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SCN1A gene sequencing in 46 Turkish epilepsy patients disclosed 12 novel mutations

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ABSTRACT

Purpose: The SCN1A gene is one of the most commonly mutated human epilepsy genes associated with a spectrum of phenotypes with variable degrees of severity. Despite over 1200 distinct mutations reported, it is still hard to draw clear genotype–phenotype relationships, since genetic and environmental modifiers contribute to the development of a particular disease caused by an SCN1A mutation. We aimed to initiate mutational screening of the SCN1A gene in Turkey and advance further our understanding of the relationship between the SCN1A sequence alterations and disease phenotypes such as GEFS+, DS and related epileptic encephalopathies.
Methods: Mutational analysis of the SCN1A gene was carried out in 46 patients with DS, late-onset DS, GEFS+ and unspecified EE using either direct Sanger sequencing of the coding regions and exon/intron boundaries or massively parallel sequencing.
Results: Nineteen point mutations, 12 of which were novel were identified, confirming the clinical diagnosis of the patients. Patients with a mutation (either truncating or missense) on linker regions had significantly later disease onset than patients with mutations in homology regions. The presence of SCN1A mutations in two clinically unclassified patients supported the association of SCN1A mutations with a wide range of phenotypes.
Conclusion: The conventional Sanger sequencing method was successfully initiated for the detection of SCN1A point mutations in Turkey in epilepsy patients. Furthermore, a modified strategy of massively parallel pyro-sequencing was also established as a rapid and effective mutation detection method for large genes as SCN1A.

1. Introduction

The *SCN1A* gene, located on 2q24.3, encodes the voltage-gated sodium channel alpha1 subunit ($Na_v1.1$, $\alpha1$). $Na_v1.1$ channels are responsible for the neuronal excitability and expressed in the central and peripheral nervous systems and in cardiac myocytes. They are located particularly in the soma and dendrites and in the axonal initial segment in a subset of inhibitory interneurons [1]. The mutations found in the *SCN1A* gene are the most common genetic cause of early epileptic encephalopathies (EE) and have been associated with a spectrum of phenotypes including Dravet Syndrome (DS), Generalized Epilepsy with Febrile Seizure Plus (GEFS+), Borderline Severe Myoclonic Epilepsy of Infancy (SMEB), Doose syndrome (MAE), infantile spasms and some other infantile epileptic disorders [2–4]. Mutations vary from point mutations to

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microdeletions, translocations and intra/intergenic duplications [2,5–7]. The *SCN1A* gene variants are listed on three webpages [8–10]. While gain-of-function mutations lead to increased neuronal excitability, loss-of-function mutations reduce the inhibitory action of GABAergic interneurons [11]. DS is a common form of EE and about 80% of DS patients have an *SCN1A* mutation approximately 90% of which arising *de novo*. DS is characterized by infantile onset, fever-sensitive, treatment resistant seizures and intellectual disability [12]. Seizure onset is in the first year of life, generally at around 6 months. At onset, seizures can be generalized or unilateral, clonic and tonic-clonic. During the course of the disease afebrile, myoclonic or focal seizures or atypical absences can also occur. Until seizure onset, development is normal. However, developmental delay and other neurological defects begin during the second year of life [1].

Despite the identification of many mutations in the *SCN1A* gene, the genotype–phenotype correlation is still not clear suggesting the involvement of either genetic or environmental modifiers in the development and progression of various diseases [13,14]. Mutations causing truncation of the channel protein and missense mutations affecting voltage sensitivity and/or pore regions are more frequent in DS patients and missense mutations affecting channel function less severely are more frequent in GEFS+ [15].

In order to initiate mutational screening of the *SCN1A* gene in Turkey and advance further our understanding of the relationship between the *SCN1A* sequence alterations and disease phenotypes such as GEFS+, DS and related epileptic encephalopathies, the conventional Sanger sequencing method and a modified strategy of massively parallel pyro-sequencing for rapid detection of mutations were established in this study.

2. Materials and methods

2.1. Subjects

Forty-six Turkish patients suspected to have DS, late-onset DS (LO-DS), unspecific EE (UEE) and GEFS+ were included in this study with the informed consent of their families. The study was conducted with the approval of the Institutional Review Board for Research with Human Subjects.

