variants, variants with a VAF ≥ 10% were assessed via Sanger sequencing, followed by MEMO-PCR and parental genetic tests. Primers targeting the locus containing each variant of interest were designed, and subsequent gene amplification was performed. The amplicons were subjected to Sanger sequencing by using a 3730 DNA Analyzer with a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA).Variants with a VAF < 10%, which can be missed by Sanger sequencing, were confirmed via NGS (coverage > 10,000 x) and mutant enrichment with 3'-modified oligonucleotides-PCR (MEMO-PCR) followed by Sanger sequencing. Specific primers and blockers per variant of interest were designed for the MEMO-PCR, which was then performed as previously described.[15]

If available, variants were also detected from other specimens, including brain and muscle tissues. Brain specimens were obtained from previous epilepsy surgeries. Parent testing

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was also performed. Sequence variants were interpreted according to the recent recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[16]

3. Ethical Considerations

This study was reviewed and approved by the Institutional Review Board of Yonsei University Health System (4-2021-0740, 2022-0744-001). The study was conducted in accordance with good clinical practices (national regulations and ICH E6) and the principles of the Helsinki Declaration. Written informed consent was obtained from the parents or legal guardians of the patients prior to sample collection following a detailed explanation of the schedules and contents of the study.

III. RESULTS

1. Mosaic Variants Detected via Routine Molecular Analyses

Overall, 2,162 patients with NDDs underwent target gene NGS panel testing, and causative pathogenic or likely pathogenic variants were identified in 686 cases (diagnostic yield 31.7%). Germline variants were identified in 670 (31%) cases, and 16 cases had mosaic variants (2.3% of the positive cases, 0.7% of the total cases). The average depth of the detected mosaic variants was 621 (51–1695), and the VAF ranged from 8.8% to 29.8%. The genetic test results are shown in Figure 1.

In total, 16 mosaic variants were detected in 14 different genes, namely *STXBP1, SCN2A, SCN1A, PURA, POGZ, PAFAH1B1, NF1, KIF21A, KCNQ2, EEF1A2, CDKL5*, and *ARID1B*. The mosaic variants of *TSC2* and *GABRA1* were detected twice. The detailed information is provided in Table 1.

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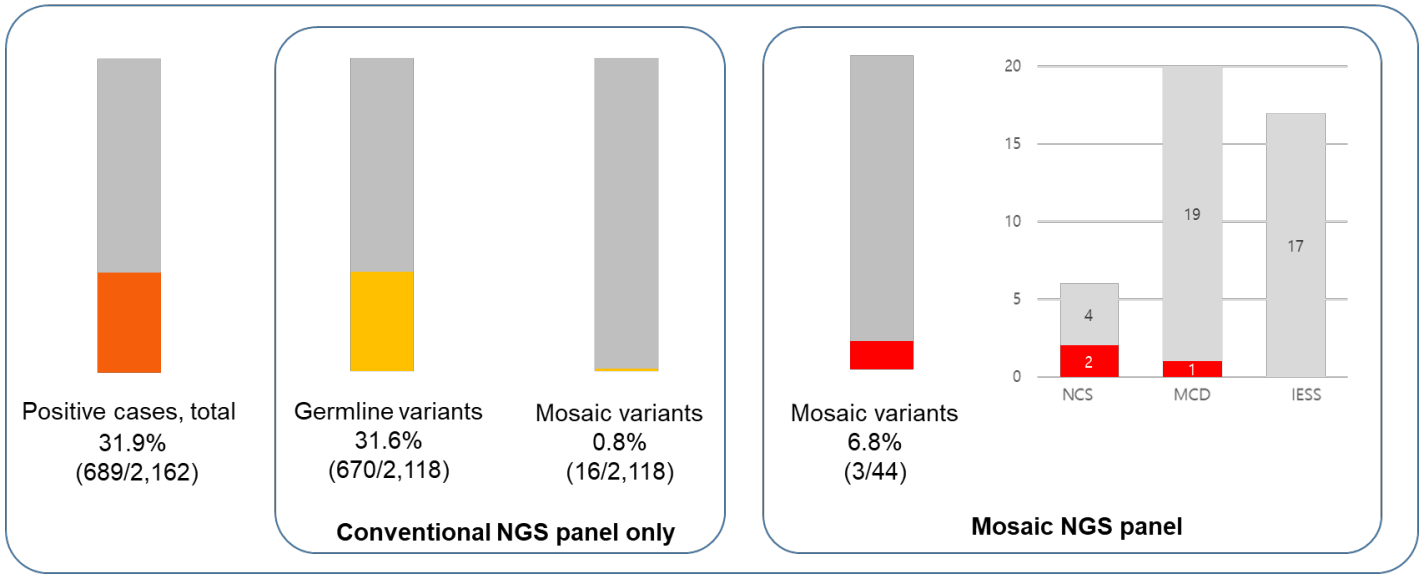

