Detection of low level somatic mutation in pediatric epilepsy and genotype-phenotype correlation

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Detection of low level somatic mutation in pediatric epilepsy and genotype-phenotype correlation

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ABSTRACT

Detection of low level somatic mutation in pediatric epilepsy and genotype-phenotype correlation

Epilepsy is a neurological disorder characterized by unprovoked seizures. Around one-third of epilepsy patients have intractable epilepsy, or drug resistant epilepsy (DRE). Recent studies have identified that somatic variants in the brain can cause focal malformations, which are linked to DRE. Although many somatic mutations have been identified, their detection requires extensive analysis of brain tissue and blood samples. Even with such efforts, some samples have low variant allele frequencies (VAFs) that may be considered mutation-negative. Furthermore, the clinical features of patients with these somatic mutations remain poorly understood.

The purpose of our study was to investigate the clinical phenotypes of a large cohort of patients with intractable epilepsy who underwent epilepsy surgery. We aimed to detect somatic mutations in tissues previously deemed mutation-negative, and compare efficacy of mutation detection in brain tissue alone versus paired brain-blood samples.

Our study focused on pediatric patients with drug-resistant epilepsy who underwent surgery in a tertiary epilepsy center from 2004 to 2022. We performed deep sequencing on both brain tissues alone and paired with peripheral blood samples.

Among 238 patients who underwent epilepsy surgery and had brain tissues evaluated for genetic variants, we identified somatic mutations in six genes (*MTOR, SLC35A2, PIK3CA, TSC1, TSC2, AKT3)* in 65 patients and germline mutations in the *DEPDC5* or *NPRL3* genes in 15 patients. We could detect the same variant in both brain-only and matched brain-blood studies. In detecting the somatic mutations, we identified somatic mutations of the *SLC35A2* gene in two patients whose brain tissues had very low VAFs. We used fluorescence activated nuclei sorting to extract nuclei with specific genetic activities, thereby enhancing the VAF.

Among the 80 patients, the most frequent histopathologic findings in the brain tissues were focal cortical dysplasia types IIa (FCDIIa) and IIb (FCDIIb). Brain samples in patients with *MTOR* mutations predominantly showed FCDIIa and FCDIIb, while brain tissues in patients with *SLC35A2* mutations frequently presented mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy. Patients with identical nucleotide mutations exhibited different pathologies, suggesting genotype-phenotype heterogeneity.

By analyzing a large cohort, we were able to provide a comprehensive clinical profile of patients

with somatic or germline mutations with intractable epilepsy. Our findings suggest that examining brain tissue only can be as effective as paired brain-blood testing for detecting genetic mutations. As methods for identifying low-VAF mutations evolve, we anticipate advancement in the genetic characterization of patients with intractable epilepsy, discovery of targeted therapies in genetic epilepsy and better patient outcomes.

Key words : somatic mutation, drug resistant epilepsy, cortical malformation, focal cortical dysplasia.

I. INTRODUCTION

1.1. Somatic variants in epilepsy

Variants that cause diseases can arise during the mitotic cell divisions that take place as the embryo develops after fertilization and the formation of the zygote. Unlike germline variants that are found in every cell, postzygotic variants lead to somatic mosaicism, where different cell lineages within the same individual have different genotypes. The timing and the specific cell lineage in which the somatic mutation occurs can result in different patterns of mosaicism. For instance, a somatic variant that occurs early post-zygotically may impact multiple organs across the body, whereas a somatic variant that arises later in development may be confined to neural progenitors, leading to lineage-specific expression, such as being limited to the brain.[1,2]

Over the past decade, it was revealed that somatic variants are the cause of epileptogenic brain lesions, including various malformations of cortical development (MCD), hypothalamic hamartoma, long-term epilepsy-associated tumors (LEATs), epileptogenic vascular malformations, and temporal lobe epilepsy with hippocampal sclerosis (Table 1). These epileptogenic brain disorders have significant clinical implications, as they are particularly associated with drug-resistant epilepsy (DRE). Indeed, focal MCD including focal cortical dysplasia (FCD) represents the most common etiology of epilepsy among children undergoing resective epilepsy surgery.[3]

1.2. PI3K-AKT-mTOR pathway and epilepsy

The phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT)–mechanistic target of rapamycin (mTOR) pathway is crucial for regulating cellular processes like growth, survival, metabolism, and synaptic plasticity in neurons (Figure 1). Dysregulation of this pathway has been implicated in epileptogenic disorders such as FCD, tuberous sclerosis complex (TSC), and LEATs.[4]

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[1] Gooley, S., Perucca, P., Tubb, C., Hildebrand, M. S., & Berkovic, S. F. (2024). Somatic mosaicism in focal epilepsies. *Current opinion in neurology*, *37*(2), 105–114.

[2] Poduri, A., Evrony, G. D., Cai, X., & Walsh, C. A. (2013). Somatic mutation, genomic variation, and neurological disease. *Science (New York, N.Y.)*, *341*(6141), 1237758.

[3] Blumcke, I., Spreafico, R., Haaker, G., Coras, R., Kobow, K., Bien, C. G., Pfäfflin, M., Elger, C., Widman, G., Schramm, J., Becker, A., Braun, K. P., Leijten, F., Baayen, J. C., Aronica, E., Chassoux, F., Hamer, H., Stefan, H., Rössler, K., Thom, M., … EEBB Consortium (2017). Histopathological Findings in Brain Tissue Obtained during Epilepsy Surgery. *The New England journal of medicine*, *377*(17), 1648–1656.

[4] Russo, E., Citraro, R., Constanti, A., & De Sarro, G. (2012). The mTOR signaling pathway in the brain: focus on epilepsy and epileptogenesis. *Molecular neurobiology*, *46*(3), 662–681.

Table 1. Epileptogenic brain disorders with known associations to somatic variants

Epileptogenic brain lesion	Associated genes	Molecular function
Focal cortical dysplasia II	*AKT3, DEPDC5, MTOR, NPRL2, NPRL3, PIK3CA, RHEB, TSC1, TSC2*	PI3K-AKT-mTOR pathway
Hemimegalencephaly	*AKT1, AKT3, DEPDC5, MTOR, NPRL3, PIK3CA, PTEN, RHEB*	PI3K-AKT-mTOR pathway
Tuberous sclerosis complex	*TSC1, TSC2*	PI3K-AKT-mTOR pathway
Polymicrogyria	*PIK3CA, PIK3R2*	PI3K-AKT-mTOR pathway
Mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE)[5]	*SLC35A2*	N-glycosylation
Subcortical band heterotopia	*DCX, LIS1*	Neuronal migration
Periventricular nodular heterotopia	*FLNA*	Neuronal migration
Ganglioglioma	*BRAF, FGFR1, FGFR2, KRAS*	Ras-Raf-MAPK pathway
Dysembryoplastic neuroepithelial tumor	*FGFR1*	Ras-Raf-MAPK pathway
Temporal lobe epilepsy with hippocampal sclerosis	*BRAF, KRAS, NF1, PTPN11, SOS1*	Ras-Raf-MAPK pathway
Cerebral cavernous malformation	*CCM, MAP3K3, PIK3CA*	Ras-Raf-MAPK pathway
Arteriovenous malformation	*KRAS*	Ras-Raf-MAPK pathway

In FCD or hemimegalencephaly (HME), mutations that activate the PI3K-AKT-mTOR pathway results in abnormal development of the cortical layers. Hyperactivation of mTOR complex 1 (mTORC1) in neural progenitor cells during brain development can cause disorganized cortical architecture and the formation of dysplastic neurons, which are prone to hyperexcitability and seizure generation. In TSC, germline loss of function mutations in the *TSC1* or *TSC2* genes, which negatively regulate mTORC1, lead to constitutive mTORC1 activation, resulting in the formation of

[5] Bonduelle, T., Hartlieb, T., Baldassari, S., Sim, N. S., Kim, S. H., Kang, H. C., Kobow, K., Coras, R., Chipaux, M., Dorfmüller, G., Adle-Biassette, H., Aronica, E., Lee, J. H., Blumcke, I., & Baulac, S. (2021). Frequent SLC35A2 brain mosaicism in mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE). *Acta neuropathologica communications*, *9*(1), 3.

cortical tubers. The PI3K-AKT-mTOR pathway is also involved in the development of LEATs, such as gangliogliomas and dysembryoplastic neuroepithelial tumors. These tumors can harbor somatic variants that lead to the activation of this pathway, contributing to both tumor growth and the development of seizures.

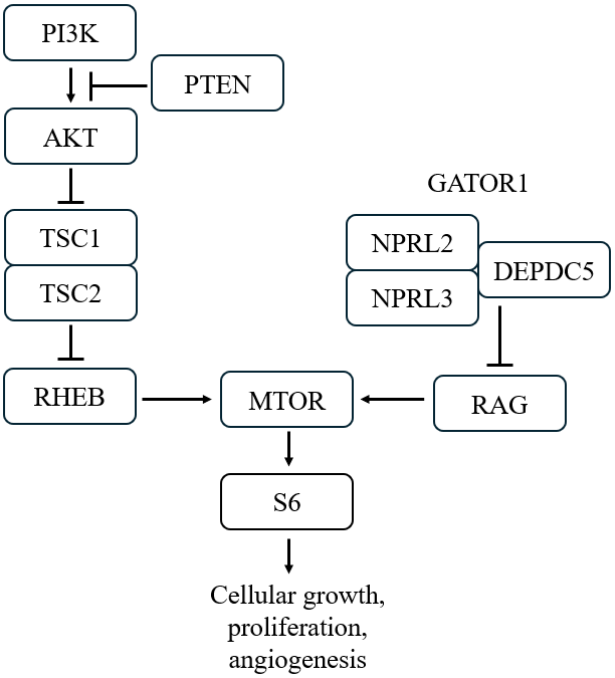

Figure 1. PI3K-AKT-mTOR pathway. GATOR1: GAP activity toward RAGs 1.

1.3. *SLC35A2* and epilepsy

The *SLC35A2* gene encodes a uridine diphosphate galactose (UDP-galactose) translocator, which is responsible for moving UDP-galactose across subcellular membranes into organelles such as the Golgi apparatus and endoplasmic reticulum. Germline mutations in the *SLC35A2* gene lead to *SLC35A2*-related congenital disorders of N-glycosylation. The most common clinical manifestations of this disorder include epilepsy, particularly developmental and epileptic encephalopathy (DEE), such as infantile epileptic spasms syndrome (IESS), as well as developmental delays.

Recently, it has been discovered that somatic variants of *SLC35A2* are also implicated in MCD associated with drug-resistant epilepsy. Upon re-evaluation of brain tissue from patients with somatic *SLC35A2* variants, the findings were consistent with the clinico-pathological entity known as mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE).

The histopathological features of MOGHE include: (1) clusters of increased oligodendrocytes near the grey-white matter junction and in the deep white matter, (2) increased densities of heterotopic neurons within the deep white matter, and (3) patchy areas of hypomyelination in the white matter.

Somatic mutations in the *SLC35A2* gene lead to a loss of function in the UDP-galactose translocator, likely causing abnormal glycosylation. However, the precise mechanisms by which this aberrant glycosylation results in oligodendroglial hyperplasia, hypomyelination, and epilepsy remain unclear.

1.4. Limitations of somatic variant analysis in clinical settings

Despite the growing number of reports on somatic variant detection in epileptogenic human brain tissue, there are no studies that comprehensively compare clinical features based on specific pathogenic somatic variants in epilepsy patients, aside from isolated case reports or series. A major challenge in detecting somatic variants in epilepsy patients is the limited availability of brain tissue, which is typically only obtainable through epilepsy surgery. As a result, genetic diagnoses are usually made post-surgery, meaning that diagnosis occurs only after treatment and, therefore, does not guide clinical management.

Based on current knowledge, DRE due to focal cortical malformation is likely attributable to somatic variants in mTOR pathway genes or *SLC35A2*. If the likely pathogenic genes could be predicted before surgery, patients with mTOR pathway somatic variants might benefit from mTOR inhibitor therapy,[6] while those with somatic *SLC35A2* variants could potentially be treated with galactose supplementation.[7] However, as mentioned above, there are no studies that collectively

[6] Shiraishi, H., Teramoto, T., Yokoshiki, S., Tohyama, J., Ueda, Y., Egawa, K., Sato, N., Manabe, A., & Kato, M. (2023). Efficacy of sirolimus for epileptic seizures in childhood associated with focal cortical dysplasia type II. *Brain & development*, *45*(6), 343–347.

[7] Aledo-Serrano, Á., Valls-Carbó, A., Fenger, C. D., Groeppel, G., Hartlieb, T., Pascual, I., Herraez, E., Cabal, B., García-Morales, I., Toledano, R., Budke, M., Beltran-Corbellini, Á., Baldassari, S., Coras, R., Kobow, K., Herrera, D. M., Del Barrio, A., Dahl, H. A., Del Pino, I., Baulac, S., … Gil-Nagel, A. (2023). D-galactose Supplementation for the Treatment of Mild Malformation of Cortical Development with Oligodendroglial Hyperplasia in Epilepsy (MOGHE): A Pilot Trial of Precision Medicine After Epilepsy Surgery. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*, *20*(5), 1294–1304.

compare clinical features by genotype,[8,9,10] making pre-surgical clinical diagnosis currently unfeasible.

1.5. Objectives of this research

The objectives of this study were threefold: first, to establish an efficient workflow for somatic variant detection from brain tissue obtained after resective epilepsy surgery; second, to build a robust cohort of patients with somatic variants associated with epilepsy; and third, to identify the characteristics associated with different genotypes in epilepsy patients who have undergone epilepsy surgery, with particular attention to those harboring somatic variants in their epileptogenic brain tissue.

[8] Khoshkhoo S, Wang Y, Chahine Y, et al. Contribution of Somatic Ras/Raf/Mitogen-Activated Protein Kinase Variants in the Hippocampus in Drug-Resistant Mesial Temporal Lobe Epilepsy. *JAMA Neurol*. 2023;80(6):578-587.

[9] Bedrosian TA, Miller KE, Grischow OE, et al. Detection of brain somatic variation in epilepsy-associated developmental lesions. *Epilepsia*. 2022;63(8):1981-1997.

[10] López-Rivera JA, Leu C, Macnee M, et al. The genomic landscape across 474 surgically accessible epileptogenic human brain lesions. *Brain*. 2023;146(4):1342-1356.

II. METHODS

2.1. Patients

We included all patients who were diagnosed with epilepsy aged 0 to 18 who were on over three anti-seizure medications (ASMs) and went through resective epilepsy surgery between 2004 and 2022 at Severance Children's Hospital, Seoul, Korea.

