

Identification of genetic variants in a pedigree associated with epilepsy by using whole exome sequencing and whole genome sequencing

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Epilepsy is a common heterogeneous group of neurological disorders including electroencephalographic and brain imaging. We used whole exome sequencing and whole genome sequencing to identify variants in a pedigree associated with epilepsy. Cranium CT scan showed that the lateral right parietal lobe was hyperdense, and there were no clear boundaries with brain tissue in affected cases. Using WES, one exclusive nonsynonymous mutant in gene TSC2 (Chr16:2138307; c.5240 T > G; p.Ile1747Ser) was involved in this disease. Further analysis showed that *de novo* variant in TSC2 was high conserved across different species. Moreover, the two affected sisters and their father had the same compound heterozygous variants in TSC2, while the father had no epilepsy but depigmentation. These variants demonstrated that variant in TSC2 may result in epilepsy with incomplete penetrance in humans, and the CNV and SV variants we identified probably be involved in this disease.

Keywords

Epilepsy; Variant; TSC2; Sequencing

1. Introduction

Epilepsy is a heterogeneous disease, and genetic cases are observed in more than 50% of patients [1]. Approximately 1000 genes are involved in this disorder whereas additional variants associated with epilepsy are required further verification in the clinical practice [2]. Development of next-generation sequencing, including whole exome sequencing (WES) and whole genome sequencing (WGS), has led to identify lots of disease-causing genes and variants in inherited pattern. The genome-wide association study (GWAS) was specifically used to find some novel loci with genome-wide significance [3]. Tuberous sclerosis complex (TSC) is an autosomal dominant hereditary disorder and patients with TSC mutations exhibit earlier onset of tuberous sclerosis, intellectual disability and epilepsy [4].

This work aims to describe novel variants that include copy number variation and structural variation to identify its genetic clues and provide functional insight into mechanism in a pedigree associated with epilepsy. In this study,

we first performed whole exome sequencing (WES) to identify variants in a single family. A unique heterozygous variant (Chr16:2138307; c.5240 T > G; p.Ile1747Ser) in TSC2 was identified in two sister patients with epilepsy, which involved in multiple clinical disorders and may contribute to this epilepsy. The WGS expanded the involvement of CNV and SV in human genetic disorders by highlighting a role in brain function. Then, focusing on the protein TSC2, which harbored a novel mutant in this family, we found that TSC2 defected variants cause tuberous sclerosis complex. However, it cannot be ruled out that TSC2 variant exacerbates tuberous sclerosis complex, resulting in seizure.

2. Materials and methods

2.1 Clinical features

From May to July in 2015, a family including the normal parents and their two affected daughters with epilepsy were received at Zhengzhou children hospital and Beijing children hospital in China. The two sisters with epilepsy were conceived naturally by non-consanguineous parents. The elder sister was diagnosed at the age of 2.5 years because of froth at the mouth accompanied by seizure every day (duration from 30 s to 1 min). After treatment with Oxcarbazepine (150 mg/day), recurrent symptoms happened every 2–3 days and mental development was slightly affected. Moreover, the MRI analysis was abnormal. The younger sister was diagnosed because of seizure at 3 months. Like the elder sister, she was epilepsy, tuberous sclerosis complex and psychomotor retardation (**Supplementary Table 1**). Furthermore, she was explained as walking problems until now.

2.2 DNA isolation

Considering the effect of antiepileptic drug on the development of infants, none of clinical treatment was implemented to her. 4–10 µg extracted DNA was isolated from patients' peripheral blood for exome enrichment with Quick-DNA™ Miniprep Kit (D3024, Irvine, CA, USA). Quantita-

tive method was tested by qubit fluorometer and agarose gel electrophoresis.

2.3 The whole exome sequencing

DNA libraries were prepared according to the Illumina library generation protocol. Each sample was tagged within the Illumina adaptor, pooled, captured by Exome Enrichment Kit and was sequenced using HiSeq 2000 (Illumina, CA, USA) following the manufacturer's protocol. Briefly, the regions of genomic DNA were captured using Agilent SureSelect Human All Exon 50 Mb kit-based capture system (Agilent Technologies, Santa Clara, CA, USA) according to the Clusters generated by isothermal bridge amplification on Illumina cBot station.

2.4 The whole genome sequencing

Extracted DNA from patients' peripheral blood was performed as previously described in whole exome sequencing (WES in **Supplementary Figs. 1,2**), and followed steps were performed to whole genome sequencing by Illumina HiSeq System for mutant spectrum and distribution of In/del length (**Supplementary Fig. 3**). In this work, the two patients are girls (Fig. 1A).