Figure 1. Study design, and summary of the somatic variants detected in blood.

IESS, infantile epileptic spasms syndrome; NCS, neurocutaneous syndrome; NGS, next-generation sequencing; MCD, malformation of cortical development.

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Table 1. Mosaic pathogenic variants detected in blood via routine NGS panel testing

Age	Sex	Gene	Accession	Nucleotide	Amino acid	VAF, %	Inter-pretation	Cover-age	Clinical impact
1y	M	*TSC2*	NM_000548.5	c.610_611del	p.Leu204AlafsTer30	9.8	LP	1235	GDx
9m	F	*TSC2*	NM_000548.5	c.976-15G>A		17.4	P	69	GDx
9y	F	*STXBP1*	NM_003165.6	c.923_933del	p.Lys308IlefsTer2	14.4	LP	180	GDx
2m	F	*SCN2A*	NM_001040142.2	c.4499C>T	p.Ala1500Val	15.7	LP	1191	GDx, Tx
28y	F	*SCN1A*	NM_001165963.4	c.4934del	p.Arg1645GlnfsTer5	21.5	P	279	GDx, Tx
1y	M	*PURA*	NM_005859.5	c.72dup	p.Gly25ArgfsTer176	23.5	LP	51	GDx
8m	M	*POGZ*	NM_015100.4	c.2517_2518del	p.His840GlnfsTer23	26.2	LP	360	GDx
8y	M	*PAFAH1B1*	NM_000430.4	c.1019G>A	p.Trp340Ter	11.0	LP	1692	GDx
2y9m	M	*NF1*	NM_001042492.3	c.334C>T	p.Gln112Ter	17.9	P	151	GDx
4y6m	F	*KIF21A*	NM_001173464.2	c.387dup	p.His130ThrfsTer5	8.8	LP	203	GDx
7m	F	*KCNQ2*	NM_172107.4	c.1687G>A	p.Asp563Asn	26.6	LP	741	GDx, Px
6y7m	F	*GABRA1*	NM_000806.5	c.839C>T	p.Pro280Leu	29.8	LP	131	GDx, Px
1y	M	*GABRA1*	NM_000806.5	c.134T>C	p.Ile45Thr	27.9	LP	466	GDx, Px
1y1m	M	*EEF1A2*	NM_001958.5	c.46G>T	p.Val16Leu	22.8	LP	600	GDx
11y4m	F	*CDKL5*	NM_003159.2	c.2684C>T	p.Pro895Leu	12.7	LP	1405	GDx
5y1m	F	*ARID1B*	NM_020732.3	c.1389_1398dup	p.Gln467GlyfsTer71	24.2	LP	120	GDx

GDx, genetic diagnosis; F, female; LP, likely pathogenic; M, male; m, months; P, pathogenic; Px, prognosis prediction; Tx, Treatment adjustment; y, years; VAF, variant of allele frequency.

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All the patients had NDDs, including intellectual disabilities. Common comorbidities included epilepsy (10/16, 62.5%), MCDs (2/16, 12.5%), and tuberous sclerosis (2/16, 12.5%). The most frequent variation type was frameshift (7/16, 43.8%), followed by missense (6/16, 37.5%) and nonsense (2/16, 12.5%). One variant was a pathogenic intronic variant, which resulted in aberrant splicing. Parental genetic tests were performed in 7 of 16 patients and revealed that the variants were *de novo*.

