**Functional studies of *scn1a* mutations**

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**Introduction**

Mutations in *SCN1A*, a gene that encodes the α-subunit of the sodium channel voltage-dependent (Nav1.1), impair the flow control of sodium ions, resulting in abnormal sodium influx into neurons that causes a disruption in the channel activity, neuronal hyper excitability and epilepsy (1). Previous studies carried out by our group found that 81% of patients with Dravet syndrome (DS) have de novo mutations in *SCN1A* (2). Interpretation of the mutation impact in protein function is usually performed using pathogenicity prediction software’s. Therefore, functional studies are important both to confirm results from *in silico* analysis and to better characterize the deleterious effects of mutations.

**Objective**

To evaluate the molecular pathogenesis of SCN1A gene mutation in a Tsa 201 cell line using electrophysiology techniques.

**Method**

Mutation c.5329delG was selected following two parameters: 1) mutations that are not described in literature and 2) scores obtained by bioinformatics tools as SIFT and Polypen2 showing that variations are damaging to protein function. The wild forms of *SCN1A* and the genes encoding auxiliary β-subunits *SCN1B*, *SCN2B* (wild-type genes) were cloned by recombinant DNA technology. Recombinant plasmids were isolated, purified and submitted to sequencing on a Miseq (Illumina) in order to assure that sequences were properly cloned. Primers for Site-Directed Mutagenesis were designed using the PrimerX software. Site-Directed Mutagenesis was performed using GeneArt PLUS Kit ®Site-Directed (Thermo Fisher Scientific) to obtain the mutant plasmid (pSCN1A-mut). The pSCN1A-mut was submitted to sequencing on a Miseq to confirm if mutation c.5329delG was properly inserted. Thereafter, the wild type *SCN1A*, *SCN1B* and *SCN2B* recombinant plasmids were co-transfected into the TSA201 cell line. In addition, pSCN1A-mut was co-transfected with wild type *SCN1B* and *SCN2B* plasmids into the TSA201 cell line.

**Relevance**

Nav1.1 functional studies (as well as of other ion channels) are important both to confirm results that were previously predicted exclusively by in silico analysis and to characterize mutations which could not be classified by bioinformatics tools. We expect that our results will not only help to improve molecular diagnosis of patients with DS an, but will also help us to better understand the repercution of genetic variability (both normal and pathological) in the functionl aspects of Nav1.1 sodium channels and other epilepsy-related ion channels.

**References:** 1. Meng H, et al. Hum Mutat 6(6):573-80, 2015.

2. MCa G, et al. J Epilepsy 18(2):60-2, 2012.