Turkish Journal of Pediatric Disease
Türkiye Çocuk Hastalıkları Dergisi

Administration Of *SCN1A* Genetic Testing As A Pre-Prognostic Indicator in Early Onset Recurrent Febrile Seizures

Erken Başlangıçlı Tekrarlayan Ateşli Nöbetlerde Pre-Prognostik Bir Gösterge Olarak ***SCN1A*** Genetik Testinin Uygulanması

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ABSTRACT

Objective: To determine whether the epileptic predispositions of recurrent febrile seizures (onset of age 1 and below) could be predicted earlier using analysis of *SCN1A* gene sequencing.

Material and Methods: The study included 55 patients aged between 0-18 who were admitted to pediatric emergency service with a febrile seizure. Patients were selected based on the criteria of presenting recurrent (two or more) febrile seizures with the onset of age one and below, having normal cranial imaging and central nervous system infections being ruled out. *SCN1A* gene sequence analysis was performed using the next-generation sequencing method.

Results: The c.1738C>T and c.4181C>T were the previously reported whereas the c.2914-1G>A and c.473A>G were novel *SCN1A* heterozygous disease-causing variants which were identified in five of 55 patients from 55 unrelated families (9.09%). The patients with c.1738C>T, c.2914-1G>A, and c.4181C>T variants presented probable Dravet syndrome or Dravet syndrome phenotype, but then the other two with c.473A> G demonstrated genetic epilepsy with febrile seizure plus.

Conclusion: Beforehand administration of *SCN1A* genetic testing in early-onset febrile seizures could be a more significant indicator rather than the clinical risk factors for determining the prognosis and designing the long-term follow-up.

Key Words: Dravet syndrome, Febrile seizures, *SCN1A*

ÖZ

Amaç: 1 yaş ve altı tekrarlayan ateşli nöbeti olan çocukların epileptik yatkınlıklarının *SCN1A* gen dizi analizi kullanılarak daha erken tahmin edilip edilemeyeceğini belirlemek.

Gereç ve Yöntemler: Çalışmaya çocuk acil servisine ateşli nöbet ile başvuran 0-18 yaş arası 55 hasta dahil edildi. Hastalar, bir yaş ve altında tekrarlayan (iki veya daha fazla) ateşli nöbetleri olma, normal kraniyal görüntülemeye sahip olma ve merkezi sinir sistemi enfeksiyonları ekarte edilme kriterlerine göre seçildi. *SCN1A* gen dizi analizi, yeni nesil dizileme yöntemi kullanılarak gerçekleştirildi.

Bulgular: c.1738C> T ve c.4181C> T daha önce bildirilmişken, c.2914-1G> A ve c.473A>G, yeni heterozigot hastalığa neden olan *SCN1A* varyantlarıydı. 55 akraba olmayan aileden 5 çocuk için (% 9.09) c.1738C> T, c.2914-1G> A ve c.4181C> T varyanta sahip olan hastalar olası Dravet sendromu veya Dravet sendromu, ancak c.473A> G olan diğer ikisi ateşli nöbet artı genetik epilepsi fenotipi gösterdi.

ALBAYRAK HM : 000-0001-5624-3878
PERK P : 000-0002-0778-056X
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Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: The study has been approved by the ethics committee of Gaziantep University with the 2018/189 registration number.

Contribution of the Authors / Yazarların katkısı*: *ALBAYRAK HM: Constructing the hypothesis or idea of research and/or article, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***PERK P:*** Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results. ***KIRAT E:*** Planning methodology to reach the Conclusions,

How to cite / *Atıf yazım şekli* : Albayrak HM, Perk P, Kirat E. Administration Of *SCN1A* Genetic Testing As A Pre-Prognostic Indicator in Early Onset Recurrent Febrile Seizures. Turkish J Pediatr Dis 2021;15:476-481.

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Received / Geliş tarihi : 12.10.2020
Accepted / Kabul tarihi : 04.12.2020
Online published : 11.03.2021
Elektronik yayın tarihi
DOI: 10.12956/tchd.809273

Sonuç: Erken başlangıçlı ateşli nöbetlerde *SCN1A* genetik testinin önceden uygulanması, prognozu belirlemek ve uzun dönem takibi tasarlamak için klinik risk faktörlerinden daha önemli bir gösterge olabilir.