2. Mosaic Variants Detected via Mosaicism-Specific Target Panel Analysis

Overall, 49 samples from 44 patients with NDDs were included in the analysis. From all the 44 patients, peripheral blood samples were obtained for analysis. Additionally, brain samples from 4 of the patients were analyzed. Among the 44 patients, 20 had MCDs and 7 had neurocutaneous syndromes, including 3 patients with tuberous sclerosis complex (TSC), 2 patients with café-au-lait spots, and 2 patients with Sturge-Weber syndrome. Most patients (40/44) had epilepsy. Specifically, 17 patients had IESS, 11 patients had Lennox-Gastaut syndrome (LGS), and 1 patient had Doose syndrome.

By using the panel designed to detect mosaicism, 3 mosaic variants were identified from the blood samples of the patients. These variants were in 3 different genes, namely *NF1, TSC2,* and *AKT3* (diagnostic yield 7.3%) (Table 2). The mean read depth was 50,170 (3615–130,792), and the VAF of the detected variants ranged from 1.7% to 10.7%, indicating that variants with a low VAF could be detected via the mosaicism-specific target panel analysis. Overall, 1 missense, 1 intronic deletion, and 1 frameshift were detected.

Regarding etiology, in neurocutaneous syndrome, the diagnostic yield was high (2/7, 28.6%). In MCD, the diagnostic yield was low (5%, 1/20). No mosaic variant was detected among the 17 patients who had IESS based on MRI (0%, 0/17).

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Table 2. Mosaic pathogenic variants detected in blood via mosaicism panel testing

Age	Sex	Co-morbidity	Sample	Gene	Accession	Nucleotide	Amino acid	VAF, %	Inter-pretation	Coverage
2y	F	NCS	Blood	*NF1*	NM_001042492.3	c.1527+4_1527+7del	-	10.7	P	497
9y3m	M	NCS	Blood	*TSC2*	NM_000548.5	c.4297_4298dup	p.Gly1434ArgfsTer43	3.3	LP	6722
8y2m	M	MCD	Blood	*AKT3*	NM_005465.7	c.863C>T	p.Thr288Ile	1.7	LP	3948

LP, likely pathogenic; MCD, malformation of cortical development; m, months; NCS, neurocutaneous syndrome; P, pathogenic; y, years.

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For neurocutaneous syndrome, first, a pathogenic *NF1* variant with a VAF of 10.7% was detected in a patient with multiple café-au-lait macules. Second, a frameshift variant of *TSC2* with a VAF of 3.3% was detected in a patient diagnosed with tuberous sclerosis. The latter patient had seizures since the age of 6 years. The patient was diagnosed with tuberous sclerosis at the age of 7 years via imaging studies, including brain MRI and kidney sonography, which revealed multifocal tubers in addition to calcified subependymal nodules, and angiomyolipoma, respectively. Previous evaluations of the *TSC1* and *TSC2* genes via Sanger sequencing yielded negative results.

Regarding MCD, a mosaic variant of *AKT3* (c.863C>T, p.Thr288Ile, with a VAF of 1.7%) was found in a patient. This patient had drug-resistant epilepsy, developmental epileptic encephalopathy, delayed development, dolichocephaly, and macrocephaly. Seizures started 5 days after birth. Brain MRI showed diffuse bilateral cortical malformations, including hemimegalencephaly, pachygyria, and multifocal dysmyelination of the cerebral white matter. Since pathogenicity of the variant is unknown, further validation was performed. The variant was confirmed via Sanger sequencing, which showed that the variant existed at a much higher frequency in the brain than in the blood (Figure 2). The variant was also found to be *de novo* via parental testing. The clinical phenotype was reviewed and compared with that previously reported by other studies. *AKT3* encodes a kinase involved in various biological processes, including cell regulation, proliferation, differentiation, apoptosis, tumorigenesis, glycogen synthesis and glucose uptake. Overgrowth syndrome and/or cerebral malformations due to abnormalities in mTOR pathway genes (OCMMPG) is the main phenotype. Accordingly, the pathogenicity of the variant could be confirmed.

Additionally, two mosaic variants were identified in the brain (50%, 2/4). A mosaic variant of the *AKT3* gene was detected in the brain and blood as above.