2.5 The information of data

dbSNP: <ftp://ftp.ncbi.nih.gov/snp/database/>;
1000genome: <ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/>;
NCBI: <ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/>;
Human reference genome (NCBI) at UCSC: hg19: <http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/>; The latest CCDS database version was released at March 27, 2009: ftp://ftp.ncbi.nlm.nih.gov/pub/CCDS/current_human/;
RefSeq gene was extracted from refGene.txt file from the UCSC.

3. Results

3.1 Scan of CT and MRI

Using the Cranium CT scan, our results showed that the lateral right parietal lobe is hyperdense, and there were no clear boundary with brain tissue in affected individual (II:2 in Fig. 1B). MRI scan of the representative individual (II:1 in Fig. 1C, A–B and II:2 in Fig. 1C, C–D) were also checked for tuberous sclerosis complex. As the Fig. 1 showed, the scan of fluid-attenuated inversion recovery imaging (FLAIR) showed some tubers were spots or triangular configuration with the apex pointing toward the ventricle, which are not clear boundary with brain tissue (arrows). In individual II:2 (Fig. 1C), cranium MRI scan showed hyperintense in T1-weighted imaging and hyperintense in T2-weighted imaging.

3.2 De novo variants

De novo variants of SNPs and In/Dels (Insert and Deletion) in the patients with epilepsy were identified by WES and WGS. Unique mutant of SNP in the WES and 25 mutant sites in the WGS were presented in this study (Fig. 2A). The heterozygous mutated condition for c.5240 T > G was identified in individuals with TSC2 by using WES and other variants were listed in panel B–C through WGS (Fig. 2B).

Using the WGS, we identified more variants in two patients, such as ATP7B. 13 *de novo* variants of variants in the patients were ATP7B, TSC2, UGT1A3 *et al.* as listed in Fig. 1B, and 12 variants of In/Dels, AMY2B, AL160286.1, GDF11 *et al.* as listed in Fig. 2C. The variants PDE4DIP and IQSEC3 were duplication while variants TRIM48 and SMR3A were deletion. Meanwhile, we identified the two filters of SV were located in Chr12 (Inserted size, 49883497) and Chr7 (Deleted size, 1267471), especially. The filter of CNV was listed Table 1, and filter of SV was listed Table 2. More information were showed in **Supplementary Tables 2,3** and **Supplementary Fig. 2**.

The WES has identified novel genetic variants in many neurological disorders, including epilepsy. Through WES, we identified a TSC2 variant in patients' samples. The underlying genetic causes are considered as the major factors for epilepsy or seizure.

4. Discussion

In the past two decades, WES was used as a powerful tool to identify candidates that have been reported to be associated with many neurological disorder including epilepsy, such as Lis1, TSC1/2 and members of potassium/sodium voltage-gated channel [2, 4, 5]. At present, variants of genome-wide significance have been identified by using the genetic/genomic technology in clinical cases, for example, the largest genome-wide association study (GWAS). Genetic determinants underlying the common epilepsies remain largely unknown because of complex inheritance. Although one exclusive nonsynonymous variant in gene TSC2 is involved in this study, no clinical case demonstrates TSC2 1747 SNP that acted in a seizure manner. Our results describe a new locus and provide further evidence on genetic architecture of this disorder. Genetic determinants underlying the common epilepsies remain largely unknown because of complex inheritance. Although the etiology of epilepsy is complex, there is no doubt that WES is a tool for Mendelian disease or complex diseases to discovery candidate genes based on pedigree strategy [6]. In this study, a unique heterozygous mutant consisting of c.5240 T > G (Chr16:2138307; p.Ile1747Ser) in TSC (NCBI: ENST00000219476, exon 41) was found (Figs. 1,2, **Supplementary Table 2**), which involves in rapamycin (mTOR) signaling cascade and is associated with hamartomas or benign tumor [7]. Multiple strict filters were used to identify targets using WES and no In/Del variants was found in this study. Since TSC1 and TSC2 expressed in human's brain, loss of them cause neurological disability, for instance, seizures, intellectual disability, autism and focal cortical dysplasia. Herein, the mutation on TSC2 underlined epilepsy, tuberous sclerosis complex and psychomotor retardation. These results showed that the TSC2 was closely related to the regulation of tuberin in patients, resulting to epilepsy and abnormal development. This unique mutation localized in common SNP (Single Nucleotide Polymorphism) in TSC2 by searching through