All patients underwent a standardized presurgical evaluation protocol at Severance Children's Hospital, which included a comprehensive documentation of clinical history, including current and past seizure semiology, scalp video-electroencephalography (video-EEG), brain magnetic resonance imaging (MRI), brain fluorine-18 fluorodeoxyglucose positron emission tomography computed tomography (18F-FDG PET CT), interictal/ictal single-photon emission computed tomography (SPECT), and neuropsychological testing. In most cases, a two-stage surgical approach was employed—initial placement of subdural grid electrodes or stereo-EEG for intracranial EEG monitoring, followed by resective surgery. Intraoperative electrocorticography was performed for all patients during resective surgery.

The patients had their brain tissues analyzed for detection of somatic variants using deep sequencing. We excluded patients who were confirmed with non-genetic etiologies such as ischemia, intracranial hemorrhage, or infection. We also excluded patients with TSC, LEATs, or hippocampal sclerosis from current study, as these conditions have distinctive clinical features that do not align with the objectives of this study (Figure 2).

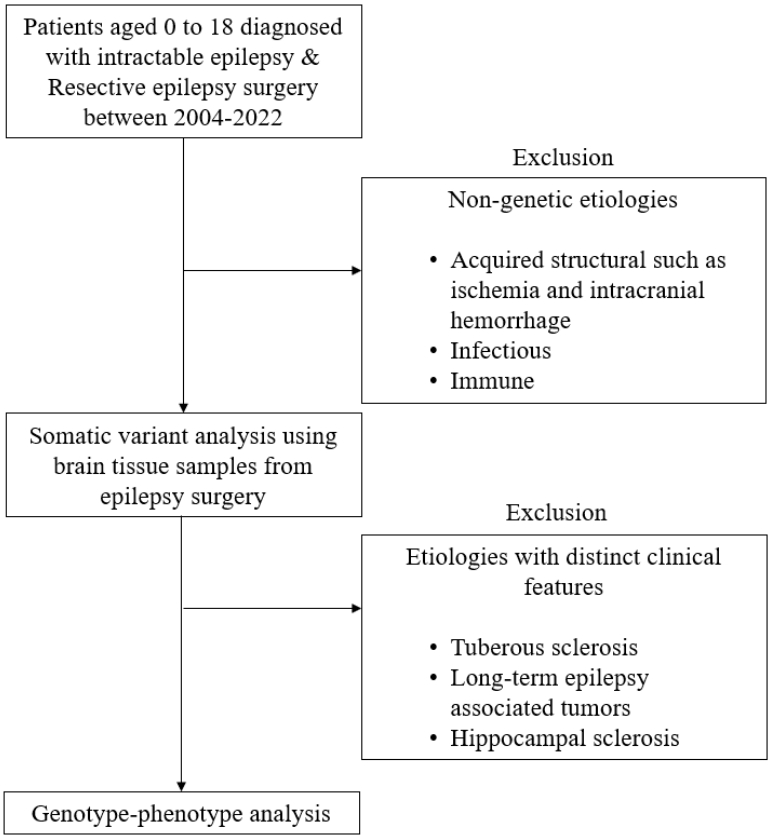

Figure 2. Patients included in this study.

For all patients subjected to somatic variant analyses, basic clinical data such as sex, age at epilepsy surgery, and other relevant details were collected. For patients identified with pathogenic variants—whether germline or somatic—more detailed clinical characteristics were gathered through a thorough review of medical records. This included data on age at seizure onset, number of ASMs, brain MRI findings, EEG findings, neuropsychological test results, epilepsy syndrome, as well as the extent and types of epilepsy surgery performed.

We also collected the data at six months, one year, two years, and three years after surgery, or until the last follow-up period if the patient visited less than three years. We evaluated seizure frequency and semiology and classified the data by International League Against Epilepsy (ILAE) classification for epilepsy surgery outcomes. We monitored number of ASMs, EEG findings, and neuropsychologic test results after surgery if available.

This study was approved by the Institutional Review Board of Severance Hospital. Informed written consent was obtained from the parents or legal guardians of the included patients for the

collection and analysis of brain tissues.

2.2. DNA extraction and targeted sequencing of the samples

2.2.1 DNA extraction from tissue samples

For all patients undergoing resective epilepsy surgery, brain tissue samples from the resected area were fresh-frozen, along with their peripheral blood samples, and stored at -80 °C and -20 °C, respectively, until DNA extraction.

For brain tissue samples weighing more than 250 mg, the phenol-chloroform DNA extraction method was used. Briefly, 250 mg of brain tissue was homogenized using a tissue grinder, then mixed with lysis buffer and incubated at 56 °C overnight. Phenol-chloroform-isoamyl alcohol (25:24:1) was added to the lysed sample, and the upper aqueous phase was obtained after centrifugation. Chloroform-isoamyl alcohol (24:1) was then added to remove any remaining phenol, and the upper aqueous phase was collected after centrifugation. Isopropanol was added, and the sample was centrifuged to obtain a pellet, which was then washed with 70% ethanol. Finally, the DNA pellet was resuspended in elution buffer.

For brain tissue samples weighing 25–250 mg, DNA was extracted from 25 mg of tissue using the QIAamp DNA Mini Kit (QIAGEN, Germany). For brain tissue samples weighing less than 25 mg, DNA was extracted from 10 mg of tissue using the QIAamp DNA Micro Kit (QIAGEN, Germany). The QIAamp DNA Blood Midi Kit (QIAGEN, Germany) was used to extract DNA from peripheral blood samples.

2.2.2 Targeted gene panel sequencing using paired brain-blood sequencing data

For paired analysis, DNA was obtained from both brain tissue and peripheral blood samples, which were then sequenced separately. The 13-gene panel, designed and manufactured by Celemics Inc. (Celemics, Korea), was used for deep hybrid capture sequencing of the new samples (Table 2). Library preparation was performed according to the manufacturer's protocol. The final libraries from the hybrid capture were sequenced using a MiSeq sequencer (Illumina, USA).

Table 2. Gene panels used in deep targeted sequencing for detection of somatic variants in epilepsy patients

Gene panel	Character	Genes	Refe-rences
6-gene panel	Genes in mTOR pathway for genetic evaluation of FCDII patients	*AKT3, DEPDC5, MTOR, PIK3CA, TSC1, TSC2*	[11],[12]
28-gene panel	Genes associated with intractable focal epilepsy	*AKT3, ALG13, BRAF, CACNA1A, CHD2, DCX, DEPDC5, DNM1, EZH2, FASN, FLNA, GABBR2, GABRA1, GABRB3, GRIN1, HNRNPU, IQSEC2, LIS1, MTOR, NEDD4L, PIK3CA, PIK3R2, PTEN, SCN1A, STRADA, TSC1, TSC2, TUBB2B*	[13],[14]
29-gene panel	Genes associated with intractable epilepsy with mMCD or without histopathological lesion	*AKT3, ALG13, BRAF, CACNA1A, CHD2, DCX, DEPDC5, DNM1, EZH2, FASN, FLNA, GABBR2, GABRA1, GABRB3, GRIN1, HNRNPU, IQSEC2, LIS1, MTOR, NEDD4L, PIK3CA, PIK3R2, PTEN, SCN1A, SLC35A2, STRADA, TSC1, TSC2, TUBB2B* (28-gene panel with *SLC35A2)*	[13],[14]
13-gene panel	Genes associated with intractable epilepsy, genes in mTOR pathway plus *SLC35A2*	*AKT1, AKT3, BRAF, DEPDC5, MTOR, NPRL2, NPRL3, PIK3CA, PIK3R2, PTEN, SLC35A2, TSC1, TSC2*	[14]

[11] Lim, J. S., Kim, W. I., Kang, H. C., Kim, S. H., Park, A. H., Park, E. K., Cho, Y. W., Kim, S., Kim, H. M., Kim, J. A., Kim, J., Rhee, H., Kang, S. G., Kim, H. D., Kim, D., Kim, D. S., & Lee, J. H. (2015). Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy. *Nature medicine*, *21*(4), 395–400.

[12] Lim, J. S., Gopalappa, R., Kim, S. H., Ramakrishna, S., Lee, M., Kim, W. I., Kim, J., Park, S. M., Lee, J., Oh, J. H., Kim, H. D., Park, C. H., Lee, J. S., Kim, S., Kim, D. S., Han, J. M., Kang, H. C., Kim, H. H., & Lee, J. H. (2017). Somatic Mutations in TSC1 and TSC2 Cause Focal Cortical Dysplasia. *American journal of human genetics*, *100*(3), 454–472.

[13] Sim, N. S., Seo, Y., Lim, J. S., Kim, W. K., Son, H., Kim, H. D., Kim, S., An, H. J., Kang, H. C., Kim, S. H., Kim, D. S., & Lee, J. H. (2018). Brain somatic mutations in *SLC35A2* cause intractable epilepsy with aberrant N-glycosylation. *Neurology. Genetics*, *4*(6), e294.

[14] Sim, N. S., Ko, A., Kim, W. K., Kim, S. H., Kim, J. S., Shim, K. W., Aronica, E., Mijnsbergen, C., Spliet, W. G. M., Koh, H. Y., Kim, H. D., Lee, J. S., Kim, D. S., Kang, H. C., & Lee, J. H. (2019). Precise detection of low-level somatic mutation in resected epilepsy brain tissue. *Acta neuropathologica*, *138*(6), 901–912.

For all sequencing data, the best practices workflow suggested by the Broad Institute was applied (http://gatk.broadinstitute.org). Raw sequences from Fastq files were aligned to the GRCh37/hg19 or GRCh38/hg38 assembly of the human genome reference sequence using BWA-MEM. Somatic variants found exclusively in the brain, and not in blood or saliva, were identified using Mutect,[15] Strelka,[16] and Mutect2 variant callers. All identified mutations were annotated using the SnpEff program.[17] The following exclusion criteria were applied: 1) variants registered in the public database (dbSNP); 2) variants with a low SnpEff impact score; 3) variants with PolyPhen or SIFT scores indicating that they are not damaging, and a phastCons score of <0.9; and 4) variants with an allele frequency of >0.1% in the ExAC database for minor allele frequencies in East Asian populations.[18]

For all candidate somatic variants, validation amplicon sequencing was performed. Briefly, region-specific primers were designed with the Illumina single index and target sequences were PCR amplified using PrimeSTAR DXL DNA polymerase (Takara, Japan). For a second PCR amplification, 20 ng of purified PCR products from the first amplification was annealed with both Illumina adaptor and index sequences. To verify fragment sizes and the quality of the amplified libraries, individual aliquots were run on a 2100 Bioanalyzer (Agilent, USA). Libraries were pooled and sequenced on a MiSeq sequencer (Illumina, USA). The sequenced data was then manually inspected using the integrative genomics viewer (IGV).

To detect germline mutations, we used GATK HaplotypeCaller. The same filtering process was applied to candidate variants as was used for the candidate somatic variants described above. American College of Medical Genetics and Genomics (ACMG) classification system was used for the interpretation of sequence variants.[19] Variants classified as pathogenic or likely pathogenic

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[15] Cibulskis, K., Lawrence, M. S., Carter, S. L., Sivachenko, A., Jaffe, D., Sougnez, C., Gabriel, S., Meyerson, M., Lander, E. S., & Getz, G. (2013). Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nature biotechnology*, *31*(3), 213–219.

[16] Saunders, C. T., Wong, W. S., Swamy, S., Becq, J., Murray, L. J., & Cheetham, R. K. (2012). Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics (Oxford, England)*, *28*(14), 1811–1817.

[17] Cingolani, P., Platts, A., Wang, leL., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., & Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly*, *6*(2), 80–92.

[18] Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., O'Donnell-Luria, A. H., Ware, J. S., Hill, A. J., Cummings, B. B., Tukiainen, T., Birnbaum, D. P., Kosmicki, J. A., Duncan, L. E., Estrada, K., Zhao, F., Zou, J., Pierce-Hoffman, E., Berghout, J., Cooper, D. N., … Exome Aggregation Consortium (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, *536*(7616), 285–291.

[19] Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine : official journal of the American College of Medical Genetics*, *17*(5), 405–424.

according to the ACMG guidelines were selected as causative variants.

2.2.3 Targeted gene panel sequencing using brain tissue only

In this study, patients were simultaneously subjected to unpaired analyses using DNA from brain tissue only. This approach was employed to evaluate and compare the efficacy of unpaired analyses against paired analyses (Appendix 1).

For unpaired analysis, the DNA sample from each patient was divided into two, and replicate DNA samples from brain tissues were sequenced separately by the 13-gene panel. Library preparation was performed according to the manufacturer's protocol, and the libraries were sequenced using a MiSeq sequencer (Illumina, USA).

The downstream bioinformatic analysis was conducted in the same manner as the paired sample analysis described in the previous section. For variant callers, MuTect STD mode and RePlow, the computational method devised for accurate detection of low-level somatic mutation, were used.[20] Germline variant analysis was performed using HaplotypeCaller, and validation using amplicon sequencing was performed.

2.3. Establishing a large cohort of somatic variants associated with intractable epilepsy

2.3.1 Inclusion of previous somatic variant analysis data

To build a large and robust cohort of epilepsy patients with somatic variants, we collected data from patients who were enrolled in the previous studies.[11,12,13,14] The patients underwent deep sequencing analysis of brain tissue samples obtained during resective epilepsy surgery. Paired brain-peripheral tissue analyses were conducted using either peripheral blood or saliva to obtain DNA from peripheral tissues.

Deep targeted hybridization sequencing or targeted gene panel sequencing was performed using one of the following gene panels: 1) 6-gene panel, 2) 28-gene panel, 3) 29-gene panel, or 4) 13-gene panel (Table 2). The targeted gene hybrid capture sequencing panels for the 6-, 28-, and 29-gene sets (the 28-gene set and *SLC35A2*) were constructed using SureDesign online tools (SureSelect DNA standard wizard, Agilent Technologies, USA). The 13-gene targeted hybrid capture sequencing panel was designed and manufactured by Celemics Inc. (Celemics, Korea). If paired tissue samples or DNA were available, patients with a histopathological diagnosis of mild MCD or non-specific

[20] Kim, J., Kim, D., Lim, J. S., Maeng, J. H., Son, H., Kang, H. C., Nam, H., Lee, J. H., & Kim, S. (2019). The use of technical replication for detection of low-level somatic mutations in next-generation sequencing. *Nature communications*, *10*(1), 1047.

gliosis who had previously undergone 6- or 28-gene panel sequencing were reanalyzed using the 13-gene panel to search for the presence of somatic *SLC35A2* variants.[14]

Library preparation was carried out according to the manufacturers' protocols. The final hybrid capture libraries were sequenced on a HiSeq 2500 sequencer (Illumina, USA) by Macrogen (Korea) and on a MiSeq Dx sequencer (Illumina, USA) by Sovargen (Korea).