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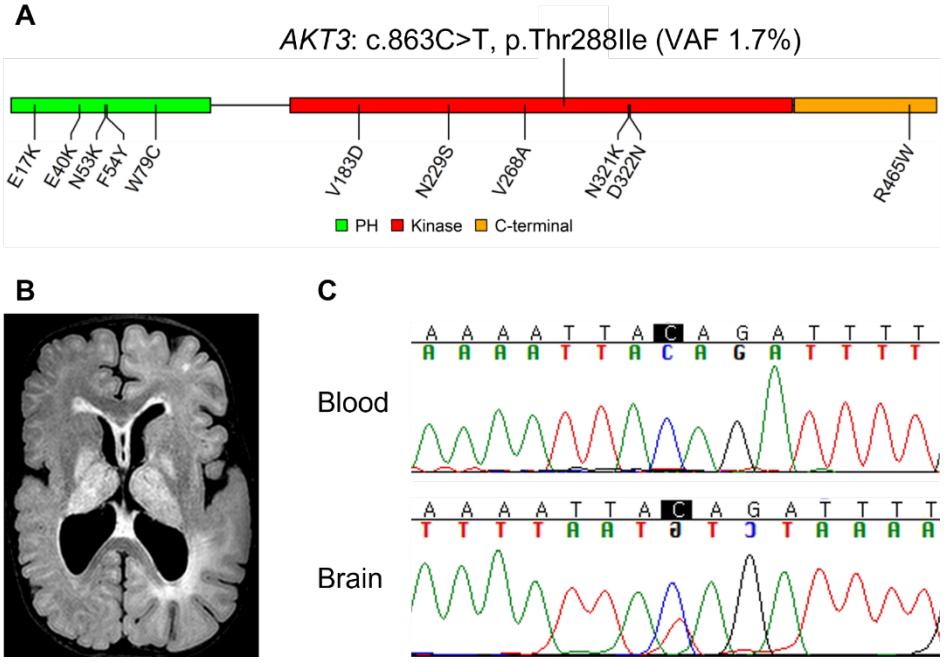

Figure 2. A patient with a likely pathogenic variant of the *AKT3* gene. A. Mosaicism-specific next-generation sequencing panel revealed a somatic variant of *AKT3* in the peripheral blood of the patient. The structure of AKT3 is shown with the detected variant and other known pathogenic variants of *AKT3*. PH; pleckstrin homology domain. Kinase; catalytic kinase domain. C-terminal; C-terminal domain. B. T2 axial view (magnetic resonance imaging) of the patient shows multifocal dysmyelination of the cerebral white matter and abnormal T2 hyperintensity of the thalamus. A combined dysplastic corpus callosum, abnormal T2 hyperintensity of the brainstem, and dentate nucleus of the cerebellum were present (not shown). An enlarged hemisphere with an associated thickened cortex, T2 hyperintensity of the peritrigonal white matter, and pachygyria suggestive of hemimegalencephaly were seen on the left hemisphere. Thickened cortex of insula and perisylvian region on the right hemisphere suggested pachygyria. C. Sanger sequencing of *AKT3* showed that the variant existed at a much higher frequency in the brain than in the blood.

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Additionally, a mosaic variant of *GNAQ* (c.548G>A, p.Arg183Gln, with a VAF of 2.6%) was detected in a patient. However, the variant was detected in the brain and not in the peripheral blood. The identified variant is a well-known causal variant of Sturge-Weber syndrome.

3. Clinical Implications

Overall, 19 (0.9%) cases with mosaic variants were identified from blood by using a conventional NGS panel and a specifically designed mosaicism panel. An accurate molecular diagnosis could be made after mosaic pathogenic variants were identified in 14 (0.6%) undiagnosed patients with EISS or MCD. For example, in 4 patients with Lennox-Gastaut syndrome, mosaic variants in various genes, such as *STXBP1, EEF1A2, CDKL5*, and *AKT3*, were identified.

In 6 (0.3%) patients, a correlation was also made between disease severity and genetic burden. Of these patients, 5 (0.2%) had neurocutaneous syndrome, and 1 patient had MCD. For example, in a patient who had multifocal pachygyria, a mosaic variant (W340X) was found in *PAFAH1B1*, a well-known causative gene for lissencephaly. The phenotype was milder than the classical lissencephaly because the VAF was low (11.0%). Additionally, 3 patients with mosaic variants of the *TSC2* gene had various VAFs ranging between 3.3% and 17.4%, and 2 patients with café-au-lait spots also showed mosaic variants with a low VAF of 10.7–17.9%.