The study cohort consisted of DS ($N = 30$), LO-DS ($N = 10$), GEFS+ ($N = 4$) and UEE ($N = 2$) patients. DS was characterized by febrile or afebrile tonic or tonic-clonic seizures with onset within the first year of life and yielding developmental stagnation and regression onwards [16]. Patients with LO-DS had the same clinic with DS patients but disease onset was beyond the first year of age. GEFS+ was characterized with febrile seizures starting early in life that continued beyond the age of six, generally with a positive family history [17]. Two patients, due to the lack of complete clinical information did not fit into definitions of DS, LO-DS and GEFS+ but were also included since they had UEE characterized by absence seizures at onset and a degree of mental retardation later.

2.2. SCN1A gene amplification

Genomic DNA was extracted from K'EDTA-treated peripheral blood samples using MagNa Pure Compact Nucleic Acid Isolation Kit Large Volume (Roche Diagnostics, Mannheim, Germany).

All 26 exons including exon/intron boundaries of the *SCN1A* gene were amplified by polymerase chain reaction (PCR) in 36 separate reactions using previously reported primer pairs [18]. For massively parallel sequencing, all amplicons were fluorometrically quantified using Quant-iT-PicoGreen dsDNA assay Kit (Invitrogen, ABD) on Light Cycler 480 (LC480II, Roche Diagnostics, Mannheim, Germany). Amplification conditions are available upon request.

2.3. Sanger sequencing

Purified PCR products were Sanger sequenced at Macrogen, Seoul, Korea. All mutations were confirmed by repeated PCR and re-sequencing. Nucleotides were numbered according to reference sequences NM_001165964.1 and NP_001159436.1 where A of the ATG start codon corresponded to nucleotide number one. Bioinformatics tools namely, Mutation Taster [19], Polyphen [20] and Provean [21] were used to evaluate the putative effects of novel mutations.

2.4. Massively parallel amplicon sequencing (MPAS)

SCN1A amplicons of each patient were pooled at equal molecule amounts and purified by MinElute DNA purification kit (Qiagen, ABD). Ten nucleotides long MID sequences specifying each patient were ligated to amplicons in each pool using GS Rapid Library Preperation Kit Lib-L (Roche, Germany) as described in the GS Junior Rapid Library Preparation Manual. Concentration of pooled amplicons was measured both by Quant-iT-PicoGreen dsDNA assay Kit (Invitrogen, ABD) and also by qPCR using KAPA NGS quantification kit (KAPA systems, ABD) on LightCycler 480II. Dilutions were made to have single fragment per bead and the sequencing library was prepared for emulsion PCR (emPCR) using GS Junior Titanium emPCR Kit Lib-L (Roche, Germany) as described in GS Junior emPCR Lib-L manual. DNA attached beads were picked up magnetically and pyrosequenced using GS Junior sequencing kit by following the instructions in the GS Junior sequencing method manual. Amplicon sequences were analyzed by Amplicon Variant Analyzer (AVA) program (Roche, Gernmany) using the *SCN1A* reference sequence, PCR primer and MID sequence information. In the result of AVA analysis, a variant list was obtained for each patient. The variants were filtered for known SNPs and unique variants were validated by Sanger sequencing.

2.5. Statistical analyses

Statistical analysis was conducted using SPSS software (Version 22) nonparametric tests module for Independent Samples Kruskal–Wallis Test with 95% confidence interval.

3. Results

A total of 46 patients with DS, LO-DS, EE and GEFS+ phenotypes were analyzed for *SCN1A* sequence variations by Sanger sequencing (30 patients) and Massively Parallel Amplicon Sequencing (16 patients) methods. Out of 46 patients, 19 (41.3%) had a sequence variation in the *SCN1A* gene, 12 of which were novel. Genetic variations and clinical information of patients are given in Tables 1 and 2, respectively. Among 30 patients with DS phenotype 12 had a mutation in the *SCN1A* gene (40%), on the other hand, only three out of 10 LO-DS patients were positive for *SCN1A* mutations (30%). Two of the four GEFS+ patients had a maternally inherited *SCN1A* mutation. Two UEE patients had a novel *de novo* frameshift mutation (2DS4) and a previously reported and paternally inherited variant (11DS23). This variant was present in the ExAC database with low frequency (0.0015).

3.1. Clinical summary of patients with SCN1A mutations

All patients except 11DS23, 29DS57 and 57DS121 had FS at disease onset (84%) and three patients had their first seizure after vaccination. All patients except 29DS57 had variable degrees of mental retardation with additional autistic features, speech delay and motor delay. Among DS patients, only 6DS12, 14DS26 and 15DS27 were pharmacoresistant (Table 1).