Anahtar Sözcükler: Dravet sendrom, Ateşli nöbet, *SCN1A*

INTRODUCTION

Febrile seizure (FS) is a seizure accompanied by a fever without central nervous system infection or neurologic dysfunction predisposing an increased risk of seizures. FSs are relatively common events in the general pediatric population, which occur in about 2-5% of children aged between 6 months to 5 years (1). *SCN1A*-related seizure disorders encompass a spectrum of phenotypes that ranges from FS, which may or may not have features suggestive of an *SCN1A*-related condition to generalized epilepsy with febrile seizures plus (GEFS+) or Dravet syndrome (DS), also known as severe myoclonic epilepsy in infancy (2).

The main clinical risk factors for developing recurrent FSs or epilepsy had been identified previously but have not been sufficient to determine prognosis and initiate antiepileptic therapy. Furthermore, genetic testing has become a powerful tool in clinical epilepsy practice in certain situations. Variants of *SCN1A* are found in 70–80% of patients with DS and up to 10% in families having generalized epilepsy with GEFS+ (3).

A study about the rate of patients with early-onset FSs who are positive for *SCN1A* genetic testing has not been previously conducted. This study aims to determine whether the epileptic predispositions of recurrent FSs (onset of age 1 and below) could be predicted earlier by using analysis of *SCN1A* gene sequencing.

MATERIAL and METHODS

Fifty-five patients aged between 0-18 years old were selected based on the criteria of presenting recurrent (two or more) FSs with the onset of age under 12 months, having normal cranial imaging, and without having central nervous system infections. The period of the patient selection time was between February 2018 to January 2019. Neither duration nor the type of seizures was disregarded in this study. *SCN1A* genetic testing was performed on all patients who had met the inclusion criteria. The patients, who were molecularly confirmed by *SCN1A* genetic testing (having a disease-causing *SCN1A* variant), were followed-up for a year after taking the consent of the families. The cognitive and neuromotor development assessment and electroencephalography (EEG) were performed once every six months by a pediatric neurologist. Antiepileptic treatment was started according to Cochrane database criteria 2017 for the prevention of FSs in *SCN1A* positive patients (4). Other essential details such as the family history of febrile and/or afebrile seizures and the response of antiepileptic treatment, were also recorded.

The study has been approved by the ethics committee of Gaziantep University with the 2018/189 registration number.

Genetic analysis

Genomic DNA was extracted from whole blood samples using an automated method (RSC whole blood DNA kit) in the Maxwell® 16 (Promega Corporation, Madison, WI) and sequenced using a custom-designed targeted panel that comprised all exons and exon-intron junctions of *SCN1A* on Ion Torrent system (Thermo Fisher Scientific).Variants were described using the Human Genome Variation Society nomenclature guidelines and checked against those available in 1.000 Genomes, dbSNP, ClinVar, and Human Genome Mutation Database. American College of Medical Genetics and Genomics Standards and Guidelines were used for the determination of variant pathogenicity (5).

RESULTS

Of the 55 patients from 55 unrelated families were included in the study, 29 were female and 26 were male. The mean age of the applicants was 57.52 (5-166) months. The mean age of the FS onset was 7.17 (1-12) months. The youngest patient in the study group was a 5-month-old girl, whose seizures started when she was 3-months-old and was *SCN1A* positive.

Table I: Review of the identified *SCN1A* variants.

Patient	Transcript	HGVS coding	HGVS protein	Coding impact	Localization	Origin	References
1	NM_006920.5	c.1738C>T	p.Arg580Ter	Missense	Topological domain (Cytoplasmic)	De novo	[6-9]
2	NM_006920.5	c.2914-1G>A	-	Splice site	Transmembrane (Domain II, segment 6)*	De novo	NR
3	NM_006920.6	c.4181C>T	p.Thr1394Ile	Missense	Topological domain (Extracellular)	De novo	[3]
4 5	NM_006920.4	c.473A>G	p.Glu158Gly	Missense	Transmembrane (DI, SII)	Maternal NA	NR

HGVS: *Human Genome Varia* , ***S:*** *Segment*, ***NR:*** *Not reported*, ***NA:*** *Not available*,****** *It is predicted to affect the segment 6 of domain II*

Table II: Clinical characteristics of the patients with pathogenic *SCN1A* variants.

