

New mutations in KCNT2 gene causing early infantile epileptic encephalopathy type 57: Case study and literature review

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Purpose. Early infantile epileptic encephalopathy (EIEE) 57 belongs to a group of encephalopathies with early-onset and characterised by severe electroencephalogram abnormalities, seizures, developmental delay and intellectual disability. **Method.** We carried out Whole Exome analysis using Next Generation Sequencing (NGS) and bioinformatic analysis performed to find mutation associated with the patient phenotypes. The effect of the mutation on protein structure analysed by PolyPhen2 and Swissmodel ExPASy. **Results.** In this study, we evaluated two unrelated Turkish males diagnosed with EIEE type 57 to investigate the genetic cause of this disease. Whole exome sequencing revealed mutations in KCNT2 gene, which is a member of Potassium channels (KCN) gene family associated with epileptic encephalopathies. Two mutations, c.545A>T (p.Asn182Ile and c.2638C>A (p.Leu880Met) were reported here as a novel mutation. **Conclusions.** Our findings implicate the genotype-phenotype correlation of these mutations. Furthermore, the computational analysis showed their effect on protein binding site and function suggesting their role in the development of early infantile epileptic encephalopathy 57.

Key words: EIEE, Encephalopathy, Seizure, NGS, KCNT2 gene, Epileptic

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Abbreviations: EE, epileptic encephalopathy; EEG, electroencephalogram; EIEE, Early infantile epileptic encephalopathy; EMG, Electromyography; ESP, exome sequencing project; ExAC, exome aggregation consortium; KCN, Potassium channels; MRI, Magnetic Imaging Resonance; NGS, Next Generation Sequencing; WES, Whole Exome Sequencing

INTRODUCTION

Early infantile epileptic encephalopathy (EIEE) is autosomal dominant and is identified by phenotypic characteristics including intractable seizures and severe cognitive impairment and/or developmental delay (Berg *et al.*, 2010). Occurrence of the EE (epileptic encephalopathy) was found frequent in some countries (Hino-Fukuyo *et al.*, 2009). Moreover, difficulties of patients' maintenance and care, high possibilities of comorbidities, and short life-span (Khan & Baradie, 2012) create devastating scenario for patients and their families. Various genetic abnormalities in regulatory proteins and ion channels have been detected as causatives of EE (McTague *et al.*, 2016). Although development of NGS (next generation sequencing) has opened new door into diagnosis of genetic impairments, more than 60% of EIEE remains

undiagnosed. Therefore, diagnosis of other genetic causes must be taken into account to facilitate therapies.

Functional abnormalities in ion channels, particularly potassium (K⁺) channels due to mutation in potassium channels gene family (KCN), are shown to contribute to impaired neurological functions (D'Adamo *et al.*, 2013). Developmental and epileptic encephalopathies in the form of refractory seizures and frequent epileptic activity have been reported with KCN mutations (Scheffer *et al.*, 2017). KCN gene family encode broad spectrum of potassium channel subunits that are KCNA1, KCNA2, KCNB1, KCNC1, KCNQ2, KCNQ3, KCNQ5 and KCNT1. 3-9 KCNT1 (Slo2.2 or Slack), together with KCNT2 (Slo2.1 or Slick), belongs to the SLO2 family of Na⁺-dependent (K⁺) channel genes, encoding for pore-forming α subunits variably gated by changes in voltage and/or concentrations of intracellular ions or second messengers (Bhattacharjee *et al.*, 2003). KCN2 has been considered as a candidate gene for epilepsy with similar symptoms as that of KCNT1 gene (Ambrosino *et al.*, 2018). Recent study on individual with West syndrome detected association of KCNT2 mutation. with the disease (Gururaj *et al.*, 2017). Although nearly 74% of the KCNT2 gene homology and hetero- tetramerization between the subunits in some brain and heart regions has been detected, no previous report indicates the connection between the variants of KCNT2 (SLICK or Slo2.1) to human phenotype (Chen *et al.*, 2009; Lim *et al.*, 2014; Lim *et al.*, 2016).

