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Determination of *SCN1A* genetic variants in Mexican patients with refractory epilepsy and Dravet syndrome

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ABSTRACT. Mutations in the *SCN1A* gene can result in syndromes associated with epilepsy, including the Dravet syndrome (DS). However, the prevalence of such mutations in these diseases varies widely between different studies, and has not been examined in Mexican patients with epilepsy. Therefore, the objective of this study was to determine the frequency of *SCN1A* mutations (in the exon 26) in a cohort of Mexican patients with DS and refractory epilepsy (RE). We recruited 24 Mexican patients (14 males and 10 females), of which 15 were diagnosed with

RE and 9 were diagnosed with DS. The *SCN1A* gene was sequenced to uncover mutations in exon 26. We detected 2 novel genotypes in 2 DS patients. One was a synonymous variant, c.5418 G > A (E1806E), and the other was a missense variant, c. 5324 T > C (L1775P). The missense mutation was predicted to be damaging with a score of 100% by the PolyPhen-2 program. The frequency of pathogenic variants was 4.17% in all the patients and 11.1% in DS patients, which, together with other publications, emphasize that specific and more severe phenotypes are associated with *SCN1A* mutations.

Key words: *SCN1A* mutations; Dravet syndrome; Refractory epilepsy

INTRODUCTION

The *SCN1A* (MIM#182389) gene encodes for type 1 subunit of the neuronal voltage-gated sodium channel, and is one of the four voltage-gated sodium channel genes that play an important role in controlling neuronal excitability (Plummer and Meisler, 1999). Mutations in this gene have been associated with a variety of neurological diseases including epilepsy, autism, and migraine. Other syndromes associated with *SCN1A* mutations include the Dravet syndrome (DS), also named severe myoclonic epilepsy of infancy (SMEI), as well as epileptic encephalopathy, partial epilepsy, generalized epilepsy, and febrile seizures (Meng et al., 2015). A total of 1727 mutations and genomic rearrangements have been reported in the *SCN1A* mutation database, with higher predominance in DS patients (58.6%). In addition, most of the reported *SCN1A* mutations (20.76%) occur in exon 26, which encodes for the pore region. This region contains the voltage sensor, whose mutations have been associated with severe disease phenotypes (Meng et al., 2015). Based on this information, our objective was to carry out a mutational screening on exon 26 in a group of Mexican patients with refractory epilepsy (RE) and/or DS in order to determine the frequency of *SCN1A* mutations in these diseases.

MATERIAL AND METHODS

We included patients with RE who were diagnosed with the following criteria: failure to control seizures despite been treated with at least two well-tolerated and properly chosen anticonvulsive drugs. Additionally, we recruited DS patients (Dravet, 2011) who attended the pediatric neurology service in a third-level public hospital between April 2012 and December 2014.

DNA was extracted from peripheral blood by the Miller technique (Miller et al., 1988). Exon 26 of the *SCN1A* gene was amplified in 3 fragments (approximately 500 bp) by conventional PCR, and was posteriorly sequenced by the Sanger technique in order to detect possible variants. The primers used to amplify exon 26 were as follows: fragment a, forward primer: 5'-AGGACTCTGAACCTTACC-3', reverse primer: 5'-ATGTTCACCACAACCAGG-3'; fragment b, forward primer: 5'-TTGTCAGTTACATCATCATA-3', reverse primer: 5'-ATAGGAGACCTTGGAAGG-3'; fragment c, forward primer: 5'-TGCTTTTACAAAGCGGGTTC-3', reverse primer 5'-GTTTGCTGACAAGGGGTCAC-3'.

Ethics

The institutional Ethical Committee approved the study protocol, and parents of the patients signed an informed consent form prior to subject enrollment.

RESULTS

Patient description

A total of 24 patients (14 males and 10 females) were included in the study; all except 2 were unrelated (these related patients presented RE). The average age was 6.6 years (min = 18 months, max = 15 years and 11 months). With respect to the diagnosis, 9 patients (37.5%) were diagnosed with DS (5 males and 4 females), and 15 (62.5%) were diagnosed with RE (9 males and 6 females). Of the patients with RE, 6 (40%) presented epilepsy of the temporal lobe, 3 (20%) presented the Lennox-Gastaut syndrome, 3 (20%) presented the Landau Kleffner syndrome, 2 (13%) presented the Doose syndrome, and 1 (7%) presented the West syndrome. Of the 24 patients, 11 (46%) had a history of febrile crisis, and of those, 8 were affected with DS. The average number of antiepileptic drugs used was 3 (min = 2, max = 5).

Molecular results

We found 2 unreported genetic variants in different DS patients. The first was discovered in a female DS patient (3 years and 3 months of age), who was presented with the change c.5418 G > A; this was a synonymous variant that does not produce a change in the amino acid sequence (E1806E). The other variant was also found in a female DS patient (3 years 8 months of age), who showed the change c.5324 T > C. This mutation led to an amino acid substitution (leucine substituted by proline; L1775P) (Figure 1). The same mutation was not detected in the mother of the patient, and could not be screened in the father, as he was unreachable. DNA sequencing with the forward and reverse primers confirmed both variants. Pathogenic prediction of this last change was carried out using the PolyPhen-2 program (Adzhubei et al., 2010). Results showed a 100% probability of the mutation being "probably damaging" (Figure 2). This indicated that when analyzing amino acid conservation and change in mutation, this had the highest likelihood calculated by the program of being pathogenic.