Patient	Gender	Current age	Age at study	Age of seizure onset	Seizure type	Epileptiform EEG	DD	AFS	Family history of FS	Family history of AFS	Antiepileptic treatment	Related phenotype
1	F	24 mo	5 mo	3rd mo	GTC	-	-	-	-	-	Single	Probable DS
2	F	25 mo	13 mo	8th mo	GTC	-	+	-	-	-	Dual	Probable DS
3	F	36 mo	27 mo	6th mo	GTC, absence	-	+	-	-	-	Dual	DS
4	F	44 mo	32 mo	4th mo	GTC	-	-	-	+	+	Dual	GEFS+
5	F	47 mo	36 mo	10th mo	GTC	-	-	-	+	+	Single	GEFS+

F:*Female,* ***mo****: Month,* ***GTC:*** *Generalized tonic clonic,* ***EEG:*** *Electroencephalography,* ***DD:*** *Developmental delay,* ***AFS:*** *Afebrile seizure,* ***FS****: Febril seizure,* ***DS:*** *Dravet syndrome;,* ***GEFS+:*** *Generalized epilepsy with febrile seizures plus*

The oldest patient was a 166-month-old girl and her seizures started at 1 month of age as febrile. When the fever-triggered seizures continued at the age of 2, antiepileptic treatment was started. She had a developmental delay, there was no family history of FS. No pathogenic variant was detected in *SCN1A* in this patient. This patient was using mono-antiepileptic and her response to treatment was good. Of the 50 *SCN1A* negative cases, five had concomitant afebrile seizures (AFS), 22 had a first-degree relative with FS, eight had a family history of AFS, and ten had developmental delay.

Pathogenic heterozygous variants in the *SCN1A* gene were detected in five of 55 patients (9.09%) (Table I). The c.1738C>T and c.4181C>T was the previously reported, disease-causing variants, whereas the c.2914-1G>A and c.473A>G variants have not been reported up to date according to our knowledge (3, 6-9). The positions of the variants on > protein are the c.1738C>T in the topological domain located in the cytoplasm, the c.4181C>T in the extracellular topological domain, and the c.473A>G variant in transmembrane domain II, segment 2.

The c.2914-1G>A variant has not been published previously as a pathogenic variant, nor has it been reported as a benign variant to our knowledge. It is a null variant (within ±2 of splice site) affecting gene *SCN1A*, which is a known mechanism of disease. It was not observed in approximately 6,500 individuals of European and African American ancestry in the NHLBI Exome Sequencing Project, indicating it is not a common benign variant in these populations. The c.2947-1 G>A splice site variant destroys the canonical splice acceptor site in intron 15. It is predicted to cause abnormal gene splicing, either leading to an abnormal message that is subject to nonsense-mediated mRNA decay, or an abnormal protein product if the message is used for protein translation.

The c.473A>G was accepted as pathogenic according to computational verdict due to 11 pathogenic predictions from DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MVP, Mutation Assessor, Mutation Taster, Primate AI, REVEL, and SIFT as opposed to no benign predictions. Another amino-acid missense variant at this position, Glu158His (chr2:166912922 C⇒G), is classified as pathogenic by a VarSome user (https://varsome.com/).

The investigation of the origin of pathogenic variants was also performed on parents of patients 2, 3, and 4. The variants, 2914-1G>A in patient 2 and c.4181C>T in patient 3 were found to be de novo; while the c.473A>G heterozygous variant was detected in the mother of case 4. The investigation of paternal origin could not be performed on patient 5.

Clinical characteristics were assessed in five patients who had disease-causing *SCN1A* variants (Table II). All of the patients were female. The mean age of the applicants was 19.25 (5-32) months. The mean age of the seizure's onset was 5.25 (3-8) months.

Patient 1 (5-months-old) with c.1738C>T heterozygous variant, had recurrent FSs with the onset of 3 months. Developmental milestones were normal. No epileptiform EEG was detected upon admission to the hospital after the first seizure. The follow-up EEGs were also non-epileptic. She had no family members with a history of FS or AFS. Phenobarbital treatment was started. During the follow up of the patient through a year, an increase in seizure intervals was observed after the antiepileptic treatment. Subsequently, no AFS was observed. She was able to walk in the 11th month. Developmental milestones were equal with her peers at the age of 12th months. She could combine sentences and speak half understandable in the 24th month.

Patient 2 (13-months-old) with 2914-1G>A heterozygous variant, had recurrent FSs with the onset of 8-month-old. Valproate (VPA) treatment was started due to having FSs with decreased recurrence intervals. Following that, levetiracetam treatment was added as the frequency of seizures didn't decrease. No epileptiform EEG was detected neither upon admission to the hospital nor during the following period. Through the one-year follow-up, the duration of the FSs decreased but the frequency of the seizures (once a month) did not respond to dual antiepileptic treatment. She started to walk

when she was 14-month-old. Although she was good at the gross motor and fine motor skills, she had few words but was not able to combine sentences at the 20th month. However, she could show four body parts and obey simple orders.

