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Short communication

A novel variant in *SCN1A* gene associated with Dravet syndrome

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1. Introduction

Epilepsies are a diverse collection of neurological disorders. Thirty percent of which have a genetic aetiology [1]. Variants in the *SCN1A*-gene have been associated with severe myoclonic epilepsy in infancy (Dravet syndrome) [2]. It is a rare autosomal dominant disorder characterized by the appearance of seizures, usually prolonged hemi-clonic or generalized tonic-clonic type, in an otherwise healthy baby which could be triggered by various stressors. Generally, they start as febrile seizures which eventually occur in the absence of fever. With the advent of new diagnostic tools such as next generation sequencing, genetic confirmation of these conditions has vastly facilitated accurate diagnosis and treatment of epilepsy.

We studied a 2.5 year old girl born to healthy non-consanguineous parents. She was diagnosed to have status epilepticus at the age of 4 months. Initially her seizures were associated with fever later she developed afebrile seizures as well. During the first 2 years of her life seizures were prolong GTC type needing rescue treatment, gradually with treatment the duration and severity of the seizures reduced. Currently she gets right sided seizures lasting from a few seconds to a couple of minutes. Her metabolic screening, full blood count, Magnetic Resonance Imaging (MRI) of the Brain and the Electro Encephalogram (EEG) were all normal. She had global developmental delay accompanied with cognitive and behavioral abnormalities including hyperactive episodes. Throughout this period, a cocktail of antiepileptic drugs including levetiracetam, sodium valproate, clobazam and acetazolamide have been used to control the severity of seizures. However, her best response is to a combination therapy of clobazam and acetazolamide.

Her genomic DNA was extracted from peripheral venous blood leukocytes and was subjected to whole exome sequencing on an Illumina HiSeq platform. Extracted DNA was tagged with adapters and libraries were prepared. Libraries were enriched with Agilent SureSelect Human All Exon + UTR kit which enabled highly uniform coverage of the exome and each individual targeted exon. Analysis was performed using an in-house bioinformatics pipeline. Variants were identified by mapping paired end sequencing data with the GrCh37 human reference sequence using BWA-mem algorithm and Genome Analysis Tool Kit (GATK). The VCF file was annotated using SNP-eff with Refseq, clinical databases and population frequency databases. The variants in a virtual gene panel consisting of genes which were known to cause epilepsy were called and the benign variants were filtered out. The gene panel consisted of the following genes. *ADSL,ALDH5A1,ALDH7A1,ALG13,ARHGEF9,ARX,ATP1A2,ATP1A3,ATRX,BRAT1,C12orf57,CACNA1A,CACNA2D2,CASK,CDKL5,CHD2,CHRNA2,CHRNA4,CHRNB2,CLCN4,CLN2,TPP1,CLN3,CLN5,CLN6,CLN8,CNTNAP2,CSTB,CTSD,DEPDC5,DNAJC5,DNM1,DOCK7,DYRK1A,EEF1A2,EFHC1,EHMT1,EPM2A,FOLR1,FOXG1,FRRS1,GABRA1,GABRB3,GABRG2,GAMTGATM,GLRA1,GNAO1,GOSR2,GRIN1,GRIN2A,GRIN2B,HCN1,HNRNPU,IER3IP1,IQSEC2,ITPA,KANSL1,KCNA2,KCNB1,KCNC1,KCNH2,KCNJ10,KCNQ2,KCNQ3,KCNT1,KCTD7,KIAA2022,LGI1,LIAS,MBD5,MECP2,MEF2C,MFSD8,MTOR,NEDD4L,NGLY1,NHLRC1,NRXN1,PACS1,PCDH19,PIGA,PIGN,PIGO,PLCB1,PNKD,PNKP,PNPO,POLG,PPT1,PRICKLE1,PRRT2,PURA,QARS,ROGDI,SATB2,SCARB2,SCN1A,SCN1B,SCN2A,SCN3A,SCN8A,SCN9A,SERPINI1,SGCE,SLC12A5,SLC13A5,SLC19A3,SLC25A22,SLC2A1,SLC35A2,SLC6A1,SLC6A8,SLC9A6,SMC1A,SNX27,SPATA5,SPTAN1,ST3GAL5,STRADA,STX1B,STXBP1,SYN1,SYNGAP1,XSYNJ1,SZT2,TBC1D24,TCF4,TSC1,TSC2,UBE3A,WWOX,ZDHHC9,ZEB2*.

In silico functional prediction tools; Mutation Taster, SIFT, PolyPhen2 and Provean were used to predict the functional significance of the remaining variants. Functional impact on the protein structure and conservation of the resided region were used to further scrutinize the variants. American College of Medical Genetics and Genomics (ACMG) criteria was used to assign pathogenicity [3].