For all sequencing data, the best practices workflow suggested by the Broad Institute was applied (http://gatk.broadinstitute.org). Somatic variants found exclusively in the brain, and not in blood or saliva, were identified using Mutect, Strelka, and Mutect2 variant callers, and annotated using the SnpEff program. The following exclusion criteria were applied: 1) variants registered in the public database (dbSNP); 2) variants with a low SnpEff impact score; 3) variants with PolyPhen or SIFT scores indicating that they are not damaging, and a phastCons score of <0.9; and 4) variants with an allele frequency of >0.1% in the ExAC database for minor allele frequencies in East Asian populations.

For germline mutations, the GATK HaplotypeCaller was used. All identified variants were annotated and filtered as described above, with pathogenic or likely pathogenic variants selected according to ACMG guidelines. All candidate variants from each analysis were validated using targeted amplicon sequencing.

2.3.2. MCD gene panel test

The MCD gene panel test using peripheral blood samples was performed in a subset of patients during their initial epilepsy workup. A total of 96 genes associated with neuronal development were included in the MCD gene panel to identify the presence of germline mutations contributing to the development of epileptogenic MCD (Appendix 2).

DNA library preparation was performed according to the manufacturer's protocol. Genomic DNA was extracted from leukocytes of whole blood samples using the QIAamp Blood DNA Mini Kit (QIAGEN, Germany). After DNA extraction, each sample was fragmented and amplified using polymerase chain reaction (PCR) for library preparation. The pooled DNA library was then sequenced using the MiSeq sequencer (Illumina Inc., San Diego, CA, USA) and the MiSeq Reagent Kit v2 (300 cycles). Sequenced data were analyzed using BaseSpace (Illumina Inc., USA) and NextGENE (SoftGenetics LLC, USA), and cross-checked with the custom analysis pipeline of the Department of Laboratory Medicine at Severance Hospital. Copy number variants were also analyzed using this custom pipeline. Databases used for analysis and variant annotation were Online Mendelian Inheritance in Man (OMIM), Human Gene Mutation Database, ClinVar, dbSNP, 1000 Genome, Exome Aggregation Consortium, Exome Sequencing Project, and Korean Reference Genome Database.

ACMG classification system was used for the interpretation of sequence variants. Variants classified as pathogenic or likely pathogenic were selected as causative variants for cortical malformations, and these variants were further confirmed by Sanger sequencing.

All patients who underwent MCD panel tests with their blood samples were subjected to somatic

variant analysis, regardless of the initial result, to search for possible second-hit mutations in patients with identified pathogenic germline mutations.

2.4. Detection of low-level somatic mutation by variant enrichment for MOGHE

Previous research[21] demonstrated that variants of genes in the mTOR pathway in FCD type II (FCDII) brain tissue can be enriched by isolating phospho-S6 positive cells—indicative of mTOR hyperactivation—and then analyzing DNA from these cells. This approach allowed for the successful identification of pathogenic mutations with very low variant allele frequencies (VAFs).

In the context of MOGHE, a study by Bonduelle et al.[5] compared VAFs of somatic *SLC35A2* variants across different cell types in a MOGHE patient. In the study, Olig2-positive glial cells exhibited the highest VAF for somatic *SLC35A2* mutations. In the study, laser capture microdissection (LCM) was used to isolate different cell types. In our research, we used fluorescence-activated nuclei sorting (FANS) instead to collet Olig2-positive nuclei because of the labor-intensive and time-consuming nature of LCM.

Minced fresh-frozen brain tissue samples were lysed with lysis buffer. After fixation with 1% paraformaldehyde, the lysed tissue samples were filtered using a cell strainer and stained with mouse monoclonal anti-NeuN antibody (1:1000 dilution, Millipore, USA) and rabbit monoclonal anti-Olig2 antibody (1:500 dilution, Abcam, UK), followed by incubation at 4 ℃ overnight. The cells were then washed with phosphate-buffered saline (PBS) and labeled with anti-mouse Alexa Fluor 488 (1:500 dilution, Thermo Fisher Scientific, USA), anti-rabbit Alexa Fluor 633 (1:500 dilution, Thermo Fisher Scientific, USA), and 4', 6-diamidino-2-phenylindole (DAPI, 0.1 μg/mL).

Immunolabeled nuclei were sorted using the Moflo Astrios EQ (Beckman Coulter, USA). To set up the sorting conditions of the instrument, control samples were used: unstained, DAPI-only stained, anti-NeuN and DAPI stained, and anti-Olig2 and DAPI stained. The unstained sample was analyzed first, followed by the DAPI-stained sample. Subsequently, the anti-NeuN and DAPI-stained or anti-Olig2 and DAPI-stained samples were used to compensate for fluorescence overlap in neighboring channels. After setup, test samples were sorted, and only the nuclei that were DAPI-positive, Olig2-positive, and NeuN-negative were collected.

DNA was extracted from the collected nuclei using the QIAamp DNA Micro Kit (QIAGEN, Germany). Replicate DNA samples were then subjected to whole genome amplification, which was conducted using the ResolveOME Whole Genome and Transcriptome Amplification Kit (BioSkryb, USA). The amplified DNA samples were individually deep sequenced using the 13-gene panel. Library preparation was performed according to the manufacturer's protocol, and the libraries were sequenced using a MiSeq sequencer (Illumina, USA).

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[21] Kim, J. H., Park, J. H., Lee, J., Park, J. W., Kim, H. J., Chang, W. S., Kim, D. S., Ju, Y. S., Aronica, E., & Lee, J. H. (2023). Ultra-Low Level Somatic Mutations and Structural Variations in Focal Cortical Dysplasia Type II. *Annals of neurology*, *93*(6), 1082–1093.

The downstream bioinformatic analysis was conducted in the same manner as the paired sample analysis described in the previous section, with the exception that MuTect STD mode and RePlow were used as somatic variant callers instead of MuTect2 and Strelka. Validation using amplicon sequencing was performed on unamplified DNA.

III. RESULTS

3.1. Patients

A total of 321 brain tissue samples from resective epilepsy surgeries were analyzed for the presence of somatic variants. Patients who underwent more than one surgery were counted as a single case. Of these, 41 patients with TSC, 30 patients with LEATs, and 12 patients with hippocampal sclerosis were excluded from the present study, leaving 238 patients included in the analysis (Figure 3). Among the 41 TSC patients, 8 (19.5%) had germline *TSC1* variants, and 19 (46.3%) had germline *TSC2* variants. Of the 30 patients with LEATs, 11 (36.7%) with ganglioglioma had somatic *BRAF* p.Val600Glu (V600E) variants.

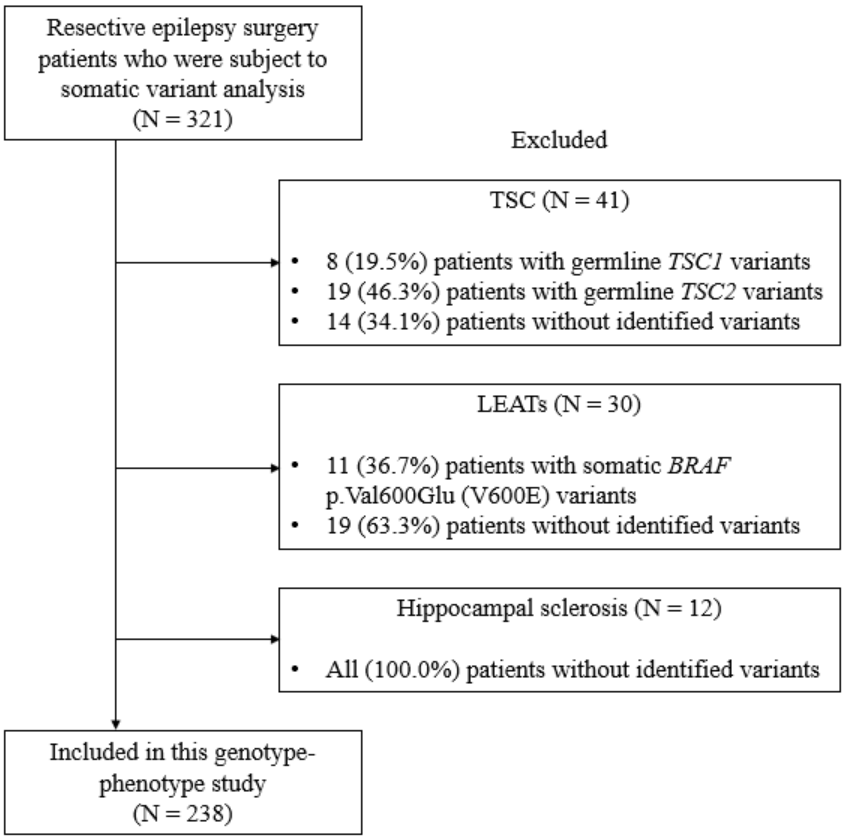

Figure 3. Overview of included and excluded patients in this study among those who underwent resective epilepsy surgery and somatic variant analysis using brain tissue

samples. TSC: tuberous sclerosis, LEAT: long-term epilepsy associated tumors.

Among the 238 patients included in this study, 132 patients (55.5%) were boys. Median age at surgery was 7.6 (interquartile range [IQR], 3.9–12.1) years. Among the 238 patients, 50 had undergone MCD gene panel testing with their peripheral blood samples prior to the presurgical evaluation for epilepsy surgery. Of these 50 patients, eight (16.0%) were identified with pathogenic or likely pathogenic variants (Table 3). Of the eight patients, four had variants in the *DEPDC5* gene, three had variants in the *NPRL3* gene, and one had a microdeletion encompassing both the *MPG* and *NPRL3* genes, with the *NPRL3* gene assumed to play a key role in the development of FCD.

Of the four patients with germline *DEPDC5* variants, three exhibited pathological findings consistent with FCD type IIa (FCDIIa), while 1 patient had HME. Similarly, all four patients with germline *NPRL3* variants were diagnosed with FCDII, with three patients having FCDIIa and one patient having FCD type IIb (FCDIIb).

As previously mentioned, all 50 patients who underwent MCD gene panel testing with their peripheral blood samples also underwent deep targeted gene panel sequencing using their brain tissue to investigate the presence of somatic variants. The eight patients identified with either *DEPDC5* or *NPRL3* germline variants were also tested with deep panel sequencing—two with the 28-gene panel and six with the 13-gene panel—but no additional somatic variants were identified in these eight patients.

Table 3. Variants identified by MCD gene panel testing using peripheral blood samples

ID	Genes	Nucleotide change	Protein change	Pathological diagnosis
S282	*DEPDC5**	Exon 1–27 deletion		FCD IIa
S321	*DEPDC5**	Exon 5 deletion		FCD IIa
S047	*DEPDC5*	c.1114C>T	p.Gln372Ter	FCD IIa
S308	*DEPDC5**	c.3589C>T	p.Gln1197Ter	HME
S254	*NPRL3**	c.188+1G>A		FCD IIa
S292	*NPRL3**	Exon 8 deletion		FCD IIa
S013	*NPRL3*	Exon 9 deletion		FCD IIb
S214	*NPRL3*	Exon 14 partial deletion		FCD IIa

*Unpublished

FCDIIa: focal cortical dysplasia type IIa, HME: hemimegalencephaly, FCDIIb: focal cortical dysplasia type IIb.

3.2. Deep targeted panel sequencing

Of the 238 patients, deep targeted hybridization sequencing was conducted on 33 patients using the 6-gene panel, 73 patients using the 28-gene panel, 18 patients using the 29-gene panel, and 114 patients using the 13-gene panel. In this study, 62 patients underwent the 13-gene panel test, and underwent both paired brain-blood sample analysis and unpaired brain sample analysis simultaneously (Figure 4).

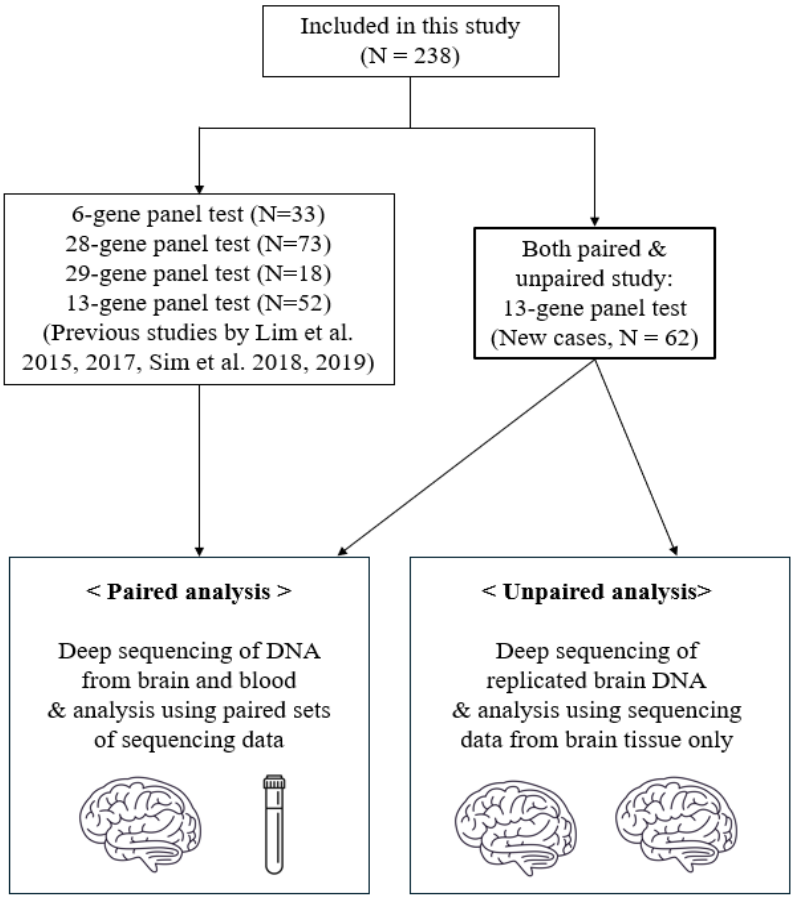

Figure 4. Deep targeted hybridization sequencing and panels used in this study.

Using the panels, 80 patients (33.6%) were identified with pathogenic variants, including eight patients identified through MCD gene panel tests prior to somatic variant analyses. In addition to the eight patients with germline variants detected by MCD gene panel tests, seven more patients

with germline variants in the *DEPDC5* gene were identified, bringing the total number of patients with pathogenic germline variants to 15—11 with germline *DEPDC5* variants and four with germline *NPRL3* variants. One patient with a germline *DEPDC5* variant also had a second somatic variant in the *DEPDC5* gene, with a VAF of 4.5%. Among the seven additional patients with germline *DEPDC5* variants, three had FCDIIa, two had FCDIIb, one had mild MCD (mMCD), and one showed gliosis on their pathology (Table 4).