In the present study, we investigated the functional impact of two de novo variants of KCNT2 gene in two unrelated patients with neurodevelopmental disorders.

PATIENTS INFORMATION

The study has been approved by the ethics committee of Biruni University. Written consent for genetic testing and related data has been obtained from each case's biological parents. However, both family refused to carry out further detailed analysis on family members.

Both individuals were born to nonconsanguineous healthy parents of Turkish origin. Individual #1 is a 6-year-old boy who was born at term with 2.90 g and normal head circumference. Individual #2 a 5-year-old boy who was born at term with 2.86 g. Individual #1 was admitted at Biruni University hospital with inability in speaking and walking which indicated delay in motor development. Magnetic Imaging Resonance (MRI) of the patient, at the age of 2, showed diffusely thin corpus callosum. Also, both of the lateral ventricles are dilated and partial colpocephaly was detected and patient was diagnosed with refractory epilepsy. Social interaction and

eye contact was reported to be normal. EEG was performed at the age of 5 and it showed sharp and slow waves in the right frontotemporal region. Further analysis with EMG at the same age detected low amplitude distal motor responses from the lower extremity. The individual #2 showed normal EEG at the age of 1. Both individuals showed reduced muscle strength (hypotonia) and delayed neural development followed by intractable seizures. Further genetic analysis was recommended to find out the causatives for the presented phenotypic characteristics.

RESULTS

Genetic analysis

To find genetic alteration associated with the patient phenotypes, we performed Whole Exome Sequencing to analyse the coding exons and exon-intron boundaries of 18,000 protein coding genes. Genomic DNA preparation, exome capture, and Illumina sequencing (NextSeq platform) were performed as recommended by manufacturer. In brief, Genomic DNA was extracted from whole peripheral blood sample using iPrep PureLink gDNA blood Kit (Invitrogen). Genome Library was prepared with Agilent SureSelect Target Enrichment system (Agilent Technologies, Inc., Santa Clara, CA, USA) and enrichment of coding exons and flanking intronic regions was performed using Agilent SureSelect Human All Exon V6 reagent following the manufacturer's protocol as previously described (Bonfond *et al.*, 2012) and

sequencing was performed using an Illumina NextSeq500 system. Sequence reads were mapped to the human genome (GRC38/hg19) using the Burrows-Wheeler Aligner (version 0.6.1; algorithm "BWA-SW"; default parameters) for targeted sequencing data. Variants with frequency more than 1% in the population were removed. Variants were annotated using Alamut visual, and allele frequency with databases, dbSNP, ExAC (exome aggregation consortium) variants and the 1000 Genomes Project. Disease causality was assessed using ClinVar and ESP (exome sequencing project) variants ExAC (exome aggregation consortium) variants, ESP (exome sequencing project) variants.

The expression of sodium activated potassium channel gene, KCNT2 (Slick), is found in heart and nervous system. Slick is activated by intracellular sodium and possesses nucleotide-binding site that maintains ATP-dependent inhibition of channel function and this characteristic affect livability of brain/heart cells which is due to vigorous activities of neurons (Salkoff *et al.*, 2006). The whole exome sequencing revealed heterozygous mutation c.545A>T (p.Asn182Ile) in transcript NM_198503.2 and c.2638C>A (p.Leu880Met) in transcript NM_198503.4 in individual #1 and #2 respectively. Based on the family histories and phenotypic characteristics of the disorder in the patients, the above mutations were suggested to be de novo missense variant.

PROTEIN ANALYSIS

Damage intensity of the mutations was analysed using PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>).

