ELSEVIER

Brain & Development xxx (2017) xxx–xxx

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Differential effects on sodium current impairments by distinct *SCN1A* mutations in GABAergic neurons derived from Dravet syndrome patients

Hyun Woo Kim [a], Zhejiu Quan [a], Young-Beom Kim [b], Eunji Cheong [c], Heung Dong Kim [a], Minjung Cho [a], Jiho Jang [d], Young Rang You [e], Joon Soo Lee [a], Ji Hun Kim [a], Yang In Kim [b], Dae-Sung Kim [e,f,**], Hoon-Chul Kang [a,*]

[a] *Division of Pediatric Neurology, Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, Yonsei-ro 50-1, Seodaemun-gu, Seoul 03722, Republic of Korea*
[b] *Department of Physiology, Korea University College of Medicine, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea*
[c] *Department of Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea*
[d] *Department of Physiology, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea*
[e] *Department of Biotechnology, BK21 PLUS Project, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea*
[f] *Department of Pediatrics, Korea University College of Medicine, Guro Hospital, 97 Gurodong-gil, Guro-gu, Seoul 08308, Republic of Korea*

Received 6 November 2017; received in revised form 4 December 2017; accepted 4 December 2017

Abstract

Background: We investigated how two distinct mutations in *SCN1A* differentially affect electrophysiological properties of the patient-derived GABAergic neurons and clinical severities in two Dravet syndrome (DS) patients.

Materials and Methods: We established induced pluripotent stem cells from two DS patients with different mutations in *SCN1A* and subsequently differentiated them into forebrain GABAergic neurons. Functionality of differentiated GABAergic neurons was examined by electrophysiological recordings.

Results: DS-1 patient had a missense mutation, c.4261G > T [GenBank: NM_006920.4] and DS-2 patient had a nonsense frameshift mutation, c.3576_3580 del TCAAA [GenBank: NM_006920.4]. Clinically, contrary to our expectations, DS-1 patient had more severe symptoms including frequency of seizure episodes and the extent of intellectual ability penetration than DS-2 patient. Electrophysiologic recordings showed significantly lower sodium current density and reduced action potential frequency at strong current injection (>60 pA) in GABAergic neurons derived from both. Intriguingly, unique genetic alterations of *SCN1A* differentially impacted electrophysiological impairment of the neurons, and the impairment's extent corresponded with the symptomatic severity of the donor from which the iPSCs were derived.

Conclusion: Our results suggest the possibility that patient-derived iPSCs may provide a reliable *in vitro* system that reflects clinical severities in individuals with DS.

Keywords: Dravet syndrome; Voltage-gated sodium channel; Induced pluripotent stem cell

* Corresponding author at: Division of Pediatric Neurology, Severance Children's Hospital, Department of Pediatrics, Yonsei University College of Medicine, Epilepsy Research Institute, Yonsei Stem Cell Institute, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea.
** Co-Corresponding author at: Department of Biotechnology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea.
E-mail addresses: sonnet10@korea.ac.kr (D.-S. Kim), hipo0207@yuhs.ac (H.-C. Kang).

https://doi.org/10.1016/j.braindev.2017.12.002
0387-7604/

1. Introduction

Dravet Syndrome (DS), a condition known as severe myoclonic epilepsy of infancy (SMEI), is a devastating early-onset neurological disorder [1]. In more than 70% of DS patients, *de novo* heterozygous mutations in the sodium voltage-gated channel alpha subunit 1 (*SCN1A*) gene result in a malfunctioned neuronal voltage-gated sodium channel. Mutations of *SCN1A* cause a wide spectrum of phenotypic severity, ranging from relatively mild genetic epilepsy with febrile seizure plus to DS and no clear correlation between mutations and symptomatic severity has been found [1]. Effects of *SCN1A* mutations on epilepsy have been revealed using *SCN1A*-specific knock-out or knock-in mice, in which the specific malfunction of inhibitory neurons appeared related to brain hyperexcitability [2–6]. Despite accumulating evidence supporting so-called *SCN1A* "loss-of-function" effects in mouse models, different model systems have provided conflicting information on the etiology of DS. For example, functional analysis of mutant channel heterologous expression in *Xenopus* oocytes and mammalian cells revealed increased persistent sodium current due to impaired inactivation of $Na_V1.1$, supporting "the gain-of-function" model [7,8].