Table 4. Additional germline variants identified by deep targeted sequencing

ID	Genes	Nucleotide change	Protein change	Pathological diagnosis
S010	*DEPDC5*	c.3021+1G>A		mMCD
S229	*DEPDC5*	c.1324+1G>A		Gliosis
S055	*DEPDC5*	c.3639G>A	p.Trp1213Ter	FCD IIa
S076	*DEPDC5*	c.3802C>T	p.Arg1268Ter	FCD IIa
S152	*DEPDC5*	c.3406A>T	p.Arg1136Ter	FCD IIa
S086	*DEPDC5*†	c.4521_4522delAA	p.Thr1508fs	FCD IIb
S296	*DEPDC5**	c.2767C>T	p.Gln923Ter	FCD IIb

*Unpublished

†This patient had a second somatic *DEPDC5* variant of c.4162_4169dupGTACTCTT (p.Phe1399fs) with a VAF of 4.5%.

mMCD: mild malformation of cortical development, FCDIIa: focal cortical dysplasia type IIa, FCDIIb: focal cortical dysplasia type IIb.

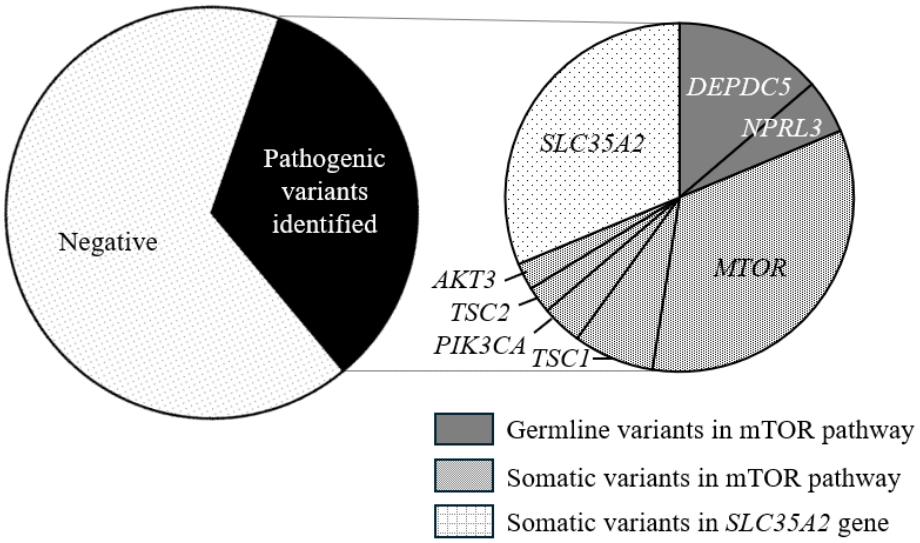

Figure 5. Pathogenic variants identified in pediatric epilepsy patients included in this study.

Among the 238 patients included in the study, 80 (33.6%) were identified with pathogenic variants—15 (6.3%) had pathogenic germline variants, as described above, and 65 (27.3%) had pathogenic somatic variants (Figure 5), excluding the patient with an additional somatic *DEPDC5* variant found concomitantly with the germline *DEPDC5* variant, as detailed in Table 4. Of the 65 somatic variants, 27 were in the *MTOR* gene, 25 in *SLC35A2*, six in *TSC1*, three in *PIK3CA*, two in *TSC2*, and two in *AKT3* (Table 5). One patient with *AKT3* mutation also had variant c.1871G>A in *MTOR* gene, and *AKT3* mutation was considered to be pathogenic for the patient's disease.

Among the 40 patients with somatic variants in mTOR pathway genes, 34 (85.0%) had a pathological diagnosis of FCDII—17 (42.5%) FCDIIa and 17 (42.5%) FCDIIb. The remaining cases included two patients (5.0%) with HME, two (5.0%) with gliosis, one (2.5%) with polymicrogyria (PMG), and one (2.5%) with mMCD. Of the 25 patients with somatic *SLC35A2* variants, 17 (68.0%) had pathology consistent with MOGHE, followed by six patients (24.0%) with gliosis, one (4.0%) with mMCD, and one (4.0%) with normal pathology.

Table 5. Somatic variants identified by deep targeted sequencing

ID	Genes	Nucleotide change	Protein change	VAF (%)	Pathology
S201	*AKT3*	c.49G>A	p.Glu17Lys	4.0	FCDIIa
S078	*AKT3*†	c.49G>A	p.Glu17Lys	0.3	FCDIIa
S094	*MTOR*	c.4348T>G	p.Tyr1450Asp	3.8	FCDIIb
S307	*MTOR**	c.4356_4361dup ACTGCA	p.Leu1453_His 1454insGlnLeu	1.1	FCDIIa
S080	*MTOR*	c.4366T>G	p.Trp1456Gly	1.7	FCDIIb
S153	*MTOR*	c.4376C>A	p.Ala1459Asp	4.7	FCDIIb
S015	*MTOR*	c.4379T>C	p.Leu1460Pro	15.2	FCDIIb
S168	*MTOR*	c.4379T>C	p.Leu1460Pro	3.8	FCDIIa
S285	*MTOR**	c.4379T>C	p.Leu1460Pro	2.8	Gliosis
S101	*MTOR*	c.4447T>C	p.Cys1483Arg	6.6	FCDIIb
S154	*MTOR*	c.4448G>A	p.Cys1483Tyr	9.8	FCDIIb
S079	*MTOR*	c.5126G>A	p.Arg1709His	1.5	FCDIIa
S265	*MTOR**	c.5930C>A	p.Thr1977Lys	3.3	FCDIIa
S090	*MTOR*	c.5930C>A	p.Thr1977Lys	2.9	FCDIIb
S111	*MTOR*	c.5930C>A	p.Thr1977Lys	1.5	FCDIIb
S068	*MTOR*	c.5930C>A	p.Thr1977Lys	1.3	FCDIIa
S065	*MTOR*	c.6400C>T	p.Arg2134Trp	1.0	FCDIIa
S066	*MTOR*	c.6577C>T	p.Arg2193Cys	1.3	FCDIIa
S295	*MTOR**	c.6644C>A	p.Ser2215Tyr	1.1	FCDIIb
S223	*MTOR*†	c.6644C>A	p.Ser2215Tyr	0.2	FCDIIb
S174	*MTOR*	c.6644C>T	p.Ser2215Phe	23.4	PMG
S248	*MTOR**	c.6644C>T	p.Ser2215Phe	8.3	FCDIIb
S149	*MTOR**	c.6644C>T	p.Ser2215Phe	3.2	FCDIIa
S109	*MTOR*	c.6644C>T	p.Ser2215Phe	2.3	FCDIIa
S081	*MTOR*	c.6644C>T	p.Ser2215Phe	2.1	FCDIIa
S037	*MTOR*†	c.6644C>T	p.Ser2215Phe	0.0	FCDIIb
S087	*MTOR*	c.7280T>A	p.Leu2427Gln	2.9	FCDIIb
S001	*MTOR*	c.7280T>C	p.Leu2427Pro	12.6	FCDIIa
S003	*MTOR*	c.7280T>C	p.Leu2427Pro	7.3	FCDIIa
S312	*PIK3CA**	c.1624G>A	p.Glu542Lys	30.1	mMCD
S241	*PIK3CA**	c.1624G>A	p.Glu542Lys	18.3	HME
S219	*PIK3CA*	c.1633G>A	p.Glu545Lys	27.0	HME
S034	*SLC35A2**	c.140A>T	p.Asn47Ile	18.8	MOGHE
S271	*SLC35A2**	c.218T>A	p.Met73Lys	26.3	Gliosis
S031	*SLC35A2*	c.275-1G>T		28.5	MOGHE

S286	*SLC35A2*[*]	c.359_360delTC	p.Leu120fs	1.8	MOGHE
S189	*SLC35A2*	c.359T>C	p.Leu120Pro	4.0	MOGHE
S317	*SLC35A2*[*]	c.436C>T	p.Gln146*	11.4	MOGHE
S249	*SLC35A2*[*]	c.443_444delTC	p.Leu148fs	5.0	MOGHE
S169	*SLC35A2*	c.502C>T	p.Gln168*	18.0	MOGHE
S300	*SLC35A2*[*]	c.502C>T	p.Gln168*	11.1	MOGHE
S163	*SLC35A2*[*]	c.502C>T	p.Gln168*	2.0	mMCD
S301	*SLC35A2*[*]	c.502C>T	p.Gln168*	1.1	Gliosis
S112	*SLC35A2*	c.553C>T	p.Gln185*	29.5	MOGHE
S255	*SLC35A2*[*]	c.553C>T	p.Gln185*	1.8	Gliosis
S293	*SLC35A2*[*‡]	c.553C>T	p.Gln185*	0.1	MOGHE
S140	*SLC35A2*	c.589C>T	p.Gln197*	27.1	MOGHE
S273	*SLC35A2*[*]	c.629G>A	p.Ser210Tyr	18.7	Gliosis
S294	*SLC35A2*[*]	c.634_635delTC	p.Ser212fs	10.3	Gliosis
S236	*SLC35A2*[*‡]	c.665_667delAGA	p.Lys222del	0.7	MOGHE
S235	*SLC35A2*	c.671T>C	p.Leu224Pro	3.7	MOGHE
S009	*SLC35A2*	c.703A>C	p.Asn235His	10.0	MOGHE
S115	*SLC35A2*	c.760G>T	p.Glu254*	15.8	MOGHE
S234	*SLC35A2*	c.842G>A	p.Gly281Asp	3.7	MOGHE
S028	*SLC35A2*[*]	c.844G>A	p.Gly282Arg	29.5	Gliosis
S269	*SLC35A2*[*]	c.844G>A	p.Gly282Arg	21.1	MOGHE
S191	*SLC35A2*[*]	c.844G>A	p.Gly282Arg	8.6	Normal
S057	*TSC1*	c.64C>T	p.Arg22Trp	2.0	FCDIIa
S073	*TSC1*	c.64C>T	p.Arg22Trp	2.0	FCDIIa
S096	*TSC1*	c.64C>T	p.Arg22Trp	1.4	FCDIIb
S042	*TSC1*	c.610C>T	p.Arg204Cys	1.0	FCDIIa
S224	*TSC1*	c.1525C>T	p.Arg509*	1.0	FCDIIb
S124	*TSC1*	c.2074C>T	p.Arg692*	3.9	FCDIIb
S274	*TSC2*[*]	c.2714G>A	p.Arg905Gln	1.2	Gliosis
S069	*TSC2*	c.4639G>A	p.Val1547Ile	1.6	FCDIIa

[*]Unpublished

[†]Variants identified after variant enrichment by collecting DNA from phospho-S6 positive neurons. The VAFs of the bulk tissue are shown in the table. The VAFs of phospho-S6 positive neurons were 9% in patient S078, 12.1% in patient S223, and 8.7% in patient S037, respectively.

[‡]Variants identified after variant enrichment by collecting DNA from Olig2-positive and NeuN-negative nuclei. The VAFs of the bulk tissue are shown in the table. The VAFs of Olig2-positive and NeuN-negative nuclei were 1.5% in patient S293 and 7.9% in patient S236, respectively.

FCDIIa: focal cortical dysplasia type IIa, FCDIIb: focal cortical dysplasia type IIb, PMG:

polymicrogyria, mMCD: mild malformation of cortical development, HME: hemimegalencephaly, MOGHE: mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy.

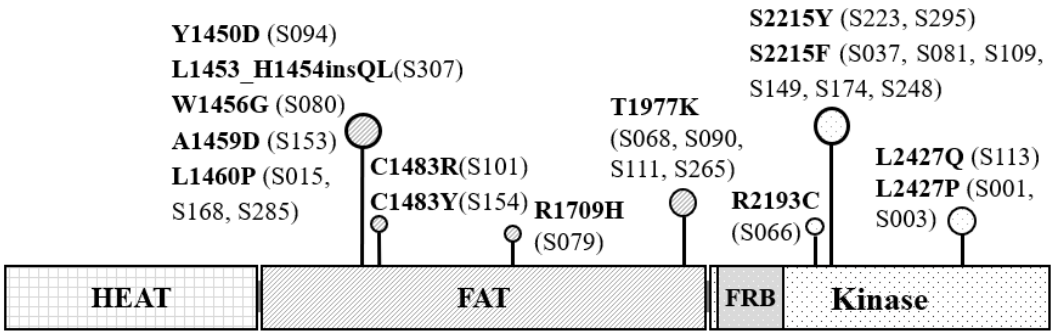

Figure 6. Mutations of the *MTOR* gene detected in 27 patients. The HEAT domain is shortened in this figure.

The somatic or germline variants of the patients are described on Table 5.

The 27 patients had somatic variants of *MTOR* gene on 13 loci. Nine loci were involved in encoding the protein's FAT domain, and four loci were responsible for encoding the kinase domain of MTOR gene (Figure 6). Among the loci, there were four hotspots where multiple patients had the mutation on the loci. Most frequent locus was c.6644C>T (six patients) and c.6644C>A (two patients). Both somatic variants are curated as pathogenic in ClinVar, and also frequently noted in previous study of Baldassari et al 2019.[22] There were four patients with c.5930C>A mutations (p.Thr1977Lys). There are multiple reports of patients with FCDII having somatic variants.[11] There were three patients with c.7280T>A or c.7280T>C. The pathogenicity of both mutations was first reported in the previous study. The variant c.7280T>C was also curated in ClinVar as pathogenic for mosaic overgrowth syndrome with or without cerebral malformations. Three patients had somatic mutations of c.4379T>C, of which the pathogenicity was previously reported in patients with FCDIIb.[23]

The 25 patients had somatic mutations of *SLC35A2* gene on 17 loci. There were three hotspots.

[22] Baldassari, S., Ribierre, T., Marsan, E., Adle-Biassette, H., Ferrand-Sorbets, S., Bulteau, C., Dorison, N., Fohlen, M., Polivka, M., Weckhuysen, S., Dorfmüller, G., Chipaux, M., & Baulac, S. (2019). Dissecting the genetic basis of focal cortical dysplasia: a large cohort study. *Acta neuropathologica*, *138*(6), 885–900.

[23] Nakashima, M., Saitsu, H., Takei, N., Tohyama, J., Kato, M., Kitaura, H., Shiina, M., Shirozu, H., Masuda, H., Watanabe, K., Ohba, C., Tsurusaki, Y., Miyake, N., Zheng, Y., Sato, T., Takebayashi, H., Ogata, K., Kameyama, S., Kakita, A., & Matsumoto, N. (2015). Somatic Mutations in the MTOR gene cause focal cortical dysplasia type IIb. *Annals of neurology*, *78*(3), 375–386.