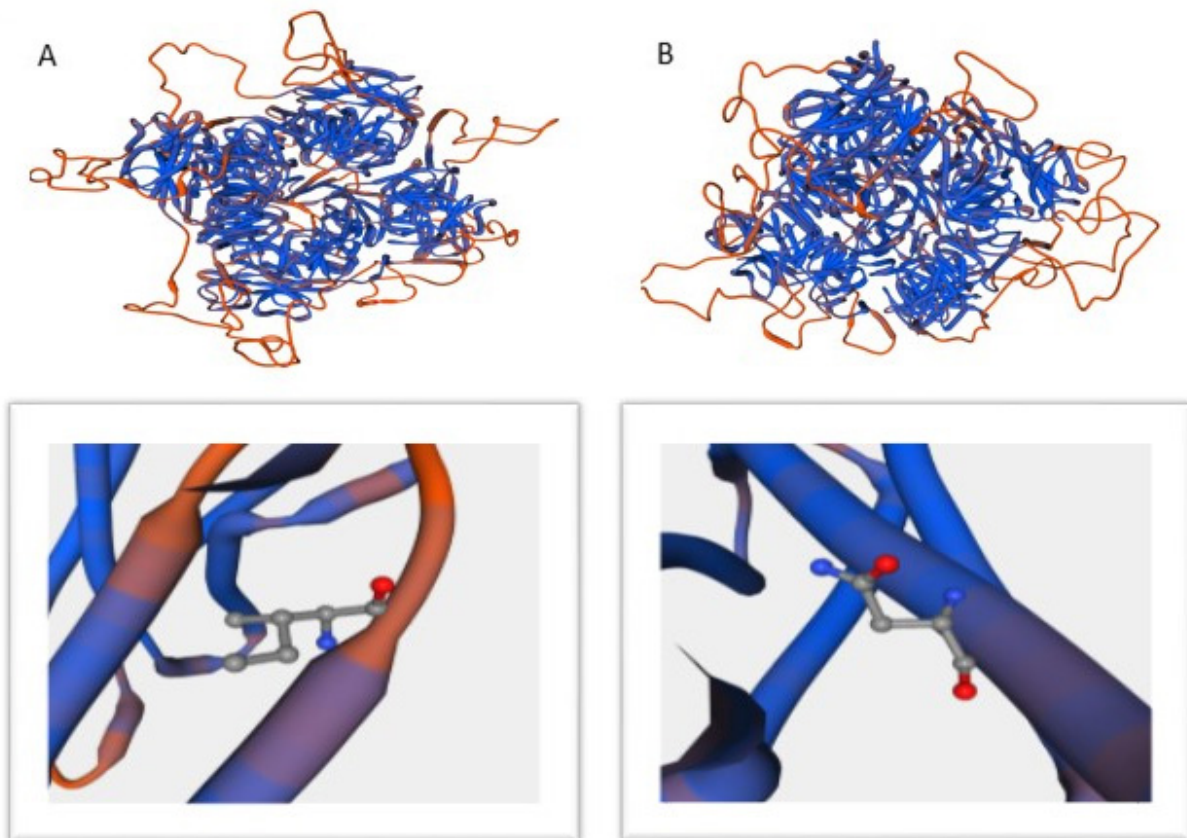


Figure 1. Protein configuration of KCNT2 gene in wild type (A) and case 1 mutations (B). Figure 1B represents protein configuration change and its binding ligand caused by substitution of Asparagine by Isoleucine.

