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A variant in *SLC12A5* for a familial benign Rolandic epilepsy

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Abstract

Benign Rolandic epilepsy (BRE) is the most common cause of epilepsy in childhood. Childhood epilepsies have high heritability, and many BRE cases show an autosomal dominant inheritance pattern. Thanks to the advancement of genomics, the causal genes of BRE were being elucidated. Although BRE is a genetic disorder, most BRE cases cannot be explained by known causal genes. Pleiotropy is a common phenomenon in genes related to epilepsy. For example, the same variant in a gene related to BRE can cause diverse epileptic syndromes from mild BRE to Landau-Kleffner syndrome, a severe form of epilepsy. Although BRE is classified as idiopathic focal epilepsy, BRE can be caused by the same genes or loci related to idiopathic generalized epilepsy (IGE). Using whole exome sequencing, we tried to find causal variants and copy number variations in the known genes for BRE and IGE. We found a novel missense variant in *SLC12A5* as a cause of a familial BRE. Although *SLC12A5* is a known causal gene for IGE, it may cause BRE, because many genes related to BRE can cause diverse epilepsy syndromes including IGE.

Keywords: Exome, next generation sequencing, benign Rolandic epilepsy, SLC12A

INTRODUCTION

Benign Rolandic epilepsy (BRE) is the most common epilepsy in childhood. BRE is highly genetic, and familial cases are often found to be autosomal dominant transmission patterns. The recent development of genomic sequencing has revealed many related genes. *BDNF*[1], *DEPDC5*[2], *ELP4*[3] *GABRG2*[4], *GRIN2A*[5], *KCNQ2*[6], *KCNQ3*[6], *PRRT*[7], *RBFOX1*[8], *RBFOX3*[8], and *SRPX2*[9] have been reported as associated genes of BRE. However, these genes do not explain most BRE cases.[4]

The genes related to epilepsies can cause a diverse clinical spectrum. For example, *GRIN2A* is associated with BRE, atypical benign partial epilepsy, Landau-Kleffner syndrome, epileptic encephalopathy with continuous spike and waves during slow-wave sleep.[5] *CLCN2*, *EFHC1*, *GABRG2*, *NRXN1*, *SLC12A5*, 1q21.1, 15q11.2, 15q13.3, 16p13.11, and 22q11.21 were related to idiopathic generalized epilepsy (IGE) and focal epilepsies.[10-12] *SLC12A5* also cause diverse epilepsy syndrome from severe epilepsies, such as epilepsy of infancy with migrating focal seizures (EIMFS) to milder forms like IGE.[13] Although GRINA2A is BRE's most common causal gene, it can be related to IGE.[14] We have found a family with BRE. We screened the known genes and copy number variations (CNVs) for BRE and IGE with exome sequencing study.

CASE REPORT

A seven-year-old boy without past medical history or febrile seizure often woke up crying at night. The symptoms occurred within two hours of falling asleep at night. He did not remember the events in the following days. Two years later, his younger brother showed the same symptoms. The two boys did not have cognitive developmental issues including language disorders. Their parents could not remember such nocturnal symptoms or febile convulsion during their childhood. On physical and neurological examination, there was no specific finding. There was no abnormal finding in the proband's magnetic resonance imaging (Supplementary Figure 1). The electroencephalography (EEG) was performed in the proband, and typical BRE