Treatment could be adjusted in 5 patients (0.2%) after mosaic pathogenic variants were detected in the *SCN2A, SCN1A, KCNQ2*, and *GABRA1* genes. In 4 (0.2%) of these patients, who had variants in genes related to the MTOR signaling pathway (such as *TSC2* and *AKT3*), an mTOR inhibitor could be proposed as a mechanism-based treatment.

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IV. DISCUSSION

Mosaic variants were identified in approximately 1% of our patients with NDDs. Mosaic variants were detected in blood and brain samples via the target-gene NGS panel testing. This finding suggests that mosaic variants might be under-reported.

Mosaic mutations have previously been detected in various diseases, such as epilepsy,[7] autism,[18] and cerebral cortical malformations.[19] The frequencies of mosaic variants differ depending on the associated disease. In the present study, mosaic variants could be detected from blood samples at a rate of 0.9% (19/2,162), which is similar to the rate in a report of mosaic variants associated with various diseases and identified via diagnostic exome sequencing.[9] Genes with recurrent mosaic variants have been reported,[9,10] and we found mosaic variants in those genes (*TSC2, SCA1A, SCN2A, NF1, KCNQ2, GABRA1*, and *CDKL5*). Notably, we additionally identified the mosaicism of genes previously not reported (or rarely reported), namely *PURA, POGZ, KIF21A, EEF1A2,* and *ARID1B*. This finding suggests that there are still unidentified genes with mosaic variants.

In a clinical laboratory, variant-calling algorithms have to exclude errors that occur during sequencing, and mosaic variants with a low VAF are usually overlooked. In addition, the low sequencing depth is usually insufficient to detect cases of low-VAF mosaicism.[20] To detect these mutations, ultra-sensitive detection methods and mosaicism-specific algorithms are required. Only a few studies have detected mosaic mutations with a low VAF.[3,5] Here, we attempted to detect low-VAF mosaicisms in the peripheral blood of NDD patients. To this end, we designed a mosaicism-specific panel, which included 41 genes that have been reported to be related to mosaicism in NDDs. By using a relatively small-size gene panel, we performed NGS followed by bioinformatic analysis, to detect true variants with a low VAF. Consistent with previous reports, the VAF of the identified

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mosaic variants was > 5% in most cases, but we could also detect low-VAF mosaic variants. To validate the noble variants with a low VAF, we performed parental trio testing and thereby confirmed the *de novo* state of the variant. Then, we conducted the same genetic testing by using DNA samples from other tissues, such as the brain. Using a MEMO-PCR, variants with a low VAF were identified with high specificity.

Mosaic variants can be categorized as obligatory somatic variants, variants following two-hit mechanisms, and variants with milder phenotypes than the germline variants, based on their genetic mechanisms.[11] Several genes with recurrent mosaic variants are associated with diseases that can be caused by both germline and somatic variants. Mosaic variants following two-hit mechanisms are also commonly detected. The VAF of these variants ranged widely between 10% and 30%. In relation to diseases caused by both germline and somatic variants, variants of the *SCA1A, SCN2A, KCNQ2, GABRA1*, and *CDKL5* genes have been identified. Regarding diseases related to two-hit mechanisms, variants of *TSC1, TSC2*, and *NF1* genes have been found. These mosaic variants could be relatively easily detected using blood samples via a conventional target-gene NGS panel testing. These findings are consistent with the previous reports. Mosaic variants can be found in the blood, saliva, angiofibroma, and skin of patients with TSC.[5,21,22]

In contrast to mosaic variants following two-hit mechanisms or to variants with milder phenotypes than the germline variants, variants associated with obligatory somatic diseases are difficultly detected using blood samples. Pathogenic germline variants of these genes are lethal, and thus disease is caused only by pathogenic somatic variants. Variants usually occur late in embryonic development, and most variants are detectable only if the brain tissue is tested. Here, a variant of the *AKT3* gene could be detected from blood samples by using a specifically designed mosaic NGS panel. The VAF was 1.7%. The AKT3 protein