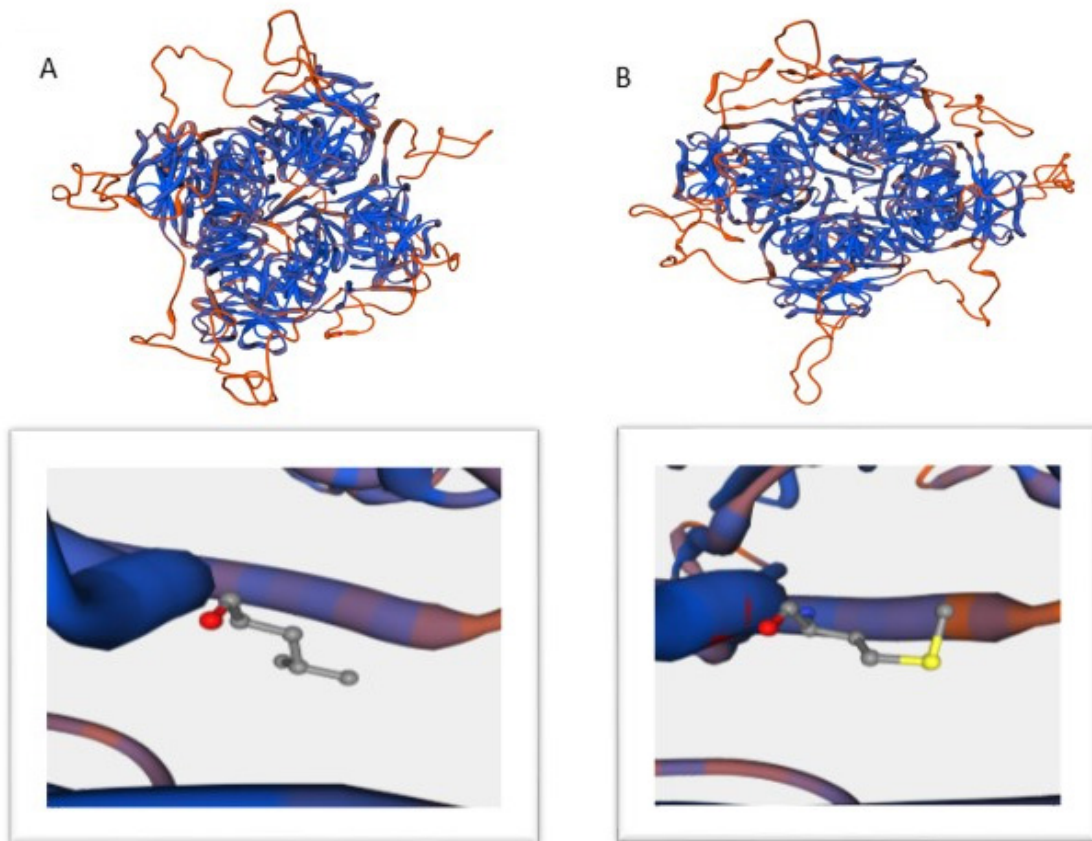


Figure 2. Protein configuration of KCNT2 gene in wild type (A) and case 2 mutations (B).
Figure 2B represents alteration in protein structure and binding sites due to substitution of leucine by methionine

N182I and L880M substitutions are shown to be probably damaging (score 0.984 and 0.989 respectively). Mutation taster program (www.mutationtaster.org/) showed that both mutations cause change in protein function and are disease causing. Following the findings, 3D protein structure analysis by swissmodel expasy (<https://swissmodel.expasy.org/interactive>) showed conformational changes in protein and its binding sites (Fig. 1 and Fig. 2). The position was shown in S4-S5 for both mutations which could possibly affect regulators of conductance of potassium (RCK) domains which were previously introduced (Bhattacharjee *et al.*, 2003; Salkoff *et al.*, 2006).

DISCUSSION

Early Infantile Epileptic Encephalopathy (EIEE) is characterized as neurological disorder and patients show seizures and tonic spasms at the first few months of life. EIEE type 57 has been previously reported in patients with KCNT2 gene mutations (Gururaj *et al.*, 2017). In this paper, we reported two patients of similar phenotypes with different mutation locations on KCNT2 gene. Although the variants' position was not same (N182I and L880M), computational analysis of data from Whole Exome sequencing presented possibly similar damaging effects on protein function and its binding sites. As both patients presented epileptic seizure and developmental delay in nervous system, we deduced the phenotypic abnormality to have been caused by ATP-dependent potassium channels' dysfunction. Although the clinical data

due to lack of patients' family cooperation for further studies are not sufficient to present strong case, previous findings on other patients showed connection between KCNT2 gene impairment and EIEE57. Therefore, further investigation of the novel mutations in relation with the development of EIEE57 and patients' clinical outcome is strongly recommended.

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