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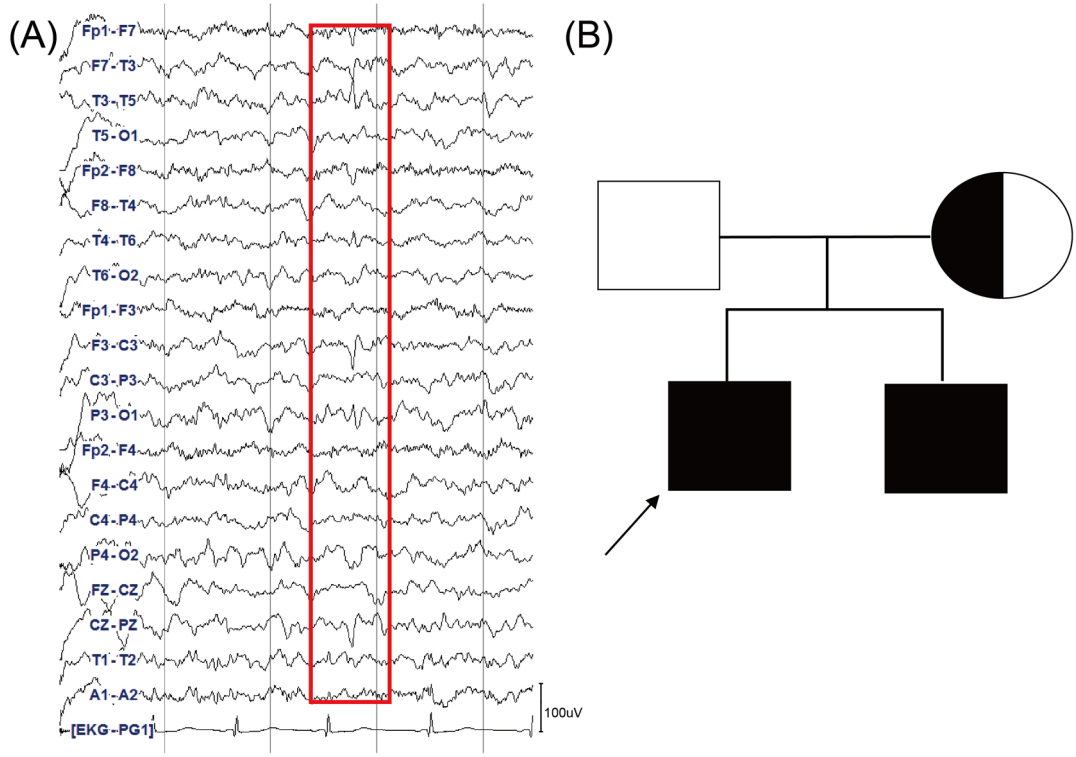

Figure 1. (A) EEG findings of the first son. The EEG had 1 Hz low-frequency filter and 70 Hz high-frequency filter. The spikes showed negatively maximal power in C3 and T3. The montage of EEG was the "double-banana run" montage. (B) A pedigree.

findings aggravated during sleep, were shown on EEG (Figure 1A, Supplementary Figure 2, and Supplementary Figure 3). The two sons started levetiracetam 250mg only at nighttime and their seizure frequencies decreased. The diagnosis of epilepsy was favored over sleep disorder due to the distinctive EEG patterns and the positive response to levetiracetam treatment.

We performed whole exome sequencing. TheragenEtex Bio Company performed the exome analyses. Genomic DNA was extracted from blood, and the libraries were prepared using a SureSelect Human All Exon Kit (version 5, Agilent Inc., Santa Clara, CA, USA). The DNAs were sequenced using HiSeq2500 (Illumina Inc, San Diego, Calif., USA). The sequencing depth was ×100. We used GATK (version 3.8, BROAD Institute) for variant calling to the hg19 reference human genome.

We tried to find variants in *BDNF*, *DEPDC5*, *ELP4*, *GABRG2*, *GRIN2A*, *KCNQ2*, *KCNQ3*, *PRRT2*, *RBFOX1*, *RBFOX3*, *SRPX2*, *CLCN2*, *EFHC1*, *NRXN1*, and *SLC12A5* which are reported to be related to BRE and IGE. Among variants with maternal or paternal segregation patterns, we selected nonsynonymous variants with less than 1% minor allele frequencies (MAF) in all ethnicities. The variant frequencies were derived from gnomAD (https://gnomad.broadinstitute.org) and the Korean exome study of TheragenEtex Bio Company. We used R (version 4.0.5) when applying filters. For CNV analyses, the depths of genetic regions were calculated using GATK. By the hidden Markov model of xhmm[15], we selected ≥ 10kb CNVs with maternal or paternal segregation patterns. We selected CNV according to read depths with a default setting of xhmm.[16] We used the exome data of 138 Koreans without epilepsy for the calculation of the frequencies of the CNVs.

Among two nonsynonymous rare variants, the variant (Arg702His) in *SLC12A5* originated from the mother (Figure 1B). The variant was not previously reported in gnomad and Korean. InterVar which automatically Interprets variants according to the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guideline[17], determined the variant as "likely pathogenic".