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is a member of the AKT serine/threonine protein kinase family. *AKT3* pathogenic variants cause overgrowth syndrome, megalencephaly, hydrocephalus, and polymicrogyria. Mosaic pathogenic variants of *AKT3* have been reported in patients with hemimegalencephaly.[24,25] In all cases, mosaic variants were only detected in the brain and skin (not detected in the blood). Our findings suggest that a mosaic somatic variant of *AKT3* can be detected in the peripheral blood of patients if a specifically designed mosaic NGS panel is used. additionally, a variant of *GNAQ* was detected in a patient with Sturge-Weber syndrome. However, the variant was detected in the brain and not in the peripheral blood. The VAF was as low as 2.6%.

It is generally challenging to detect mosaic variants by using blood samples in patients with no organ involvement other than the brain. In our study, mosaic variants with a low VAF could be frequently found in blood in patients with neurocutaneous syndrome, who had multiple-organ involvement. In contrast, mosaic variants were rarely found from blood in patients with MCDs who had diffuse pathology in the brain, but no other organ was affected. No mosaic variant with a low VAF could be detected in blood in patients who had IESS with negative MRI findings. This finding also supports the previous reports showing that variants that arise late in embryonic development are difficult to detect in blood. In previous studies, most disease-causing mosaic variants were detected only in the brain of patients with focal cortical dysplasia and not in their blood.[4,23]

There are some limitations to the presented study. We used a specific gene panel for high-sensitive mosaicism detection. This gene panel enabled us to perform NGS for the detection of low-level mosaicism from blood. However, because only a limited number of genes were included in the analysis, mosaic variants of other genes might have been missed. Thus, an effective method that covers numerous NDD-related genes with sufficient sensitivity to

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detect variants present at low levels may increase the detection rate. Second, we used peripheral blood for the detection of mosaic variants. Mosaic variants could be detected in cerebrospinal fluid (CSF) in other studies.[26,27] A CSF analysis may increase the detection rate. However, collecting peripheral blood samples is easier and less invasive than collecting brain or CSF samples.

V. CONCLUSION

Low-VAF mosaicism in NDD patients, especially in patients with neurocutaneous syndromes or MCDs, can be detected using blood samples and a highly sensitive method, whereby an accurate molecular diagnosis and treatment adjustment can be made.

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ABSTRACT (IN KOREAN)

신경발달장애의 모자이크 변이 발견

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신경발달장애는 인지와 사회성에 영향을 주는 발달 과정에서 발생하는 질환이다. 신경발달장애가 모자이크 유전자 변이에 의해 발생한다는 연구결과가 자주 보고되고 있다. 하지만, 대립 유전자 빈도가 5% 미만으로 낮은 모자이크 변이를 발견하는 것은 쉽지 않다. 본 연구에서는 특수하게 제작된 유전자 패널 검사를 사용하여 말초혈액에서 대립유전자빈도가 낮은 모자이크 변이를 혈액에서 찾고자 하였다. 기존의 유전자 검사를 사용하여 2,162명의 환자 중 686명(31.7%)의 환자에서 유전자 변이 이상을 발견하였다. 이들 중 16명(0.7%)의 환자에서 모자이크 변이가 확인되었다. 유전자 변이가 밝혀지지 않은 44명의 환자에서 특수 제작된 유전자 패널검사를 시행하여 모자이크 변이를 가진 3명(0.2%)의 환자를 추가로 진단할 수 있었다. 이들은 *NF1, TSC2, AKT3* 유전자에 모자이크 변이를 가지고 있었다. 본 연구에서 신경발달장애 환자 중 총 1%에 달하는 환자가 모자이크변이를 가지고 있음을 확인하였다. 이 결과는 신경발달장애에서 모자이크 변이가 현재까지 보고된 것보다 실제로 더 많이 존재할 수 있음을 시사한다. 앞으로 발달된 검사 방법을 통해 더 많은 신경발달장애 환자에서 모자이크 변이를 확인할 수 있을 것으로 예측된다.

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핵심되는 말: 신경발달장애, 모자이크 변이, 체세포, 유전, 차세대염기서열분석, 발달 지연, 뇌전증