2. Results and discussion

In this proband, we identified a novel missense variant in exon 16 of the *SCN1A* gene [ENST00000303395: c.2963 T > G] in a heterozygous state. It was confirmed by bi-directional Sanger sequencing (Fig. 1).

At protein level this variant results in the substitution of Leucine by Argnine at position 988 of the amino acid sequence [p.Leu988Arg]. This variant has not been described in scientific literature or clinical databases Clinvar [http://www.ncbi.nlm.nih.gov/clinvar/] or Human Gene Mutation Database; HGMD [http://www.hgmd.cf.ac.uk/ac/index.php] previously nor was listed in any of the population frequency databases such as 1000 Genomes Database [http://www.

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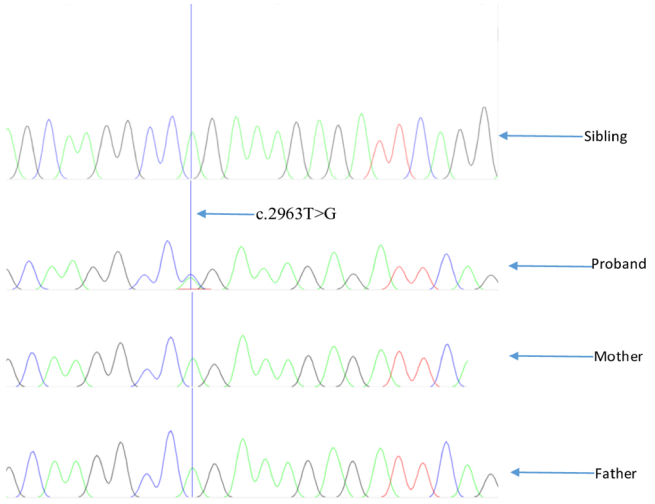

Fig. 1. Sanger sequencing results of the Sibling (homozygous for the ancestral allele), Proband (heterozygous for the variant), Mother (homozygous for the ancestral allele) and Father (homozygous for the ancestral allele) respectively.

Human	V	I	G	N	L	V	V	L	N	L	F	**L**	A	L	L	L	S	S	F	S	A	D	N	L
Chimpanzee	V	I	G	N	L	V	V	L	N	L	F	**L**	A	L	L	L	S	S	F	S	A	D	N	
Rhesus macaque	V	I	G	N	L	V	V	L	N	L	F	**L**	A	L	L	L	S	S	F	S	A	D		
House mouse	V	I	G	N	L	V	V	L	N	L	F	**L**	A	L	L	L	S	S	F	S	A	D	N	
Tiger puffer fish	V	I	G	N	L	V	V	L	N	L	F	**L**	A	L	L	L	S	S	F	S	A	D	N	
Zebrafish	V	I	G	N	L	V	V	L	N	L	F	**L**	A	L	L	L	S	S	F	S	A	D	N	
Proband	V	I	G	N	L	V	V	L	N	L	F	**R**	A	L	L	L	S	S	F	S	A	D	N	

Fig. 2. Evolutionary conservation of the mutated residue.

1000genomes.org/] and The Exome Aggregation Consortium (ExAC) [http://exac.broadinstitute.org/]. It was also not present in any other exome of our in house Sri Lankan exome database. Rare variants with a minor allele frequencies of < 1% are considered to be likely pathogenic.

This variant [p.Leu988Arg] causes a non-conservative substitution of an amino acid sequence of the SCN1A protein. Substitution of Leucine by Arginine may result in a significant alteration in the structure of the protein resulting in psychochemical changes. This variant is located within the DII S6 domain of the SCN1A protein. This domain is highly conserved among the α-subunits of human and other species. This too predicts its deleterious effect on the SCN1A protein that leads to its pathogenicity (Fig. 2).

We utilized four bioinformatics algorithms to predict the functional significance of this variant. The variant was predicted to be damaging by all four algorithms: Mutation Taster - Disease Mutation; Provean - Deleterious; Polyphen2 - Damaging; SIFT - Damaging. Variants predicted to be damaging by In Silico functional prediction tools are likely to be pathogenic.

Variants in the *SCN1A* gene coding for the α-subunit of neuronal sodium channel is associated with a spectrum of seizure related disorders in human, such as Dravet syndrome, febrile seizures and other rare early onset epileptic encephalopathies. Majority of these variants follow an autosomal dominant pattern of inheritance while some show reduced penetrance. In most instances these variants occur *de novo* [4]. The variant found in this child was a *de novo* variant as the parents and her sibling did not have the variant.

3. Conclusion

In this patient Dravet syndrome was caused by a novel *de novo* variant in the *SCN1A* gene. She responded well to a combined therapy which consisted of clobazam and acetazolamide.

Conflicts of interest

There was no funding for this study and all authors declare that there are no conflicts of interest

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