We selected CNVs with maternal or paternal transmission. We could not detect CNVs in BRE

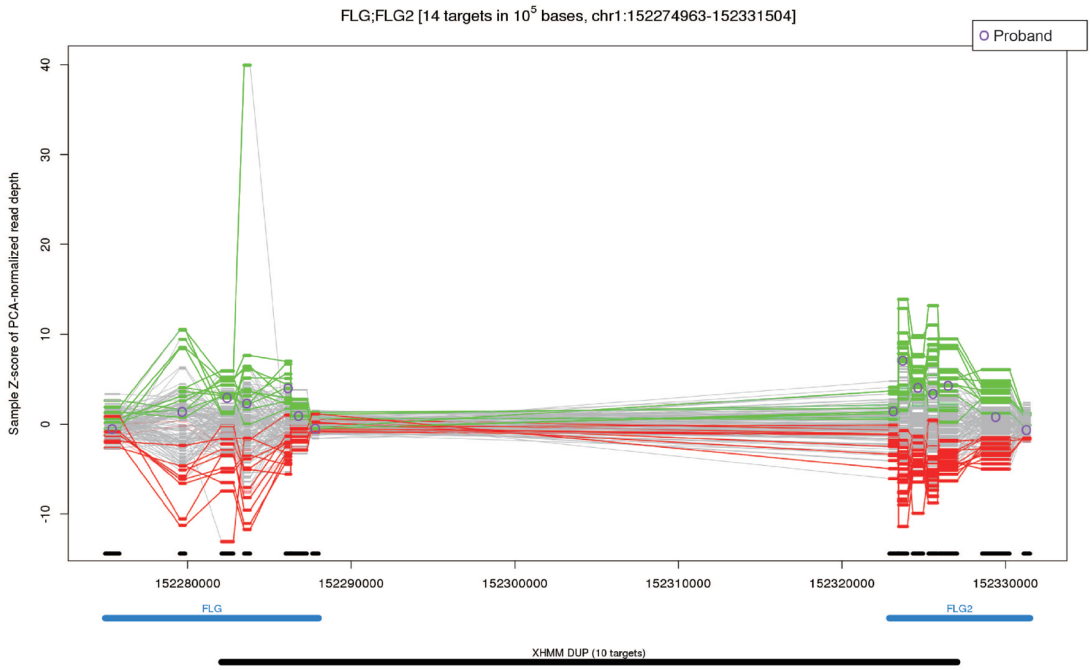

Figure 2. A common CNV with the maternal transmission in the gene potentially related to epilepsy.

and IGE genes. We were able to find duplication in FLG, which is proposed by Wang *et al*.[18] as a potentially epilepsy-related gene (Figure 2). However, the duplication CNV was a common CNV found in 23 (16.8%) out of 138 controls.

DISCUSSION

Solute Carrier Family 12 Member 5 (*SLC12A5*) is mainly expressed in the brain acting as K+/Cl- cotransporter. It is related to the fast hyperpolarization of *GABA*.[19] It was reported that *SLC12A5* variants could cause IGE, epilepsy of infancy with migrating focal seizures (EIMFS), febrile seizure, schizophrenia, and autism spectrum disorder.[13] Considering that genes are related to diverse epilepsy symptoms, *SLC12A5* can cause BRE.

SLC12A5 consists of 1399 amino acids. The variant found in this study replaces the 702nd arginine located in the large C-terminal cytoplasmic domain with histidine. Four programs consistently predicted this variant's nature as being deleterious (Table). While the variants in the N-terminal direction of *SLC12A5* tend to cause EIMFS, the variants in the C-terminal direction of *SLC12A5* cause mild epilepsies such as various IGE and febrile seizure (Figure 3).[13]

This study has a limitation: the other maternal family members did not report symptoms of BRE. The de novo variant might

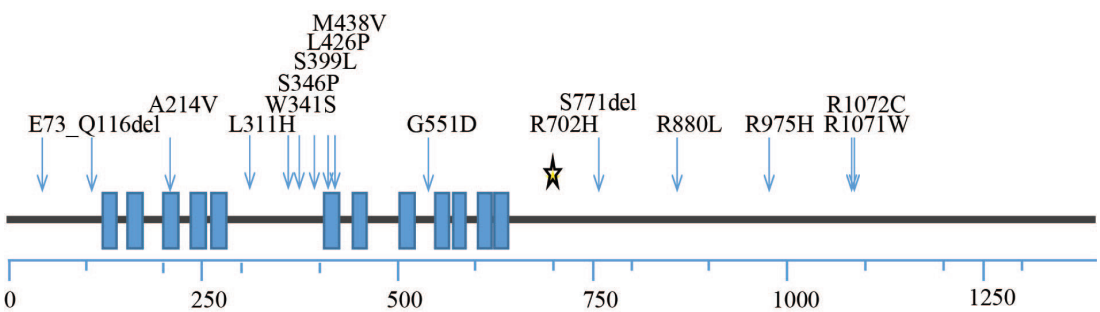

Figure 3. The schematic structure of SLC12A5. The bars represent the transmembrane domains. We showed the locations of the pathologic mutations with arrows.[12] The variant marked with a star is discovered in this study.