In patient 3 (27 months old) with c.4181C>T heterozygous variant, VPA treatment was started at the age of 8 months due to having recurrent seizures within 24 hours, and then levetiracetam was added by the 12th month. She had no epileptiform EEGs. She was able to walk in the 12th month. Developmental milestones were equal comparing her peers at the age of 12th months. When she was 3-years-old she could only name nine familiar things but was not able to carry on a conversation using two to three sentences. Levetiracetam was stopped after having a year of the seizure-free period at the age of 3. Unfortunately, she developed absence seizures during the febrile periods just after discontinuing levetiracetam.

In case 4 (32-months) with c.473A>G heterozygous variant, FSs started at 4th months and VPA treatment was started when she was 1-year-old. Phenobarbital was insufficient to take the seizures under control which was added to the treatment as monotherapy. She had no epileptiform EEGs. Developmental milestones did not show regression during the one-year follow-up. The 33-year-old mother had seizures which were started as febrile in infancy and then have continued as afebrile up to now. The c.473A>G variant was also detected in the mother. She was rarely suffering from relapsing of seizures during the levetiracetam treatment if she forgot to take her pills regularly.

47-months-old patient 5 with the c.473A>G heterozygous variant had recurrent FSs starting at 10th month. She also developed breath-holding spells by the 12th month. She started to walk at the age of 1. There was more than one family member with recurrent FS and AFS on the paternal side. The father did not permit genetic testing. She could balance each foot 4-5 seconds, dress without help, knew 3-4 colors, speak all understandable, and make short term conversations. Regular EEG screenings did not show abnormalities. She has been seizure-free for the last one year with the treatment of VPA.

DISCUSSION

The *SCN1A* gene has 26 coding exons, responsible for the encoding of the alpha subunit of the voltage-dependent neuronal sodium channel (Nav1.1 channel protein). The Nav1.1 channel protein has four homologous domains (DI-DIV), and each domain has six transmembrane segments (10). Since the discovery of the association of *SCN1A* in GEFS+ in 2000 and DS in 2001, 1470 known pathogenic variants have been reported to our knowledge (11).

Among the *SCN1A*-related disorders, DS is the severest phenotype whereas FS or FS+ is the mildest. Most variants are novel and when an infant presents with FS it is challenging to predict which phenotype will develop (3). A total of 55 pediatric patients with early-onset, recurrent FSs were enrolled and investigated of probable DS or GEFS+ phenotype. Five of them (9.09%) had identified to be pathogenic variants (three missense, one splice site) of SCNA1 which were proven to have disease-causing alterations of *SCN1A*-related disorders. Two in four of the variants were previously described in the literature, and two were found as novel alterations.

A seizure which is associated with a fever above 38°C rectally or tympanically, without central nervous infection in a child aged 6 months to 5 years is a common definition of simple FS according to the American Academy of Pediatrics (12). Therewithal, Capovilla et al. (13) suggested that FS could start even at two months of age. In our study, the initial FS of P1 has identified at 3 months that the seizure onset of this patient was smaller than the age range defined by the American Academy of Pediatrics. The c.1738C>T variant has been previously identified in two patients, who progressed to febrile/afebrile generalized tonic-clonic seizures, hemiconvulsion, focal seizure, absence, status epilepticus up to 4-20 years, following the recurrent FSs period with age onset of 5-7 months (6). One had an abnormality on EEG, while the other did not. P1 with c.1738C>T did not demonstrate the aforementioned cumulative features simultaneously. Due to fact that the seizures of P1 were very early-onset, we accepted the patient as a probable DS. However, long-term follow-up is required to establish a suitable genotype-phenotype correlation.

Domain II segments 5 and 6 can be considered as a hotspot where the *SCN1A* variants are most frequently clustered (11). Zuberi et al. (3) showed that amino acid changes in the functionally important domains, S4 and S6 are associated with a severe phenotype more specifically, whereas changes in the transmembrane segments S1-S3 are not. The c.2914-1G>A variant was found in P2 which is probably affecting the transmembrane domain II, segment 6. This splice site variant was not previously reported. However, it was classified as likely pathogenic, and criteria were provided by a single submitter in Clinvar. P2 with this novel variant had early-onset recurrent FSs characterized by resistance to VPA and levetiracetam without epileptiform activities on EEG. Mild speech delay was also noted. Despite being a clinically undefined variant in literature, having 2914-1G>A variant could be a risk factor for progressing to probable DS phenotype.