To date, several studies have attempted to challenge DS pathophysiology by deriving neurons from iPSCs with a nonsense [9] or missense [10] mutation in *SCN1A* and have presented evidence of epileptic-like hyperexcitability in the patient-specific neurons. In contrast, other studies reported that mutations in *SCN1A* caused functional decline in GABAergic neurons [4,11–13]. Such inconsistent results may result from the different mutations in *SCN1A* and neuronal subtypes examined in each study.

To challenge these inconsistencies, we generated iPSC lines from two DS patients carrying a missense or nonsense mutation in *SCN1A* and subsequently differentiated them into forebrain GABAergic neurons, a primarily afflicted cell type in DS. We then investigated how two distinct mutations in *SCN1A* differentially affect electrophysiological properties of the patient-derived GABAergic neurons and clinical severities in these two DS patients.

2. Materials and methods

2.1. Patient selection and SCN1A gene analysis and interpretation of related variants

Two patients, DS-1 and DS-2, were clinically diagnosed as typical DS in a tertiary epilepsy center according to the following criteria; 1) prolonged febrile and non-febrile seizures within the first year of a child's life, 2) many different seizure types including myoclonic seizures, 3) frequent seizures when children are ill or have a fever, 4) normal development in the early years but then progressive developmental delay.

Direct sequencing of all coding exons and flanking intronic sequences of the *SCN1A* gene was performed using primer pairs designed by the authors. Sequence variations were analyzed via comparison with the wild-type sequence. In addition, massive targeted sequencing with 172 epileptic encephalopathy-related gene candidates (Table 1) has been executed to evaluate modifier genes along with mutations in *SCN1A*. A library preparation from genomic DNA of a whole blood sample and massive parallel sequencing on the MiSeq System (Illumina) were done. Sequence analysis and quality control was done using the BaseSpace (Illumina) and NextGENE (SoftGenetics) software. Copy number variation was examined using the institutional analysis pipeline. The interpretation of variants followed the 5-tier classification system recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) using a step-by-step approach. Variants of unknown significances (VOUSs), especially missense variants, were prioritized according to population frequency, ACMG score, and the patient's clinical phenotype. A parental study was scheduled to detect *de novo* occurrence.

2.2. Generation of iPSC lines from skin fibroblasts of two donors with different SCN1A mutations

All experiments were conducted under supervision of the Human Research Protection Center, Yonsei University College of Medicine and followed guidelines of the Institutional Review Board (Approval No. 4-2013-0570). Human fibroblast cell lines were isolated from the patients' skin biopsies carrying a *SCN1A* missense mutation of c.4261G > T [GenBank: NM_006920.4] (designated DS-1) or nonsense mutation of c.3576_3580 del TCAAA [GenBank: NM_006920.4] (designated DS-2). The two DS patients' fibroblasts, along with BJ fibroblasts as unaffected controls (CRL-2522; ATCC, Manassas, VA), were reprogrammed to iPSCs using the Sendai virus system (CytoTune®-iPS 2.0 Sendai Reprogramming Kit, Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Human embryonic stem cell (hESC)-like colonies emerged 2–3 weeks after viral infection were manually picked, and expanded with mitotically-arrested mouse embryonic fibroblast (STO) feeder cells (CRL-1503, ATCC) as described previously [14]. Established iPSC lines were characterized by alkaline phosphatase staining (Merck, Darmstadt, Germany; Cat. No.: SCR004), immunocytochemistry, and teratoma formation assays. Karyotyping using a standard G-banding technique for genomic stability during expansion and sequencing

Please cite this article in press as: Kim HW et al. Differential effects on sodium current impairments by distinct *SCN1A* mutations in GABAergic neurons derived from Dravet syndrome patients. Brain Dev (2017), https://doi.org/10.1016/j.braindev.2017.12.002