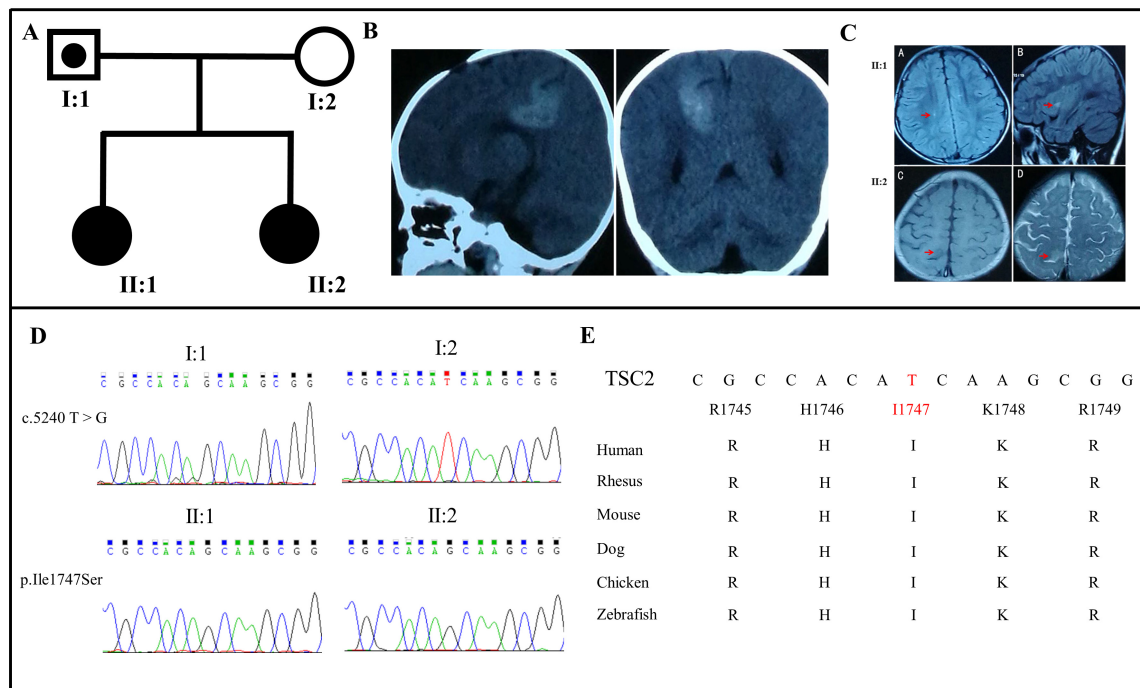


Fig. 1. The feature in the epilepsy pedigree. A pedigree and the patients II:1 and II:2 with the presence of epilepsy (A). Cranium CT (panel B) and MRI (panel C) scan of the family representative individual (arrows), Cranium CT scan showed that the lateral right parietal lobe is hyperdense and there are no clear boundaries with brain tissue. A *de novo* variant in TSC2 was identified and a highly conserved domain (D–E).

Table 1. CNV Filtered information by whole genome sequencing.

Gene	Sample	Chr	Start	End	CNV type	Size	Normalized RD
PDE4DIP	II:1; II:2	chr1	144831901	144894300	duplication	62400	2.10225, 2.14575
TRIM48	II:1; II:2	chr11	55031701	55038600	deletion	6900	0.586812, 0.0152614
IQSEC3	II:1; II:2	chr12	145801	177300	duplication	31500	2.48339, 2.10328
SMR3A	II:1; II:2	chr4	71227501	71250000	deletion	22500	0.530721, 0.502036

UCSC and Genbank databases. Although cannabidiol was a useful drug to reduce seizure frequency than placebo [8], rapamycin might serve as a therapeutic option for reducing the frequency of epilepsy among children via the mTOR, S6K1 and GAP pathway in this family [9].

Multiple strict filters were used to identify *de novo* variants in this study, in the regions targeted by WES capture, the mean numbers of single nucleotide variants (SNVs) and insertions/deletions (In/Dels) detected sample were 1 and 0 for WES, and 24 and 6 (Tables 1,2) for WGS, respectively. These data provided difference between WES and WGS. Although the WGS provided massive data, it is not prominent in the accuracy of single nucleotide polymorphism [10]. However, WGS is more powerful than WES for detecting potential disease-causing mutations within SNVs and SVs. Our results describe a new locus and provide further evidence on genetic architecture of this disorder (copy number variation, CNV and structure variation, SV). Variants of CNV and SV are likely to make a significant contribution to human diversity and play an important role in disease susceptibility [11], affecting the normal processes of neurodevelop-

ment, systemic abnormalities and seizures. Understanding the mechanisms underlying CNV and SV formation is helpful for understanding mutation mechanisms in determining human genetic and phenotypic variation, thereby shedding light on evolution, genomic disorders and complex human diseases [12, 13]. Given the limitation and uncertainty of the WES, the abnormal CNV (longer than 1 Kb) and SV variants in this study were identified by using WGS, and we got the novel variants in our patients.

5. Conclusions

This study showed that a novel nonsynonymous mutation in TSC2 causes tuberous sclerosis complex and epilepsy with incomplete penetrance, and the mutants had no report on epilepsy in previous studies. This association expanded the involvement of SNP in human genetic disorders by highlighting a role in brain function, and it cannot be ruled out that other CNV and SV variants exacerbates tuberous sclerosis complex, resulting in seizure. However, due to the small sample size, more pedigrees will be our next steps to identify causative genes and possible mechanisms.