Four patients had mutation c.502C>T (p.Gln168*). There is no report on pathogenicity of this locus, yet there is one case of germline mutation c.500_509del related to epileptic encephalopathy (ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/variation/374238/). Three patients had mutation on c.553C>T (p.Gln185*), of which there is no previous report. Three patients had mutation on c.844G>A (p.Gly282Arg), of which the germline mutation (c.844G>C, p.Gly282Arg) had pathogenicity confirmed in patients with IESS.

The somatic mutation c.49G>A on *AKT3* gene of two patients is curated as pathogenic in ClinVar database for mosaic overgrowth syndrome with or without cerebral malformations due to abnormalities in MTOR-pathway genes. Also, the mutation was reported in several patients with HME.

Three patients had somatic *PIK3CA* mutations across two loci. Two patients had c.1624G>A (p.Glu542Lys) and one patient had c.1633G>A(p.Glu545Lys). Both somatic mutations had pathogenicity confirmed in PIK3CA related overgrowth syndrome and the latter mutation is cited in previous studies for the cerebral involvement of the mutation.

Four patients had somatic mutations of *TSC1* gene and two patients had somatic mutations of TSC2 gene. The somatic mutations of *TSC1* c.64C>T and c.610C>T, and of *TSC2* c.4639G>A, were reported as pathogenic in previous study of our data.[12] There are no other reports of pathogenicity regarding the somatic mutations of these genes.

3.3. Validation of unpaired analysis for somatic variant detection

In this study, 62 patients underwent simultaneous paired and unpaired deep targeted sequencing (Figure 4). Identical pathogenic variants were identified in 25 patients in both paired and unpaired analyses. The unpaired analyses produced more raw variant calls, with a total of 91 raw calls (364%) compared to 65 raw calls (260%) in the paired analyses. However, after validation with amplicon sequencing, sequencing errors and artifacts were successfully removed, resulting in identical final calls of pathogenic variants in both analyses. Therefore, the performance of the unpaired analysis matched that of the paired analysis, validating its effectiveness in detecting low-frequency somatic variants present in brain tissue in pediatric epilepsy patients with MCD.

3.4. Detection of low-VAF *SLC35A2* variants after variant enrichment

To enhance the detection of low-VAF *SLC35A2* variants, a variant enrichment strategy was employed. This strategy involved isolating specific cell populations that are more likely to harbor somatic mutations with low VAF. As previously published, in patients S037 and S223, low-VAF *MTOR* somatic variants were identified after variant enrichment by collecting phospho-S6 and NeuN positive cells. The VAFs for the identified *MTOR* variants were 0% in bulk tissue but increased to 8.7% after enrichment in patient S037, and 0.2% in bulk tissue compared to 12.1%

after enrichment in patient S223.

For the enrichment of *SLC35A2* variants, we referred to the previous study by Bonduelle et al.,[5] which compared VAFs of *SLC35A2* variants in a patient with MOGHE. In that study, cells were selected using LCM, and the VAF was highest in Olig2-positive glial cells, followed by other glial cells, heterotopic neurons, bulk tissue, and was lowest in cortical neurons. Since cell collection via LCM is time-consuming and labor-intensive, an alternative method was developed for *SLC35A2* variant enrichment by collecting Olig2-positive cells.

A method for collecting DAPI-positive, Olig2-positive, and NeuN-negative nuclei was developed using fresh frozen brain tissue (Figure 7). After dissociating the brain tissue into nuclei and performing immunolabeling, FANS was employed to select Olig2-positive nuclei using the following gating strategy: 1) nuclei were gated using side scatter (SSC) and forward scatter (FSC), 2) Singlet nuclei were gated using the nuclei's height and area, 3) Nuclei were gated with DAPI staining using, 4) NeuN-negative nuclei were gated, 5) Olig2-positive nuclei were gated, 6) finally, Olig2-positive and also NeuN-negative nuclei were gated using Alexa Fluor 488-labeled nuclei as X-axis and Alexa Fluor 633-labeled nuclei as Y-axis (Figure 8).

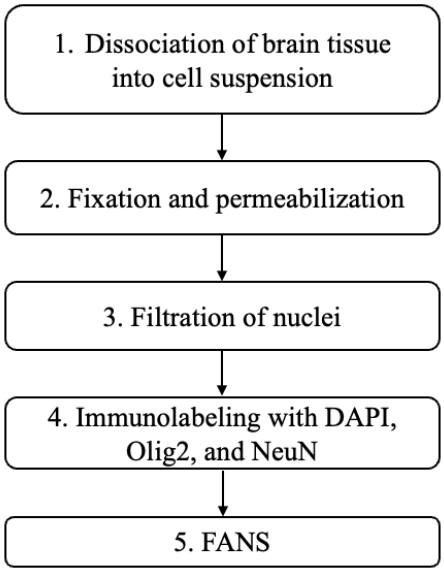

Figure 7. Optimization of tissue preparation.
DAPI: 4', 6-diamidino-2-phenylindole, FANS: fluorescence-activated nuclei sorting.

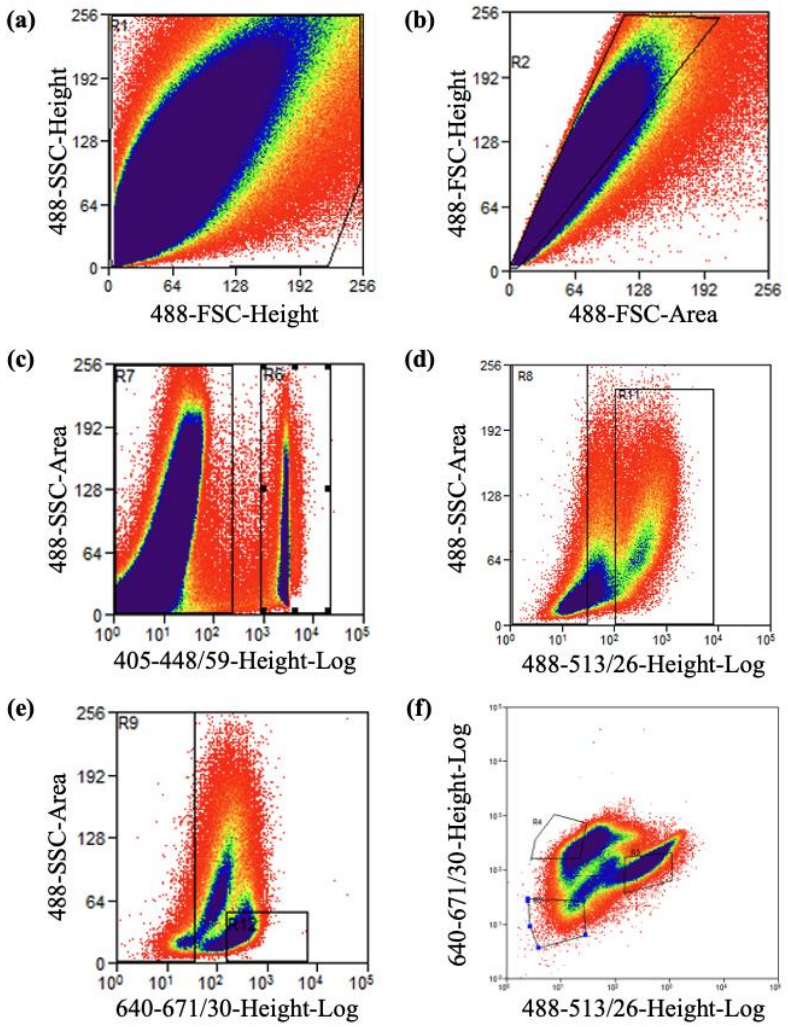

Figure 8. Gating strategy for dissociated nuclei from the brain tissue of patient S140. (a) Linear plot of all events, where nuclei are gated using forward scatter (FSC) and side scatter (SSC). (b) Singlet gating, with singlet nuclei gated using FSC height and area. (c) 4', 6-diamidino-2-phenylindole (DAPI) gating, selecting DAPI-positive nuclei from the singlet nuclei. (d) Neuron gating, with Alexa Fluor 488-labeled NeuN-positive nuclei (X-axis) gated within singlet DAPI-positive nuclei. (e) Oligodendrocyte gating, with Alexa Fluor 633-labeled Olig2-positive nuclei (X-axis) gated within singlet DAPI-positive nuclei. (f) Olig2-positive and NeuN-negative nuclei gating, visualizing and selecting Olig2-positive and NeuN-negative nuclei using Alexa Fluor 488-labeled NeuN-positive nuclei (X-axis) and Alexa Fluor 633-labeled Olig2-positive nuclei (Y-axis).

This method was validated using brain tissue from two MOGHE patients with known somatic *SLC35A2* variants—patients S140 and S169. For each patient, Olig2-positive (DAPI-positive and NeuN-negative), NeuN-positive (DAPI-positive and Olig2-negative), and Olig2-negative and NeuN-negative (DAPI-positive) nuclei were collected, and the VAFs of *SLC35A2* variants in each nuclear population were compared (Figure 9). For patient S140, with a VAF of 23.0% for the somatic *SLC35A2* variant (c.589C>T; p.Gln197*) in the bulk tissue, Olig2-positive nuclei showed an increased VAF of 40.5%, while NeuN-positive and Olig2-negative/NeuN-negative nuclei showed decreased VAFs of 1.6% and 1.4%, respectively. Similarly, for patient S169 (*SLC35A2* c.502C>T; p.Gln168*), with a bulk VAF of 18.0%, the VAF in Olig2-positive nuclei increased to 35.2%, while NeuN-positive and Olig2-negative/NeuN-negative nuclei showed decreased VAFs of 0.4% and 4.6%, respectively. Therefore, the variants were successfully enriched in both patients.

		Collected nuclei	Acquired DNA	VAF (%)
S140	**Bulk tissue**			23.0
	Olig2(+)	18,351	41.4 ng	40.5
	NeuN(+)	36,997	87.0 ng	1.6
	Olig2/NeuN(-/-)	10,591	26.7 ng	1.4

		Collected nuclei	Acquired DNA	VAF (%)
S169	**Bulk tissue**			18.0
	Olig2(+)	21,752	60.6 ng	35.2
	NeuN(+)	51,034	96.0 ng	0.4
	Olig2/NeuN(-/-)	10,244	30.6 ng	4.6

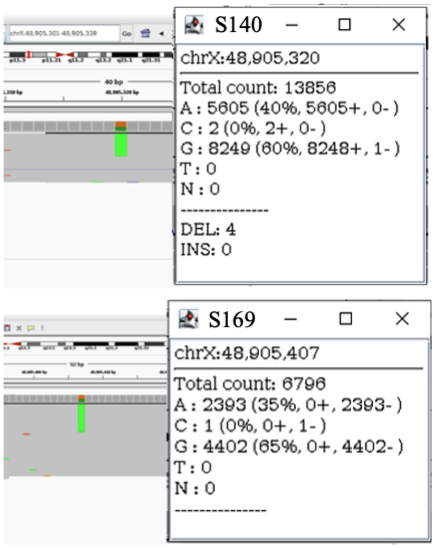

Figure 9. Validation of somatic *SLC35A2* variant enrichment method.

Five patients with a pathological diagnosis of MOGHE, but without identified pathogenic variants, were subjected to variant enrichment. Of these five patients, two (40.0%) were further identified with pathogenic somatic *SLC35A2* variants after enrichment—patients S236 and S293. In patient S236, a somatic c.665_667delAGA; p.Lys222del variant was identified in Olig2-positive nuclei with a VAF of 7.9%, while the VAF from bulk tissue was 0.7%. In patient S293, a somatic *SLC35A2* c.553C>T; p.Gln185* variant was identified in Olig2-positive nuclei with a VAF of 1.5%, while the VAF from bulk tissue was 0.1%.

3.5. Clinical phenotypes of the patients with mutations

Among the 80 patients with the mutations, there were 43 males (54%, Table 6). Patients had median seizure onset at 0.68 years old (range 0.01-11.5) and had epileptic surgeries at median age of 5.5 years old (range 0.13-30.3). Most patients had focal seizures (56 patients, 70%) with or without spasms, clustered brief tonic seizures, or atonic seizures. Additionally, one-third of the patients (30 patients, 37.5%) had spasms, clustered brief tonic, or atonic seizures. The focality of seizures were confirmed by caregiver report and EEG. Spasm, clustered tonic, and atonic seizures were categorized separately because they were associated with DEE. Among the 31 patients, seven patients had generalized ictal EEGs and interictal features of epileptic encephalopathy.

Regarding types of epilepsy, 36 patients (45%) had focal epilepsy. Majority of patients (43 patients, 54%) had diagnosis of DEE, including Lennox-Gastaut syndrome (LGS, 27 patients, 34%), IESS (15 patients, 19%), and early infantile developmental and epileptic encephalopathy (EIDEE, one patient, 1.3%). The patients had median 75 seizures per month as per caregivers' report, and were on median number of three ASMs (range 1-5, IQR 1). Before the surgeries, 17 patients (21%) had previous epilepsy surgeries. Seven patients (8.8%) had previous corpus callosotomy, and ten patients (13%) had previous resective surgery.

Table 6. Clinical characteristics of the patients (n=80)

General Characteristics	Total
Number of males:females (ratio)	43:37 (1.2)
Median age of seizure onset (y)	0.68
Median age of epileptic surgery (y)	5.5
Median interval between seizure onset and surgery (y)	4.0
Seizure semiology (multiple choice)	
Focal motor/focal to bilateral (%)	50 (62.5)
Focal non-motor (%)	13 (16.3)
Spasm/clustered brief tonic/atonic (%)	30 (37.5)
Epileptic syndromes as diagnosed before epileptic surgery (multiple choice)	
EIDEE (%)	1 (1.3)
IESS (%)	15 (19)
LGS (%)	27 (34)
Focal epilepsy (%)	36 (45)
Median seizure frequency per month (range, IQR)	75 (0.3-7500, 120)
Median number of anti-seizure medications (range, IQR)	3 (1-5, 1)
History of two or more epilepsy surgeries (%)	17 (21)
Corpus callosotomy (%)	7 (8.8)
Resective surgery (%)	10 (13)

EIDEE: early infantile developmental and epileptic encephalopathy, IESS: infantile epileptic spasms syndrome, LGS: Lennox-Gastaut syndrome, IQR: interquartile range

Table 7. Presurgical evaluation (n=80)

Evaluation	Number of patients (%)
EEG	80 (100)
MRI	
Normal MRI	14 (18)
Cortical dysplasia	42 (53)
Hemimegalencephaly	9 (11)
Polymicrogyria	1 (1.3)
Brain atrophy	7 (8.8)
Others	5 (6.3)
PET-CT	74 (93)
Neuropsychological tests (multiple choices)	63 (79)
Bayley scales of infant development	29 (36)
Wechsler preschool and primary scale of intelligence	13 (16)
Wechsler intelligence scale for children	20 (25)
Wechsler adult intelligence scale	5 (6.3)

The patients went through extensive presurgical evaluation as shown in Table 7. All 80 patients had EEG and MRI done. All patients had abnormal EEG findings as characterized by slow and disorganized background with frequent epileptiform discharges. Among the patients, 66 (82%) had abnormal MRI findings. Causative structural abnormalities included cortical dysplasia (42 patients, 53%), HME (nine patients, 11%), and PMG (one patient, 1.3%). Most patients (74 patients, 93%) had PET-CT done. Sixty-three patients (79%) had evaluation of developmental milestones before the surgery. Most common neuropsychological study was Bayley scales of infant development (29 patients, 36%), followed by Wechsler intelligence scale for children (20 patients, 25%).