The c.4181C>T missense variant localized to extracellular topological domain IIIS5-S6 was identified in P3. This variant was previously described in two patients with the phenotype of DS (3, 14). Due to localization of the variant and having the clinical features such as early-onset recurrent febrile seizures, speech delay we classified the phenotype of P3 in DS regardless of epileptiform discharges in EEG.

Although the missense c. 473A>G variant on DIS2 has not been previously reported, some de novo splice site alterations c.473+1G>A, c.473+1G>C, c.473+1G>T, and c.474-1G>A which were close to this region were described in DS patients (9,15,16). A total of 40%–50% of variants associated with the Dravet phenotype are missense (10). However, almost all the variants associated with GEFS+ phenotypes are missense (17,18). P4 in whom c.473A>G variant was also detected in her epileptic mother, had first FS at 4 months. Although the absence of developmental delay and without having abnormalities on periodic EEG controls, P4 was classified in GEFS+ and monitored closely due to having a family history of FS in more than one family member. P5 with presented early-onset FS, had a good response to monotherapy. Breath-holding spell attacks were also defined as concomitant. We excluded the DS and classified P5 in GEFS+ due to having mild clinic presentations and the history of two family members with FS. Previous studies exhibited that the same *SCN1A* variants can cause significant clinical variability including moderately to severely affected patients in the same family (19). For this reason, we suggested that the patients with c.473A>G variant should be follow-up long term.

SCN1A testing is recommended for infants who have recurrent prolonged febrile/afebrile hemi-clonic seizures or generalized status epilepticus even if they are developmentally normal according to the International League Against Epilepsy (20). *SCN1A*-related disorders cause progressive regression in cognitive and motor skills. Villeneuve et al. (21)studied on developmental milestones of children with DS and showed that the mean walking age was 16±2 months while mean time of speaking the first word was 20±4 months. In our study, P2, and P3 started to speak the first word before the 24th month but they all showed remission to make proper sentences after then.

Early diagnosis and appropriate treatment in early-onset recurrent FS suggesting *SCN1A*-related disorders may prevent progress epileptic encephalopathy (22). Sodium channel agents such as carbamazepine, oxcarbazepine, phenytoin, and lamotrigine, are frequently used antiepileptics which can usually aggravate the seizures and develop status epilepticus in patients with *SCN1A* variants (23). The other medications which may lead to increase seizures are vigabatrin and rufinamide (24). Despite a previous study suggested avoidance of maintenance phenobarbital, two of our patients received phenobarbital and they were followed as seizure-free (25). It may not be claimed contrary to the recent studies in the literature due to the small number of our samples. VPA and clobazam are the most frequently used drugs for the treatment of DS, either individually or in combination, even though they have many side effects. Topiramate, stiripentol are used for second-line therapy while controlling seizures in DS (21). Levetiracetam, zonisamide, ethosuximide, and bromides are later agents for add-on-therapy. In our study, all patients maintain activities of daily living while receiving oral medications (Phenobarbital, VPA, benzodiazepine, and levetiracetam).

Sequence analysis of *SCN1A* detects small intragenic deletions/insertions and missense, nonsense, and splice-site variants; typically, exon or whole-gene deletions/duplications are not detected. Intragenic deletions or duplications are consists of 8%-27 among *SCN1A* negative individuals (2). The limitation of our study is not performing multiplex ligation-dependent probe amplification synchronous to *SCN1A* sequencing analysis which is a gene-targeted microarray designed to detect single-exon deletions or duplications.

Consequently, it should be kept in mind that pediatric patients with early-onset FSs may be candidates for DS. Antiepileptic drugs that act on the sodium channel may aggravate the clinical course if the patient has an *SCN1A* variant. It should be also considered more before choosing the sodium channel blockers such as intravenous phenytoin to control prolonged seizures in patients with a history of early-onset recurrent FSs even if *SCN1A* testing was not performed yet. Beforehand usage of *SCN1A* genetic testing in early-onset FSs could be a more significant indicator rather than the clinical risk factors for determining the prognosis and designing the long-term follow-up. It has also considerable importance for genetic counseling.

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