Table 1: Nonsynonymous mutations less than 1% MAF in the genes related to BRE or IGE

Gene*	Amino acid	All pop†	EAS†	Korean†	InterVar‡	CADD§	In silico‖	GERP++¶
NRXN1	Ala1239Thr	2.00×10^{-4}	0.0037	9.39×10^{-3}	Uncertain	20.3	B	Conserved
SLC12A5	Arg702His	.	.	.	Likely pathogenic	24.8	D	Conserved

* The locations are 2:50318584:G:A in *NRXN1*, and 20:44678284:G:A in *SLC12A5* according to the hg19 genome reference
† Korean data were derived from the Korean Exome database. Other data were derived from gnomAD database (https://gnomad.broadinstitute.org). All pop means the average MAF of all populations, and EAS means the MAF of East Asian
‡ Interprets variants according to the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guideline[17, 22]
§ A mutation with the CADD score > 20 indicates the 1% most deleterious mutation
‖ metaSVM23, MutationTaser24, Polyphen225, and SIFT26 predicting the effects of the missense mutations, whether the mutations' characters are damage (D) or benign (B). The results in this table of the four tools were the same.
¶ An algorithm inferring the functional significance of variants by their conservation across species[27]

occur in the proband's mother. According to trio studies, the de novo variant is responsible for multiple focal and generalized epilepsies and neuropsychiatric disorders.[20] The mother's symptom was subclinical, considering that the grandparents could not remember the mother's epileptic symptoms. Genetic cofactors may affect the penetrance of the variant. In addition, the variant in *SLC12A5* may be a risk variant or has low penetrance. A further limitation is that the diagnosis was based solely on clinical symptoms and the response to levetiracetam treatment, as there was no EEG conducted for the second son.

We could not find candidate causal CNVs for BRE. Only common CNVs with the same segregation pattern have been found in the genes potentially related to epilepsy. Screening CNVs is essential in finding genetic causes of epilepsy because as much as 16% of childhood-onset epilepsy are caused by CNVs.[21] Algorithms that can detect CNV using NGS data are being developed. We used xhmm using a hidden Markov model algorithm, which has 84.9% validation accuracy.[15]

It is the first small study to show the relationship between BRE and *SLC12A5*. Since the BRE associated with *SLC12A5* has not been reported, confirmation should be made as an additional report. It would be important to identify more BRE-related genes by gathering family cases to identify more genes and the pathogenesis of BRE. Studying the genetic cofactors by which the same gene exhibits various epilepsy symptoms is also necessary.

DISCLOSURE

Financial support: This study was supported by an intramural fund from Ilsan Hospital, National Health Insurance Service.

Conflict of interest: None

Ethics: This study protocol was reviewed and approved by the institutional review boards of Ilsan hospital, approval number [NHIMC 2014-12-002]. The parents gave written and informed consent for the publication of the information about their medical case and any accompanying images.