Presurgical evaluation showed different findings per genetic variants of the patients. (Table 8) The overall male-to-female ratio was 43:37. Patients with *TSC1* (six patients), *TSC2* (two patients), or *AKT3* (two patients) mutations had equal numbers of males and females. All patients with *PIK3CA* (three patients) mutations were male. There was male predominance among patients with *NPRL3* (male-to-female ratio 3:1), *SLC35A2* (16:9), and *DEPDC2* (6:5) mutations. In contrast, patients with *MTOR* gene mutation had female predominance (10:17).

Table 8. Preoperative clinical features and study results per somatic variants (n=80)

Features	Total	*MTOR*	*SLC35A2*	*DEPDC5*	*TSC1*
N	80	27	25	11	6
Sex (M:F, ratio)	43:37 (1.2)	10:17 (0.59)	16:9 (1.8)	6:5 (1.2)	3:3 (1.0)
Median age of seizure onset (yr)	0.68	1.25	0.59	0.10	5.5
Median age of epileptic surgery (yr)	5.5	7.1	5.2	1.4	12.3
Median interval between onset and surgery (yr)	4.0	4.5	3.9	1.3	6.0
Median seizure frequency (/month)	75	60	60	90	97.5
Median number of antiepileptic medications	3	4	3	3	2.5
Seizure semiology (multiple choice)					
Focal motor/focal to bilateral (%)	50(62.5)	25 (92.6)	9(36)	7 (63.6)	3(50)
Focal non-motor (%)	13(16.3)	4 (14.8)	3(12)	3 (27.2)	3(50)
Spasm/clustered brief tonic/atonic (%)	30(37.5)	3 (11)	18 (72)	5 (45.5)	0(0)
Epileptic syndromes (%)					
EIDEE	1 (1.3)	0 (0)	0 (0)	1 (9)	0 (0)
IESS	8 (10)	2 (7.4)	4 (16)	2 (18)	0 (0)
IESS→ LGS	7 (8.8)	1 (3.7)	5 (20)	1 (9)	0 (0)
LGS	20 (25)	5 (18.5)	12 (48)	1 (9)	0 (0)
Focal epilepsy	40 (50)	21 (77.8)	4 (16)	5 (45)	4 (67)
Focal→LGS	2 (2.5)	0 (0)	0 (0)	0 (0)	2 (33)
Abnormal MRI (multiple choices, %)	64 (80)	24 (88.9)	15 (60)	9 (82)	5 (83)
FCD	42 (53)	19 (70.4)	7 (28)	7 (64)	4 (67)
HME	9 (11)	2 (7.4)	0 (0)	1 (9)	0 (0)
PMG	1 (1.3)	0 (0)	1 (4)	1 (9)	0 (0)
Others	5 (6.3)	3 (11.1)	4 (16)	0 (0)	1 (17)

EIDEE: Early onset developmental and epileptic encephalopathy, IESS: Infantile epileptic spasms syndrome, LGS: Lennox-Gastaut syndrome, FCD: focal cortical dysplasia, HME: hemimegalencephaly, PMG: polymicrogyria

Table 8. (continued)

Features	*NPRL3*	*PIK3CA*	*TSC2*	*AKT3*
N	4	3	2	2
Sex (M:F, ratio)	3:1 (3.0)	3:0 (N/A)	1:1 (1.0)	1:1 (1.0)
Median age of seizure onset (yr)	0.02	0.10	3.4	0.01
Median age of epileptic surgery (yr)	0.50	0.88	7.8	0.93
Median interval between onset and surgery (yr)	0.49	0.84	4.5	0.92
Median seizure frequency (/month)	105	180	34	62
Median number of antiepileptic medications	4	3	4	5
Seizure semiology				
Focal motor/focal to bilateral (%)	3(75)	0 (0)	1(50)	2 (100)
Focal non-motor (%)	0(0)	0 (0)	0(0)	0(0)
Spasm/clustered brief tonic/atonic (%)	1(1)	3(100)	1(50)	0(0)
Epileptic syndromes (%)				
EIDEE	0 (0)	0 (0)	0 (0)	0 (0)
IESS	1 (25)	1 (33)	0 (0)	0 (0)
IESS→ LGS	0 (0)	1 (33)	0 (0)	0 (0)
LGS	0 (0)	1 (33)	2 (100)	0 (0)
Focal epilepsy	3 (75)	0 (0)	0 (0)	2 (100)
Focal→LGS	0 (0)	0 (0)	0 (0)	0 (0)
Abnormal MRI (multiple choices, %)	3 (75)	3 (100)	2 (100)	1 (100)
FCD (%)	3 (75)	1 (33)	1 (50)	1 (50)
HME (%)	0 (0)	3 (100)	0 (0)	1 (50)
PMG (%)	0 (0)	0 (0)	0 (0)	0 (0)
Others (%)	0 (0)	0 (0)	1 (50)	0 (0)

EIDEE: Early onset developmental and epileptic encephalopathy, IESS: Infantile epileptic spasms syndrome, LGS: Lennox-Gastaut syndrome, FCD: focal cortical dysplasia, HME: hemimegalencephaly, PMG: polymicrogyria

The median age of seizure onset was 0.68. Median age of seizure onset was youngest in the patients with *AKT3* mutation (both age 0.01 year), followed by patients with *NPRL3* mutations (median age 0.02 year). Patients with *DEPDC5* or *PIK3CA* mutations both had seizure onset at median age 0.10. Patients with *SLC35A2* mutations had median seizure onset age of 0.59. Patients

with *MTOR* mutations had seizure onset at median age 1.25. Patients had *TSC2* and *TSC1* somatic mutations had later onset of seizures at age of 3.4 and 5.5, respectively.

The median age of epileptic surgery was 5.5, and the median interval between seizure onset and epileptic surgery was 4.0 years. Patients with earlier seizure onset had shorter median interval between onset and surgery. Patients with germline *NPRL3* mutations had the earliest surgeries at median age of 0.50, with the shortest median of seizure-to-surgery interval (0.49 year). Patients with *PIK3CA* mutations had surgeries at median 0.88 year old with a median interval of 0.84 year. The patients with *AKT3* mutation who had neonatal onset of seizures at 0.01 year old had surgeries at 0.72 and 1.15 years old each (median 0.93 year, median interval 0.92 year). Patients with *DEPDC5* mutations had surgery at median 1.4 year old. (interval 1.3 year) Patients with *SLC35A2* and *MTOR* patients had surgery at 5.2 year old (interval 3.9 years) and 7.1 year old (interval 4.5 years) respectively. Patients with somatic *TSC2* mutations had surgery at 7.8 years old (interval 4.5 years). Patients with somatic *TSC1* mutations had the latest age of surgery at 12.3 years old, with the longest median interval of 6.0 years from seizure onset to surgery.

The median seizure frequency of 80 patients was 75 seizures per month. Patients with *PIK3CA* mutations had the highest median frequency of 180 seizures a month followed by patients with *NPRL3* mutations (median 105 seizures/month). All patients had median seizure frequency of over 30 seizures per month.

The median number of ASMs was three. Patients with somatic *TSC1* mutations had the lowest median of 2.5 ASMs. Both patients with *AKT3* mutation were on five ASMs, of which was the median was higher than any other genes. Patients with *MTOR, NPRL3*, and *TSC2* mutations had median of four ASMs.

The focality of seizure semiology differed among genetic variants. Most patients with *MTOR* mutations (24 patients, 89%) had focal seizures only, while three patients had spasm, atonic, or myoclonic seizures. One patient with confirmed spasms had ictal focality confirmed in video EEG. All patients with somatic *TSC1* mutation (two patients) and *AKT3* mutation (two patients) had focal seizures only. Among patients with *DEPDC5* mutation, nine patients (82%) had focal seizures, but three patients (27%) also had spasms. Two patients (18%) had spasm-only seizures. Three patients with *NPRL3* (75%) mutations had tonic seizures, while one other patient (25%) had axial spasm. Among *TSC2* mutations, one patient had atonic seizures, and one patient had focal tonic seizures. On the other hand, most patients with *SLC35A2* mutations had spasm, atonic, or clustered tonic seizures (18 patients, 72%). Also, all patients with *PIK3CA* mutations (100%) had spasms, clustered brief tonic, or atonic seizures.

Among the 80 patients, nearly half of the patients had focal epilepsy (42 patients, 53%). The other half of the patients had diagnosis of DEE (38 patients, 48%). Most patients with *MTOR* (21 patients, 78%) or *NPRL3* mutation (3 patients, 75%) had focal epilepsy. Both patients with *AKT3* mutations had focal epilepsy. Two patients with *TSC1* mutations had focal epilepsy, and other two patients had focal epilepsy which later evolved into LGS. Patients with *DEPDC5* had diagnosis of focal epilepsy in five patients (45%) and diagnosis of DEE with diverse age of onset in other five patients (45%). Most patients with *SLC35A2* were diagnosed with DEE (21 patients, 84%). Nine patients were diagnosed with IESS, and five of the patients later evolved to LGS. Other 12 patients

had LGS diagnosis from seizure onset. All patients with *PIK3CA* had diagnosis of DEE, one IESS, one IESS to LGS, and one LGS. Both patients with somatic *TSC2* mutations had diagnosis of DEE, notably LGS.

Most patients had abnormal MRI findings (64 patients, 80%), most commonly FCD (42 patients, 53%). All patients with *PIK3CA, TSC2* and *AKT3* mutations had abnormal MRI. Especially, all patients with *PIK3CA* mutations had HME (100%). Patients with *SLC35A2* mutations had lowest ratio of abnormal MRI findings (15 patients, 60%) with lowest ratio of FCD (7 patients, 28%). Instead, four patients (16%) had findings of white matter hyperintensity.

Patients with genetic mutations went through resective or disconnective surgeries (Figure 10). About a half of patients had surgeries restricted to extratemporal region (38 patients, 48%) and a third had surgeries including temporal cortex (25 patients, 31%). Twelve patients (15%) had functional hemispherotomy or hemispherectomy.

After epilepsy surgery, most patients had favorable seizure outcomes. Up to 71% of the patients were seizure-free at 6 months after surgery. The percentage of patients who were classified as ILAE class 1 (seizure-free and without auras), was kept above 60% at annual follow-up for three years. As most patients had decreased seizures or became seizure-free, they could be on a smaller number of ASMs than before the surgery. The median number of ASMs gradually decreased from three before surgery to one at three years after the surgery.

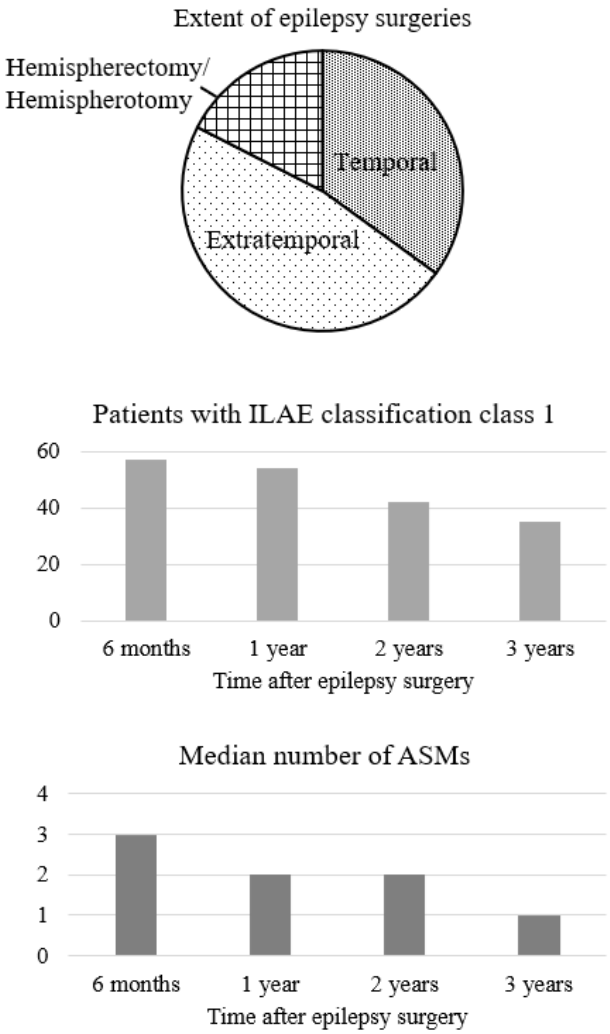

Figure 10. Extent and types of surgery and post-operative outcomes of the patients.
ILAE: International League Against Epilepsy, ASM: anti-seizure medication.

Among the 80 patients with genetic mutations, all but one patient had positive results in surgical histopathology (79 patients, 99%, Table 9). FCD was the most frequent pathologic finding. In detail, FCDIIa (26 patients, 33%) was more common than FCD type 2b (FCDIIb, 20 patients, 25%). Among FCDIIa-positive brain tissues, common mutations were *MTOR* (12 patients, 46%), *DEPDC5* (six patients, 23%), *NPRL3* and *TSC1* (three patients, 12% each). *MTOR* was also the most common genetic mutation in FCDIIb patients (14 patients, 70%). *SLC35A2* mutation was the sole mutation detected in brain tissues with MOGHE. *SLC35A2* was also the most common mutation in gliosis (seven patients, 64%). Patients with mMCD (three patients,

3.8%), HME (two patients, 2.5%), and PMG (two patients, 2.5%) had heterogenous genetic mutations, with no dominant causative genes.

