WHOLE-EXOME SEQUENCING IDENTIFIES A HETEROZYGOUS MISSENSE VARIANT IN THE *GABRB3* GENE IN A PATIENT WITH DRAVET SYNDROME

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INTRODUCTION

Dravet syndrome is a rare and severe type of epilepsy in infants. Approximately 70-80% of DS cases are caused by mutations in *SCN1A*, the gene encoding the alpha-1 subunit of the sodium channel, while some proradic cases would have variants in several other genes including but not limitted to *PCDH19, GABRG2, SCN1B, SCN9A, CHD2*. In this present study, we performed whole-exome sequencing in 6 *SCN1A*-negative patients with Dravet syndrome in order to identify other related genes for this disorder. In one affected individual, we detected a novel *de novo* heterozygous missense mutation R232Q in *GABRB3*, the gene encoding the β3-subunit of the gamma-aminobutyric acid type A (GABAA) receptor, which mediates inhibitory signalling within the central nervous system. Furthermore, a heterogeneous *SCN1A* variant that had been undetected by previous Sanger sequencing was revealed in another patient, whose father was mosaic to the variant. Our results extended the genetic basis of Dravet syndrome and confirmed the utility of whole-exome sequencing in genetic diagnosis

SUBJECTS AND METHODS

Subjects

The study subjects were 6 patients with ages ranged from two years and ten years. The study was approved by the Institutional Review Board of Children Hospital 2 (Ref. No.: CS/N2/16/01HT). The diagnostic criteria for Dravet Syndrome included the following: normal early development; seizures beginning in the first year of life in the form of generalized or unilateral clonic seizures or myoclonic seizures; sensitivity to fever; a normal interictal EEG and a normal MRI at onset; and the presence of afebrile seizures. The diagnosis of Dravet Syndrome was further confirmed with the emergence of other progressive symptoms, including slowing or arrest of cognitive development after 2 years of age, ataxic gait, pyramidal signs, persistence of clonic seizures, and continued sensitivity to fever. The 6 patients subject to WES had been previously screened for *SCN1A* point variants using Sanger sequencing. In addition, exonic delection/duplications had been excluded using *SCN1A* multiplex ligation-dependent probe amplification.

Whole-exome sequencing

Genomic DNA was extracted from probands and their parent blood. Libraries was performed on the genomic DNA using the SureSelectXT Library Prep Kit and the obtained libraries were sequenced on an Illumina HiSeq 4000. Sequences were aligned to the human reference genome (UCSC hg19) using the Burrows Wheeler Aligner (BWA) algorithm. Variants were called to SNPs and short indels using Platypus and Genome Analysis Toolkit (GATK), annotated using SNPeff version 4.1g (the impact of effects is classified into four types: high, moderate, low, and modifier) and determine minor allel frequency using three databases: 1000 genome project, ExAC database, and ESP6500 database. We used the script bayesianDeNovoFilter.py developed together with Platypus to detect de novo variants from four trios and analysed variants from these samples on 9 genes with Dravet syndrome have been reported (i.e., *SCN1A; PCDH19; TSPYL4; CHD2; GABRA1; GABRG2; STXBP1; SCN1B; SCN9A*). Variants with minor allele frequency greater than 0.01% were removed from analyses.

Sanger sequencing and variant analysis

Sanger sequencing was performed to confirm candidate variants. Variants detection analysis was performed using CLC Main Workbench (QIAGEN Bioinformatics). The candidate variants were interpreted and classified according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines (Richards, 2015).

RESULTS

- Through WES of 6 Dravet patients (designed as 4 proband-parent trios and 2 unrelated probands), we identified 2 interesting candidate variants in 2 patients, one variant occurred in GABRB3 and the another occurred in SCN1A gene.
- The variant in *SCN1A* locates in the pore-forming structure of the DIS5-S6 of the Nav1.1 while the variant in *GABRB3* locates in the extracellular domain of the protein. Both variants were absent from global human variant databases.
- In silico analysis predicted both two variant to be deleterious. Using the guidelines developed by the American College of Medical Genetics and Genomics (ACMG) for the interpretation of sequence variants, both variants were classified as “pathogenic variants”