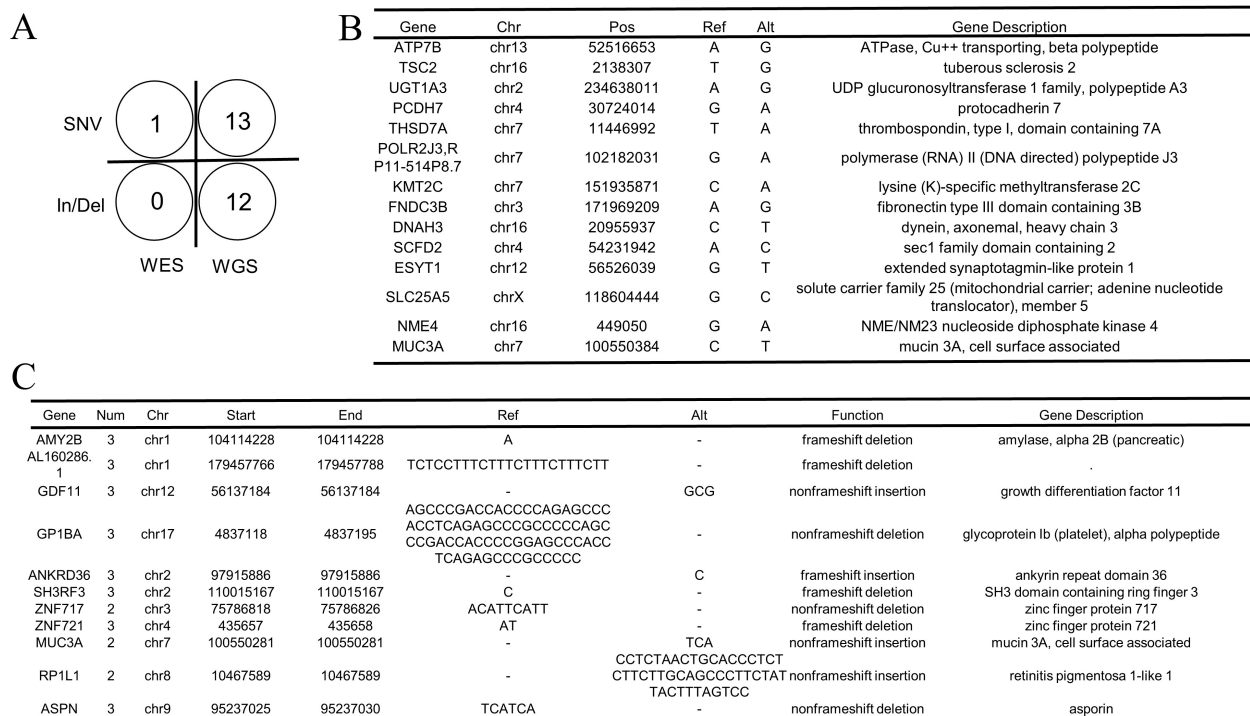


Fig. 2. The variants in this study by sequencing. *De novo* variants of SNV and In/Del in the patients with epilepsy by using the WES and WGS (A). The SNV mutated conditions by using WGS were listed in panel B and *de novo* variants of In/Del were listed in the panel C.

Table 2. SV filtered information by whole genome sequencing.

Gene	Sample	Chr	Start	End	SV type	Size
AAAS, ABCD2, ACVR1B, <i>et al.</i> See the attachment Supplementary Table 3 for details	II:1; II:2	chr12	26627438	76510935	INS	49883497
CASD1, COL1A2, PEG10, PON1, PON2, PON3, PPP1R9A, SGCE	II:1; II:2	chr7	93833712	95101183	DEL	1267471

Abbreviations

CNV, copy number variation; CT, computed tomography; GWAS, genome-wide association study; SNV, single nucleotide variants; SV, structure variation; TSC, Tuberous sclerosis complex; WES, whole exome sequencing; WGS, whole genome sequencing.

Author contributions

LA and XL conceived and the study, QZ, JZ and JG conducted the experiments, ZW analyzed the data, LA wrote the manuscript, all authors read and approved the final version.

Ethics approval and consent to participate

The parents and patients' peripheral blood were obtained with the informed consent of all participants in this study. Written informed consent was obtained from this family and this study was approved by the ethics committee of the hospital. The institutional review board of the Organization Office/Scientific Research Division/Department of Medical Administration approved TSC2 mutation cause epilepsy with incomplete penetrance, code HENU-2016036.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at <https://jin.imrpress.com/E/10.31083/jin2002039>.

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