Table 9. Genes per histopathology (n=80)

Histopathology (N, %)	Genes	Number of patients (% of pathology)
FCDIIa (26, 33%)	*MTOR*	11 (46)
	DEPDC5	6 (23)
	NPRL3	3 (12)
	TSC1	3 (12)
	AKT3	2 (7.7)
	TSC2	1 (3.8)
FCDIIb (20, 25%)	*MTOR*	14 (70)
	TSC1	3 (15)
	DEPDC5	2 (10)
	NPRL3	1 (5.0)
MOGHE (15, 19%)	*SLC35A2*	15 (100)
Gliosis (11, 14%)	*SLC35A2*	7 (64)
	DEPDC5	1 (9.1)
	MTOR	1 (9.1)
	PIK3CA	1 (9.1)
	TSC2	1 (9.1)
mMCD (3, 3.8%)	*DEPDC5*	1 (33)
	PIK3CA	1 (33)
	SLC35A2	1 (33)
HME (2, 2.5%)	*DEPDC5*	1 (50)
	PIK3CA	1 (50)
PMG (2, 2.5%)	*MTOR*	1 (50)
	SLC35A2	1 (50)
Normal (1, 1.3%)	*SLC35A2*	1 (100)

FCDIIa: focal cortical dysplasia type IIa, FCDIIb: focal cortical dysplasia type IIb, MOGHE: mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy, mMCD: mild malformation of cortical development, HME: hemimegalencephaly, PMG: polymicrogyria.

The favorable seizure outcome was consistent in patients with different germline or somatic mutations (Table 10). During the three years after surgery, over 60% of patients were seizure-free (ILAE classification class 1), and over 80% had null to rare disabling seizures (ILAE classification class 1, 2, and 3). The patients with *MTOR* or *SLC35A2* mutations had lower seizure-free rates at three years compared to patients with other mutations, but the seizure-free rates were still over 50%. Over 80% of the patients were seizure-free or had rare disabling seizures, so median number of ASMs was lowered from four to two in patients with *MTOR* mutations and three to 1.5 in patients with *SLC35A2* mutations.

Table 10. Postoperative seizure outcomes per somatic variants (n=80)

Features	Total	*MTOR*	*SLC35A2*	*DEPDC5*	*TSC1*
Number of patients	80	27	25	11	6
Types and extent of surgery					
Hemispherectomy/ functional hemispherotomy	14(18)	3(11)	4(16)	2(18)	0(0)
Temporal	28(35)	7(26)	15(60)	2(18)	2(33)
Extratemporal	38(48)	17(63)	6(24)	7(64)	4(67)
ILAE epilepsy surgery outcome classification					
Patients post-op 6m (N)	80	27	25	11	6
Class 1 (%)	58 (73)	19 (70)	20 (80)	7 (64)	4 (67)
Class 2 (%)	1 (1.3)	0 (0)	0 (0)	1 (9.1)	0 (0)
Class 3 (%)	14 (18)	6 (23)	2 (8)	3 (27)	1 (17)
Class 4 (%)	2 (2.5)	1 (3.8)	0 (0)	0 (0)	0 (0)
Class 5 (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Class 6 (%)	5 (6.3)	1(3.8)	3 (12)	0 (0)	1 (17)
Patients post-op 1y (N)	78	25	25	11	6
Class 1 (%)	54 (69)	17 (68)	17 (68)	9 (82)	4 (67)
Class 2 (%)	2 (2.6)	0 (0)	0 (0)	1 (9.1)	1 (17)
Class 3 (%)	14 (18)	6 (24)	6 (24)	0 (0)	1 (17)
Class 4 (%)	2 (2.6)	1 (4.0)	0 (0)	0 (0)	0 (0)
Class 5 (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Class 6 (%)	6 (7.7)	1 (4.0)	2 (8.0)	1 (9.1)	0 (0)
Patients post-op 2y (N)	69	22	22	8	5
Class 1 (%)	42 (62)	12 (55)	13 (59)	7 (88)	4 (80)
Class 2 (%)	5 (7.4)	1 (4.5)	1 (4.5)	0(0)	1 (20)
Class 3 (%)	9 (13)	7 (32)	1 (4.5)	1(13)	0 (0)
Class 4 (%)	1 (1.5)	0 (0)	0 (0)	0 (0)	0 (0)

Class 5 (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Class 6 (%)	4 (5.9)	2 (9.1)	0 (0)	0 (0)	0 (0)
Patients post-op 3y (N)	56	18	18	7	4
Class 1 (%)	35 (64)	9 (50)	11 (61)	5 (71)	3 (75)
Class 2 (%)	1 (1.8)	1 (5.6)	0 (0)	0 (0)	0 (0)
Class 3 (%)	14 (26)	6 (33)	5 (28)	1 (14)	1 (25)
Class 4 (%)	2 (3.6)	1 (5.6)	0 (0)	1 (14)	0 (0)
Class 5 (%)	1 (1.8)	1 (5.6)	0 (0)	0 (0)	0 (0)
Class 6 (%)	2 (3.6)	0 (0)	2 (11)	0 (0)	0 (0)
Median number of ASMs					
Pre-operation	3	4	3	3	2.5
6m	3	3	2	2.5	2.5
1y	2	2	2	2	2.5
2y	2	1.5	2	1.5	2
3y	1	2	1.5	1	2

ILAE: International League Against Epilepsy, ASM: anti-seizure medication

Table 10. (continued)

Features	*NPRL3*	*PIK3CA*	*TSC2*	*AKT3*
Number of patients	4	3	2	2
Types and extent of surgery				
Hemispherectomy/functional hemispherotomy	0(0)	3(100)	0(0)	2 (100)
Temporal	2(50)	0(0)	0(0)	0(0)
Extratemporal	2(50)	0(0)	2(100)	0(0)
ILAE epilepsy surgery outcome classification				
Patients post-op 6m (N)	4	3	2	1
Class 1 (%)	2 (50)	3 (100)	1 (50)	2 (100)
Class 2 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Class 3 (%)	1 (25)	0 (0)	1 (50)	0 (0)
Class 4 (%)	1 (25)	0 (0)	0 (0)	0 (0)
Class 5 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Class 6 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Patients post-op 1y (N)	4	3	2	1
Class 1 (%)	3 (75)	2 (67)	1 (50)	1 (50)
Class 2 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Class 3 (%)	0 (0)	0 (0)	0 (0)	1 (50)
Class 4 (%)	1 (25)	0 (0)	0 (0)	0 (0)

Class 5 (%)	0 (0)	0 (0)	0	0 (0)
Class 6 (%)	0 (0)	1 (33)	1 (50)	0 (0)
Patients post-op 2y (N)	4	3	2	1
Class 1 (%)	2 (50)	3 (100)	1 (50)	0 (0)
Class 2 (%)	1 (25)	0 (0)	0 (0)	1 (50)
Class 3 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Class 4 (%)	1 (25)	0 (0)	0 (0)	0 (0)
Class 5 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Class 6 (%)	0 (0)	0 (0)	1 (50)	1 (50)
Patients post-op 3y (N)	2	2	2	1
Class 1 (%)	2 (100)	2 (100)	1 (50)	2 (100)
Class 2 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Class 3 (%)	0 (0)	0 (0)	1 (50)	0 (0)
Class 4 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Class 5 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Class 6 (%)	0 (0)	0 (0)	0 (0)	0 (0)
Median number of ASMs				
Pre-operation	4	3	4	5
Post-op 6m	3	1	3	2
Post-op 1y	2.5	1	2.5	1
Post-op 2y	2.5	2	2.5	1.5
Post-op 3y	2.5	2.5	2.5	2

ILAE: International League Against Epilepsy, ASM: anti-seizure medication.

Table 11. Seizure outcomes per types and extent of surgery

ILAE classification 1 (% of patients with data)	Before surgery	Post-op 6 months	Post-op 1 year	Post-op 2 years	Post-op 3 years	Last follow-up
Temporal	28	19 (68)	19 (68)	15 (54)	15 (54)	19 (68)
unilobar	9	7 (78)	8 (89)	7 (78)	9 (100)	9 (100)
multilobar	19	12 (63)	11 (58)	8 (57)	6 (60)	10 (53)
Extratemporal	38	26 (69)	23 (61)	18 (47)	10 (28)	20 (53)
unilobar	32	22 (71)	19 (63)	16 (59)	10 (45)	19 (59)
multilobar	6	4 (50)	4 (67)	2 (25)	1 (33)	2 (33)
Hemispherectomy/ hemispherotomy	14	13 (93)	12 (86)	9 (64)	9 (64)	12 (86)
Total	80	58 (73)	54 (69)	42 (62)	35 (64)	51 (64)

The seizure outcomes per types and extent of surgery is shown on Table 11. There was no statistical significance among the extent and types of epilepsy surgery with seizure outcomes (p=0.056). Patients with smaller extent of surgery (unilobar) had better outcome than patients with multilobar resective surgery. Among 41 patients with epilepsy surgeries limited within a single lobe, 28 patients (68%) had ILAE class 1 at last follow-up. In contrast, 12 patients out of 25 patients with multilobar resection had ILAE class 1 at last follow-up (48%). Also, patients with hemispherectomy or hemispherotomy had favorable seizure outcomes, with 12 patients with ILAE class 1 (86%) at last follow-up (p=0.0495).

The phenotypes of the somatic and germline variants of mTOR pathways are as follows. As of genes in the upstream mTOR pathway, there were three patients with *PIK3CA* somatic gene mutation and two patients with *AKT3* somatic mutation. The patients had early seizure onset of 0.01 to 0.1 years old. The seizures were mostly tonic seizures or spasms. Patients with both mutations had median frequent seizures. All five patients went through hemispherectomy or hemispherotomy. All patients had ILAE class 1 at last follow-up of two to three years post-surgery. Among them, one patient with *AKT3* mutation had additional surgery two years after first surgery due to increase in seizures, and was seizure free after the second surgery (ILAE class 1 at 3 years after first operation). The patient had another surgery at five years after first surgery, and had additional somatic *MTOR* mutation found of which the germline variant was ACMG classified as variant of unknown significance.

Regarding the genes downstream of *AKT3* and upstream of *MTOR* genes, there were six patients with somatic *TSC1* variants and two patients with somatic *TSC2* variants. Most patients had pathology of FCD. In detail, four patients had FCDIIa (3 *TSC1*, 1 *TSC2*) and three patients had FCDIIb (all *TSC1*). Seven patients (87.5%) had positive MRI findings of FCD. The patients had seizure onset at median 5.5 years (*TSC1*) and 3.4 years (*TSC2*) old. Seven patients (87.5%) had focal epilepsy, and one other patient had LGS. Seven patients had focal resection involving single lobe, while one other patient with *TSC2* mutation had multi-lobar resection. Post-operative outcomes at two years were favorable, with ILAE seizure classification 1-2 in six patients out of seven patients with data (86%).

There were 27 patients with somatic *MTOR* variants. Most patients had pathologic findings of FCD: 14 patients (51.8%) had FCDIIb, and 11 patients (40.7%) had FCDIIa. In similar context, most patients had focal MRI findings of FCD (21 patients, 81%), and clinically focal seizures (25 patients, 92.6%).

Among the 28 patients, clinical burden of the disease correlated with VAF. The four patients with the highest VAF had MRI findings of hemimegalencephaly, while most others had focal MRI findings.

There were 15 patients with germline mutations in GAP activity toward RAGs 1 (GATOR1) complex, upstream of *MTOR* gene. Specifically, 11 patients had germline mutations in *DEPDC5* gene and four patients had mutations on *NPRL3* gene. All patients had early onset of seizures from 0.01 to 0.1 years old. All patients had frequent seizures (90-105 seizures per month), and were on three or four ASMs. Eight patients with *DEPDC5* mutations had pathologic findings of FCDIIa (six patients, 55%) or FCDIIb (two patients, 18%). All patients with *NPRL3* mutations had FCDIIa

(three patients, 75%) or FCDIIb (one patient, 25%). Consistent with pathologic results, most patients had FCD on MRI, including seven patients with *DEPDC5* mutations (63%) and four patients with *NPRL3* (100%). Patients with *NPRL3* mutations had no seizure at three years post-surgery. However, among the 11 patients with *DEPDC5* mutations, four patients had further resection, and three other patients had seizures from contralateral areas as confirmed by further EEG.

Apart from the mutations in the MTOR pathway, the gene *SLC35A2* had different clinical phenotype. Among the 25 patients with *SLC35A2* mutations, there were 15 patients with MOGHE (60%), and seven patients with gliosis (28%). MRI showed nonspecific findings in 13 patients (51%). The other 12 patients with abnormal findings had FCD, PMG, or white matter abnormality. Median seizure onset was 0.59 years old. Most patients were diagnosed with epilepsy syndromes of IESS and LGS in 21 patients (84%). Among 18 patients who had data on three years after surgery, 11 patients were seizure free.

IV. DISCUSSION

This study contributes to understanding the genetic underpinnings of epilepsy, particularly in the context of DRE caused by MCD and other epileptogenic brain lesions.

We established a large-scale cohort of 238 patients who underwent epilepsy surgeries for intractable epilepsy in a single center. This cohort is larger than recent single center studies that involved 34 to 110 patients with MCD.[9,22] Due to dispersion of patients and rarity of somatic mutations as per current technology, multicenter studies focus on 47 to 223 patients with specific mutations.[24,10] Our cohort is based at a tertiary epilepsy center with a constant pool of intractable epilepsy patients and periodic evaluation for surgical treatment. Also, we updated the gene panels associated with somatic and germline mutations, leading to accurate detection of pathogenic variants associated with intractable epilepsy.

Our cohort had 80 patients positive for genes related to FCD and MOGHE among 238 patients (33.6%). This yield is similar to the previous report of 33.6%[10] and lower than another report of 53.7%.[22] The difference in yield is because the latter study had genetic tests on brain tissues with confirmed histopathology of mMCD, FCD, or HME, while we performed tests on all brain tissues obtained from epilepsy surgeries. While pathology-matched sequencing may give higher yield, our study showed 12 cases of genetic mutations of which the pathology was nonspecific (normal or gliosis), of which 8 cases had *SLC35A2* mutations. This finding may add to the phenotype of *SLC35A2* somatic mutation.

The role of somatic mosaicism in epileptogenic brain lesions was highlighted by our findings. Pathogenic mutations can arise during mitotic cell divisions post-fertilization, resulting in somatic mosaicism where different cell lineages have distinct genotypes. The timing and lineage specificity of these somatic mutations can lead to different patterns of mosaicism, influencing the extent and nature of brain involvement. For example, a somatic mutation occurring early in development may affect multiple organs, whereas a mutation later in neural progenitor cells may be restricted to the brain. This concept was supported by our findings of somatic variants in brain tissue with low VAF, demonstrating the need for targeted enrichment strategies to identify these mutations effectively.