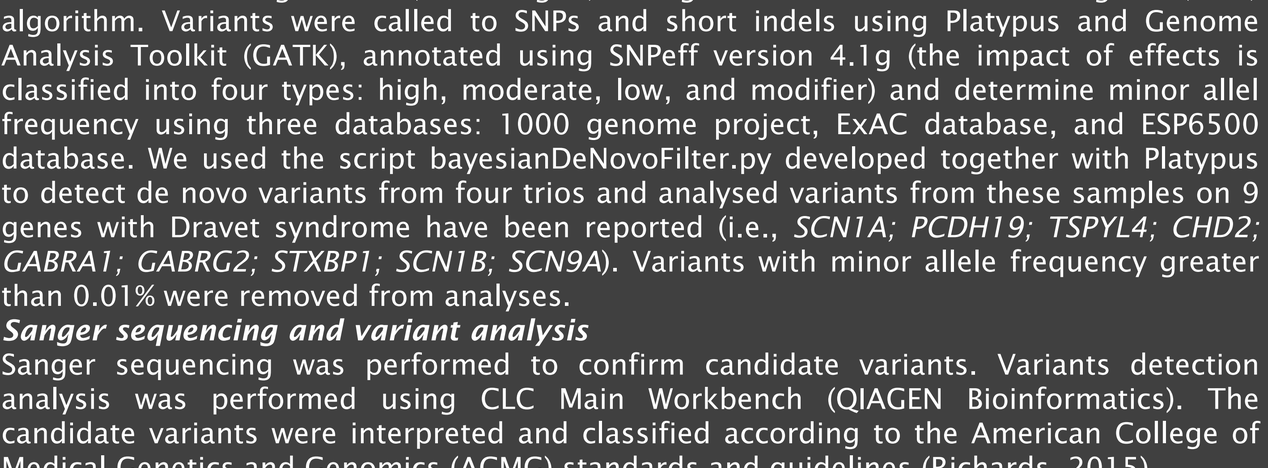

Figure 1. *Electropherograms obtained by direct sequencings on the PCR products of SCN1A coding-exon 9 (3 independent repeats) confirmed the heterozygous mutation c.1178G>A (p.Arg393His) in the patient 1 (A) and the lower levels of the mutant allele in his father (B). Electropherograms obtained by direct sequencings on the PCR products of GABRB3 coding-exon 7 amplified from genomic DNAs of patient 2 and his parents confirmed the heterozygous mutation c.695G>A (p.Arg232Gln) occurred in the patient 2(C)*

Table 1. *Two disease causing variants identified by whole-exome sequencing in two patients*

		Patient 1	Patient 2
Gene		SCN1A	GABRB3
Chromosome		2	15
Position		166903479	26812868
ID		rs121917927	.
REF		C	C
ALT		T	T
QUAL		1482	1397
DEPTH		226	369
SOURCE		Platypus	Platypus
ANOTATION		missense_variant	missense_variant
PutativeImpact		MODERATE	MODERATE
HGVS.c		c.1178G>A	c.695G>A
HGVS.p		p.Arg393His	p.Arg232Gln
Clinvar		Severe_myoclonic_epilepsy_in_infancy; pathogenic (criteria provided)	-
ExAC		NA	NA
ESP6500		NA	NA
1000G		NA	NA
CADD		NA	NA
Rank		0;0	10;78
NR:NV	Proband	92:47	126:63
	Father	49:13	109:0
	Mother	85:0	134:0

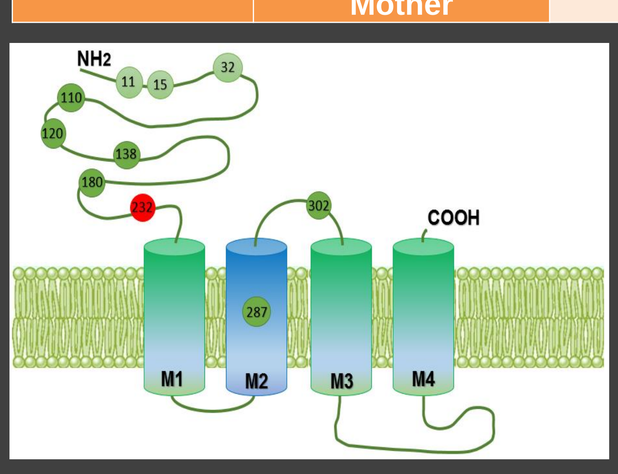

Figure 2: *The gamma-aminobutyric acid type A receptor 63 (GABRB3) subunit, including an extracellular domain, an M1-M4 transmembrane bundle and an M3-M4 intracellular loop. The de novo heterozygous variant in GABRB3 in our proband is located in amino acid position 232 which is located in extracellular domain. Previously reported variants in GABRB3 with epileptic encephalopathies in patients are located in amino acid positions 110, 120, 138, 180, 287 and 302. Related variants in childhood absence epilepsy are in positions 11, 15 and 32, closer to the N terminus.*

CONCLUSIONS AND DISCUSSION

- In this study, we identified for the first time a pathogenic variant in *GABRB3* in a patient with characteristics consistent to Dravet syndrome. In previously studies, the GABRB3 variants had been identified in Childhood absence epilepsy as well as in epileptic encephalopathies (including Lennox–Gastaut syndrome and infantile spasms). While polymorphisms and variants implicated in childhood absence epilepsy are in positions closer to the N terminus, variants in patients with epileptic encephalopathies are located in amino acid positions near and belong to the transmembrane area. The variant found in our patient also locates in the transmembrane area.
- In addition, we detected a heterogeneous *SCN1A* variant that had been undetected by previous Sanger sequencing in another patient, whose father was mosaic to the variant and, therefore, confirmed the utility of whole-exome sequencing in genetic diagnosis.

REFERENCES

1. Bahareh Rabbani, Mustafa Tekin and Nejat Mahdieh; The promise of whole-exome sequencing in medical genetics; Journal of Human Genetics 2014; 59, 5–1
2. Dravet C. The core DS phenotype. Epilepsia 2011; 52:3-9.
3. Epi4K Consortium; Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P et al. De novo mutations in epileptic encephalopathies. (2013) Nature. 2013 Sep 12; 501(7466):217-21.
4. Gemma L. Carvill (2014) GABRA1 and STXBP1: Novel genetic causes of Dravet syndrome, American Academy of Neurology 82:1–8
5. Papandreou A, McTague A, Trump N et al. (2016) GABRB3 mutations: a new and emerging cause of early infantile epileptic encephalopathy. Dev Med Child Neurol 58(4):416-20.
6. Suls A, Jaehn JA, Kecskés A, et al. (2013) De novo loss-of-function mutations in CHD2 cause a fever sensitive myoclonic epileptic encephalopathy sharing features with DS. Am J Hum Genet 93:967-975
7. Scheffer IE. Diagnosis and long-term course of DS. Eur J Paediatr Neurol 2012; 16:S5-8.
8. Xu X, Yang X, Wu Q, Liu A et al. (2015). Amplicon Resequencing Identified Parental Mosaicism for Approximately 10% of "de novo" SCN1AMutations in Children with Dravet Syndrome. Hum Mutat. 36(9):861-72.

ACKNOWLEDGEMENT

This research is funded by Vietnam National University - Ho Chi Minh City (VNU-HCM) under grant number C2016-44-04/HĐ-KHCN