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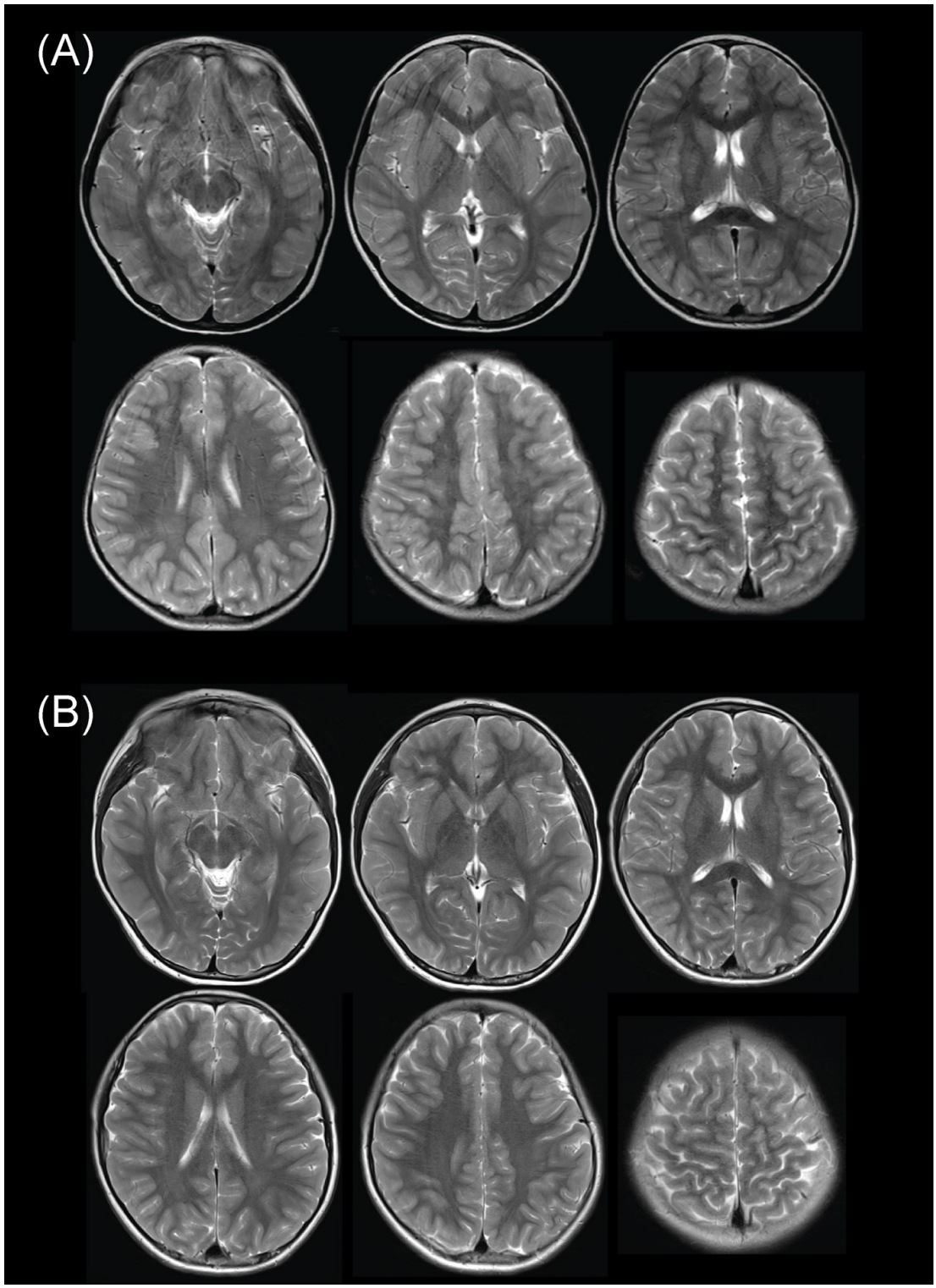
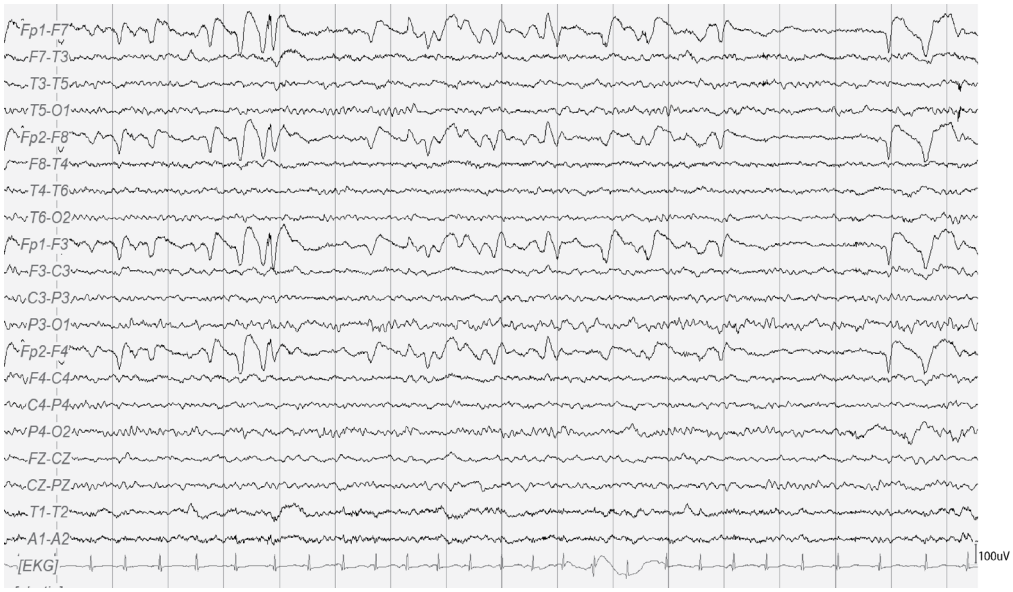
Supplementary Figure 1. Magnetic resonance imagings (A) The first son (B) the second son



Supplementary Figure 2. Awake EEG



Supplementary Figure 3. Sleep EEG.