We could evaluate 80 patients with somatic or germline mutations specifically related to MCD, FCD and MOGHE. Our study provides evidence that mutation detection in brain tissue alone is comparable to paired brain-blood sampling for identifying somatic variants. We conducted both paired and unpaired deep targeted sequencing and found that the two methods yielded identical pathogenic variant results after validation, supporting the effectiveness of brain-only analysis. This

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[24] Barba, C., Blumcke, I., Winawer, M. R., Hartlieb, T., Kang, H. C., Grisotto, L., Chipaux, M., Bien, C. G., Heřmanovská, B., Porter, B. E., Lidov, H. G. W., Cetica, V., Woermann, F. G., Lopez-Rivera, J. A., Canoll, P. D., Mader, I., D'Incerti, L., Baldassari, S., Yang, E., Gaballa, A., … SLC35A2 Study Group (2023). Clinical Features, Neuropathology, and Surgical Outcome in Patients With Refractory Epilepsy and Brain Somatic Variants in the *SLC35A2* Gene. *Neurology*, *100*(5), e528–e542.

finding has clinical implications particularly in cases where obtaining blood samples is challenging or not feasible, and it offers a flexible and practical approach for genetic diagnosis in epilepsy.

Additionally, we identified two somatic mutations in previously mutation-negative tissues with low VAF using an innovative variant enrichment strategy. Recently, Kim et al[21] detected ultra-low VAF mutations in *NPRL3* and *DEPDC5* by LCM and fluorescence-activated affected cell sorting. Similarly, we detected *SLC35A2* mutations in two patients whose brain tissues were previously mutation-negative. We detected Olig2-positive glial cells using FANS, and observed higher VAFs in Olig2-postive nuclei (7.9 and 1.5%) compared to very low VAF in bulk tissues (0.7 and 0.1% each). This approach demonstrates the potential for enhanced detection of low-VAF variants, which could significantly improve the diagnostic yield for patients with DRE who have negative results on standard genetic testing. Also, periodic re-evaluation of the tissues would yield higher rate of somatic or germline mutations in currently-gene-negative tissues of the patients.

Furthermore, we conducted an in-depth examination of genotype-phenotype correlations in patients with somatic and germline mutations. Despite the focus on somatic mutations in patients with intractable epilepsy, there are limited studies on phenotype-genotype correlations. We examined the clinical phenotypes in detail, including seizure and epilepsy types, presurgical evaluations, histopathology of brain tissues, and post-operative epilepsy prognosis across eight genes associated with MCD, FCD and MOGHE.

There is currently no report on somatic mutation burden and burden of disease in pediatric epilepsy. In our cohort, we found a trend toward increased burden of disease with higher VAF in *MTOR* somatic mutation. We noted that the three of the four patients with the highest VAF (S143, S015, S174, VAFs 9.8%-23.4%) of *MTOR* mutations had HME and one patient (S001, VAF 12.6%) had diffuse hemispheric cortical dysplasia, suggesting high burden of disease and requiring extensive surgeries such as multilobar resection or hemispherectomy. Among the four patients, only one patient had ILAE class 1 at last follow-up, while two patients had class 3, and one patient had class 6 (worsened seizures), suggesting suboptimal seizure outcomes compared to patients with lower VAF. Other patients with lower VAFs had MRI findings of focal lesions. Among them, three patients with lower VAFs had normal findings on MRI (VAF 1.1-7.3%), and one patient with VAF of 2.8% had pathologic finding of gliosis.

In patients with *SLC35A2*, there was no association between burden of disease and VAF only. Trends were noted between VAF and burden of disease at one mutation locus. The four patients with c.502C>T (p.Gln168*), two patients (S300, S301) with VAF 1.1% had gliosis, while one patient (S163) with VAF 2.0% had mMCD, and one patient with VAF of 18% (S169) had MOGHE. Pre-operative burden of seizure was higher in the two patients with higher VAFs with higher frequency (90/month and 30/month each) and more diverse semiology (eye deviation or head drops in S163, focal nonmotor, spasm, and focal to generalized motor in S169) compared to the two patients with lower VAFs (8/month and 3/month respectively, single semiology of focal non-motor and focal motor respectively). The seizure outcomes were unfavorable in patients with lower VAF (both class 6, worsened) compared to other two (class 1 and 3 each). This may be because the two patients with low VAF had longer time to treatment (10.6y, 3.5y) compared to the two patients with higher VAFs (1.4y, 1.0y). Such trend did not exist among patients with other

mutation in *SLC35A2*. There was one study in cancer where VAF of circulating tumor DNA correlated with prognosis in advanced breast cancer.[25] Further studies may confirm the association between VAF and disease burden in epilepsy.

Our study has several limitations. Although this study is based on a large cohort, several genes have small size of patients, such as two patients with *AKT3* mutations. Also, this study is based on brain tissues from refractory epilepsy patients during epilepsy surgery. There may be selection bias since patients with similar mutations of the genes with milder clinical phenotype would not have undergone epilepsy surgery, and we cannot detect somatic mutations of such patients. Also, there are missing genes in the panel, especially the most recent 13-gene panel that we used for deep sequencing. Currently, we are updating the gene panel including more genes in the PI3K-AKT-mTOR pathway such as *RHEB* or *PTPN11* gene.

Although our study contributes to a better understanding of the genotype-phenotype correlations in epilepsy, the current ability to predict specific pathogenic genes before surgery is limited. Pre-surgical identification of these genes could guide targeted therapies, such as mTOR inhibitors for patients with mTOR pathway mutations or galactose supplementation for those with *SLC35A2* mutations. However, further research is needed to validate these potential treatments and to develop reliable methods for pre-surgical genetic diagnosis.

Higher VAFs may lead to higher disease burden but may also serve as potential therapeutic targets. This parallels findings in other fields such as oncology, where high VAF in non-small cell lung cancer was correlated with treatment response to tailored drug.[26] However, since VAF can be influenced by tissue selection, further studies are needed to confirm whether VAF consistently correlates with distinct pathologies within the same mutation.

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[25] Zhong, J., Jiang, H., Liu, X., Liao, H., Xie, F., Shao, B., Jia, S., & Li, H. (2024). Variant allele frequency in circulating tumor DNA correlated with tumor disease burden and predicted outcomes in patients with advanced breast cancer. *Breast cancer research and treatment*, *204*(3), 617–629. https://doi.org/10.1007/s10549-023-07210-9.

[26] Boscolo Bielo L, Trapani D, Repetto M, et al. Variant allele frequency: a decision-making tool in precision oncology?. *Trends Cancer*. 2023;9(12):1058-1068.

V. CONCLUSION

In conclusion, our study established a robust large-scale cohort and employed advanced strategies to enhance mutation detection, providing new insights into the genetic basis of epileptogenic brain lesions. The comparable effectiveness of brain-only mutation detection versus paired brain-blood analysis, combined with detailed genotype-phenotype correlations, underscores the importance of continued research in this area to improve diagnostic and therapeutic strategies for patients with DRE.

Our study advances our understanding of the genetic factors contributing to epilepsy, particularly in the context of somatic and germline mutations. Further studies would enhance the knowledge in genetic basis and tailored treatments for patients with somatic mutations, ultimately improving outcomes for intractable epilepsy.

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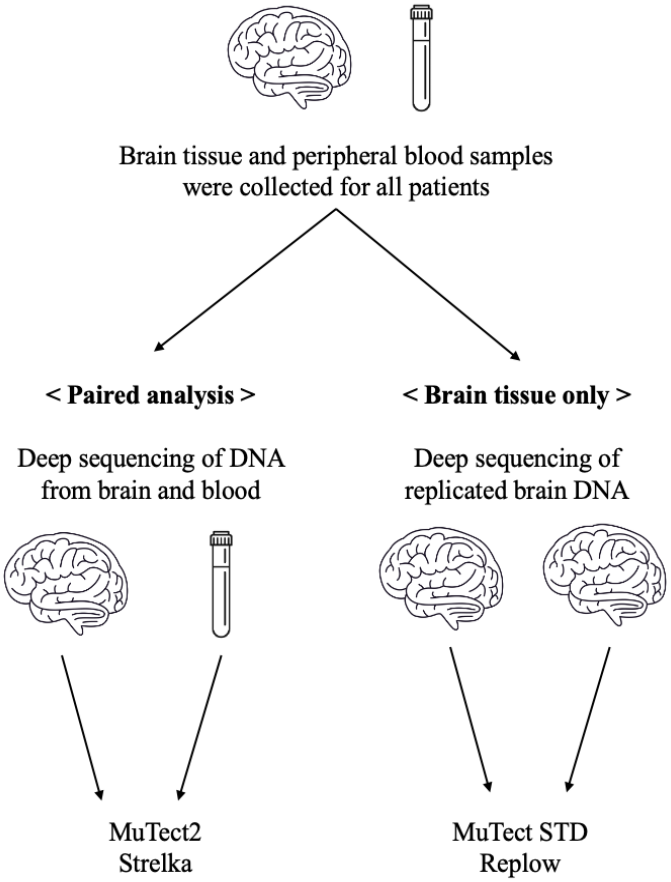
Appendices 1. Patient analysis by matched blood-brain samples and unmatched, replicated brain samples.



Appendices 2. Genes included in the MCD targeted gene panel.

ACTG1	*CEP152*	*GCSH*	*NDE1*	*POMT1*	*STRADA*
ADGRG1	*CNTNAP2*	*GLDC*	*NRXN1*	*POMT2*	*TCF4*
AHI1	*COL18A1*	*GNAQ*	*NSD1*	*PTEN*	*TMEM216*
AKT1	*COL4A1*	*GPC3*	*NSDHL*	*RAB18*	*TSC1*
AKT3	*DCX*	*HEPACAM*	*OCLN*	*RAB39B*	*TSC2*
AMT	*DEPDC5*	*HIP1*	*OFD1*	*RAB3GAP1*	*TSEN2*
ARFGEF2	*DYNC1H1*	*HSD17B4*	*ORC1*	*RAB3GAP2*	*TSEN34*
ARX	*EOMES*	*KDM5C*	*ORC4*	*RARS2*	*TSEN54*
ASPM	*ETFA*	*KIAA1279*	*ORC6*	*RELN*	*TUBA1A*
ATP6V0A2	*ETFB*	*KIF5C*	*PAFAH1B1*	*RIN2*	*TUBA8*
ATR	*ETFDH*	*LAMB1*	*PAX6*	*SHH*	*TUBB2B*
CASK	*FGFR3*	*LAMC3*	*PCNT*	*SLC25A19*	*TUBB3*
CDC6	*FKRP*	*LARGE1*	*PIK3CA*	*SLC9A6*	*UBE3A*
CDK5RAP2	*FKTN*	*MCPH1*	*PIK3R2*	*SRD5A3*	*VLDLR*
CDT1	*FLNA*	*MECP2*	*PNKP*	*SRPX2*	*WDR62*
CENPJ	*FOXG1*	*MTOR*	*POMGNT1*	*STIL*	*YWHAG*

Abstract in Korean

소아청소년 뇌전증에서 저단계 체성 변이의 확인 및 유전형에 따른 임상 양상의 분석

뇌전증은 비유발 경련을 특징으로 하는 신경학적 질환이다. 뇌전증 환자들의 약 30%는 난치성 뇌전증, 즉 약물 저항성 뇌전증으로 치료받는다. 최근 연구상 뇌의 체성 변이는 국소 발달 장애를 일으킬 수 있으며, 이는 약물 저항성 뇌전증과 관련이 있음이 밝혀졌다. 많은 체성 변이가 확인되었지만, 이른 검출하려면 뇌 조직과 혈액 샘플에 대한 광범위한 분석을 요한다. 이러한 과정 후에도 일부 조직은 변이 대립 유전자 빈도(VAF)가 낮아 돌연변이가 발견되지 않기도 한다. 나아가, 이러한 체성 돌연변이가 있는 환자들의 임상적 특징에 대해서는 정보가 부족한 상태이다.

본 연구는 뇌전증 수술을 받은 난치성 뇌전증 환자의 대규모 코호트에서 임상적 표현형을 조사하고자 하였다. 연구진은 기존에는 돌연변이 음성으로 간주된 조직에서 체성 돌연변이를 탐지하고, 돌연변이를 탐지함에 있어서 뇌 조직 단독 검사와 뇌-혈액샘플 양측 검사에서의 효용을 비교하고자 하였다.

연구 대상은 단일 삼차 뇌전증 기관에서 약물 저항성 뇌전증을 진단받고 2004년부터 2022년까지 수술을 받은 소아청소년 환자들이었다. 본 연구진은 심층 염기서열 분석을 뇌 조직 단독 분석 및 뇌 조직과 말초 혈액 샘플의 맞춤 분석 두가지로 수행을 하였다.

본 연구진은 국소 뇌 발달 장애가 확인된 238명의 환자들 중 65명의 환자들에서 6개 유전자(*MTOR, SLC35A2, PIK3CA, TSC1, TSC2, AKT3*)의 체성 변이를 확인하였으며 15명의 환자들에서 *DEPDC5* 또는 *NPRL3* 유전자의 배선 돌연변이를 확인하였다. 본 연구에서는 뇌 단독 분석과 뇌 조직-말초 혈액 맞춤 분석 두 검사 모두에서 같은 체성 변이를 검출할 수 있었다.

또한 VAF 가 매우 낮아 이전에는 돌연변이 음성으로 확인된 두 환자들을 대상으로 *SLC35A2* 유전자의 체성 변이를 확인하였다. 연구진은 형광 활성화 핵 분류 기법을 통해 특정 유전적 활동이 있는 핵을 추출하여 VAF 를 향상시켰다.

환자 80명의 뇌조직에서 가장 흔하게 확인된 조직병리 소견은 국소 피질 이형성 IIa 형(FCDIIa)과 IIb 형(FCDIIb)이었다. *MTOR* 유전자 돌연변이가 있는 환자의 뇌조직은 주로 FCDIIa, FCDIIb 소견이었으며, *SLC35A2* 유전자 돌연변이가 있는 환자의 뇌조직에서는 과립교세포 과형성을 동반한 경미한 피질발달장애 소견을 주로 보였다. 동일한 염기서열 돌연변이가 확인된 환자들에서도 다른 조직병리를 보이는 경우가 있었으며, 이는 유전형-표현형 이질성을 시사한다.

본 연구에서는 대규모 코호트를 분석하여 난치성 뇌전증이 있는 환자들 중 체성 또는 배선 돌연변이가 있는 환자들의 임상적 양상을 확인할 수 있었다. 본 연구 결과는 체성 변이 검출에 있어서 뇌 조직 단독 분석이 뇌 조직-혈액 샘플 맞춤 분석과 비슷하게 효과적임을 제시한다. 저단계 체성 변이를 식별하는 방법이

발전함에 따라 난치성 뇌전증 환자의 유전적 특성이 명확해지고 표적 치료가 정립되면 환자들의 치료 결과가 향상될 것이다.

핵심되는 말 : 체성 변이, 약물 저항성 뇌전증, 피질 발달장애, 국소 피질 이형성