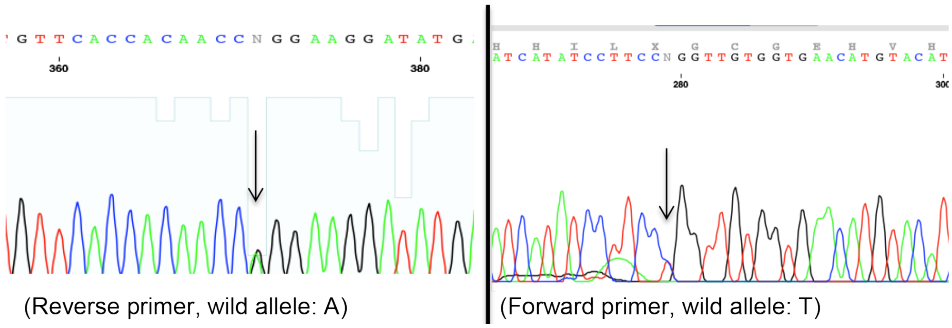

Figure 1. Electropherogram showing the mutation c.5324 T>C (L1775P). Reverse primer, wild allele: A; Forward primer, wild allele: T.

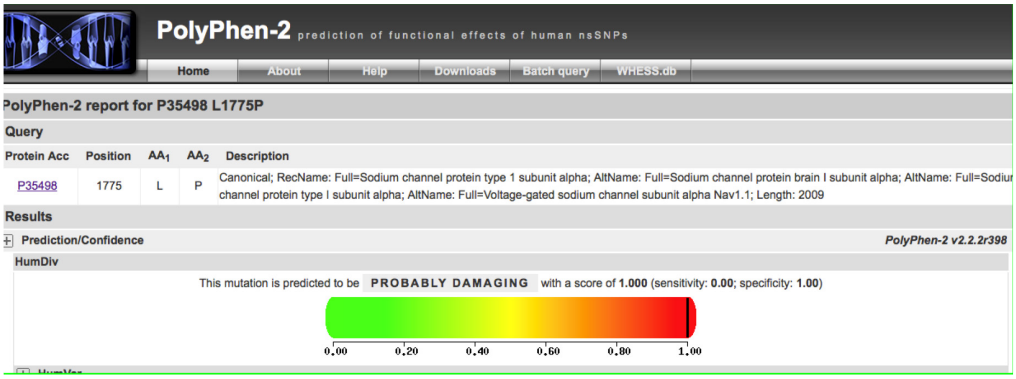

Figure 2. PolyPhen-2 analysis showing the probability of the mutation c.5324 T>C being damaging.

DISCUSSION

To the best of our knowledge, this is the first study that aimed to detect *SCN1A* mutations in the Mexican population. Of the 24 patients with RE and/or DS, 2 novel genetic variants in 2 patients with DS were found; 1 was synonymous while the other was highly likely to be pathogenic. Considering only the pathogenic variant, the percentage of mutations detected was 4.17% in all included patients and 11.1% in DS patients alone. Studies aimed to detect *SCN1A* mutations in Asians with unexplained epilepsy and encephalopathy detected them in 3-5% of the patients (Saitoh et al., 2015; Zhang et al., 2015). However, this rate was significantly lower as compared to that reported by Fujiwara et al. (2003), these mutations were present in 86.5% of the patients with intractable generalized tonic-clonic seizures and DS. This difference could be explained by differences in included cases, as specific phenotypes (such as DS and intractable generalized tonic-clonic seizures) are more likely to present *SCN1A* mutations. As we also found *SCN1A* mutations in DS patients, it is possible that this disease is more likely to be associated with *SCN1A* mutations. It has been reported that mutations in the pore region usually induce loss of function, and can be more frequently observed in patients with severe phenotypes (partial epilepsy, partial epilepsy with febrile seizures plus, epileptic encephalopathy and SMEI). Likewise, missense mutations tend to be associated with milder phenotypes, with the exception of those located at the pore region that generally produce loss of function or partial loss of function. In this case, although we detected a missense mutation, it was found in the pore region. This could lead to loss of function of protein, which may explain the severe phenotype (DS/SMEI) presented by the patient; however, only further experimental studies could determine its exact functional implications.

The overall penetrance of *SCN1A* mutations has been reported to be 91.9% in familial cases. Cases with incomplete penetrance have been associated with missense and splice site mutations, but not with genomic rearrangements and truncating mutations (Meng et al., 2015). In addition, familial cases represent only 3.7% of SME cases, explained by the decreased fertility in severe cases (Meng et al., 2015). In this case, although we could only screen the mother of the patient without detecting the mutation, it is likely that the mutation was *de novo*, considering its predicted pathogenicity and location (in the pore region).

In conclusion, we report 2 novel genetic variants of the *SCN1A* gene in patients with

DS, one was synonymous and the other pathogenic. Considering only the pathogenic variant, we detected a mutation frequency of 4.17%. However, this frequency may have been higher if we had analyzed the entire gene.

Conflicts of interest

The authors declare no conflict of interest.

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