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Ion channels: genetics and as a targets for antiepileptic drugs

Kazuhiro Yamakawa

Laboratory for Neurogenetics, RIKEN Brain Science Institute, Hirosawa, Saitama, Japan

Abstract

Dravet syndrome is caused by mutations of the *SCN1A* gene that encodes voltage-gated sodium channel alpha-1 subunit. *SCN1A*-knock-in mouse with a disease-relevant nonsense mutation that we generated reproduced the disease phenotypes. Both homozygous and heterozygous knock-in mice developed epileptic seizures within the first postnatal month. Our immunohistochemical studies showed that in wild-type mice Nav1.1 is dominantly expressed in parvalbumin-positive inhibitory interneurons (PV cells), intensely in its axons and moderately in somata, and mostly not observed in pyramidal cells nor other types of interneurons including somatostatin-positive and calretinin-positive cells. These results suggest that Nav1.1 is largely expressed in PV interneurons and plays critical roles in their spike output, and that impaired function of PV cells would be the cellular basis of Dravet syndrome. PV interneurons and Nav1.1 in those cells would be critical targets for antiepileptic drugs.

SCN1A GENE MUTATIONS IN PATIENTS WITH DRAVET SYNDROME AND RELATED EPILEPSIES

Mutations in *SCN1A* have been reported to be associated with several types of epilepsies including generalized epilepsy with febrile seizures plus (GEFS+)[1,2] and Dravet syndrome.[3-5] Approximately 10%-20% of GEFS+ patients show *SCN1A* mutations, while approximately 80% of Dravet patients show the mutations. Most exclusively GEFS+ mutations are missense, while two-third of Dravet mutations are truncation mutations such as nonsense and frameshift and remained one-third are missense. In most cases, GEFS+ mutations are inherited, while Dravet mutations are *de novo*. Further we reported microdeletion mutations affecting *SCN1A* 5' promoter region but not affecting the coding region.[6] Missense mutations in *SCN1A* were also found in 70% of patients having intractable childhood epilepsy with generalized tonic-clonic (ICEGTC), an atypical Dravet syndrome that does not show myoclonic seizures.[5] We also reported that *SCN1A* mutations associates with psychiatric phenotypes in addition to epilepsy.[7]

Dravet mutations are mostly sporadic. However, the fact that several *SCN1A* mutations were found in familial Dravet syndrome cases in that identical missense mutations were observed in family members with Dravet syndrome or ICEGTC as well as in those having idiopathic epilepsy, febrile seizures only, or even non-symptomatic[5], suggests that genetic backgrounds or environmental modifiers, or more possibly mosaicisms in parents as shown in our previous study[8] largely affects the disease phenotype. These observations further indicates that even in families with sporadic Dravet syndrome cases the Dravet syndrome risk for successive children are higher than the general population, and emphasize the importance of prenatal or preimplantation diagnoses.

IN VITRO FUNCTIONAL STUDIES ON NAV1.1 MUTANTS

A number of biophysical studies on *SCN1A* mutations have been reported[9-11], however some of those suggested increased activities of mutant channels likely to be enhancing neuronal excitability and others suggested decreased, and interpretation of obtained results still remain elusive. I previously proposed that the ultimate functional consequences of these mutations in the brain may be an overall reduction in channel activity, in which milder GEFS+ phenotypes may be explained by the intermediate reduction, complete loss of the channel activity for one allele, that means half amount of normal channel protein or haproinsufficiency, in Dravet syndrome[12], however in vivo studies were still required to prove it.

Address correspondence to: Dr Kazuhiro Yamakawa, Laboratory for Neurogenetics, RIKEN Brain Science Institute, Hirosawa 2-1, Wako-shi, Saitama 351-0198, JAPAN. E-mail: yamakawa@brain.riken.jp

MOUSE WITH SCN1A-DEFICIENCY SHOWED SEVERE EPILEPTIC SEIZURES

We then generated knock-in (KI) mice with an *Scn1a* nonsense mutation that appeared in three independent Dravet syndrome patients.[13] Both homozygous and heterozygous knock-in mice developed epileptic seizures within the first postnatal month. All homozygotes died before postnatal day 20 and averagely at 16 postnatal days. In contrast, ~25% and ~40% of heterozygotes died at one month and three months after birth respectively, and remained mice survived thereafter. In heterozygous knock-in mice, trains of evoked action potentials in these fast-spiking, inhibitory cells exhibited pronounced spike amplitude decrement late in the burst. A similar study of an Scn1a KO mouse has been reported by another group one year ahead[14], in which they also showed specific physiological dysfunction in GABAergic neurons.

NAV1.1 IS DOMINANTLY EXPRESSED IN AXONS AND SOMATA OF PARVALBUMINE-POSITIVE INHIBITORY NEURONS

Yu *et al*.[14] also reported that the Nav1.1 expression was restricted to somata of both pyramidal and inhibitory neurons as described in previous other studies.[15] In contrast, in our study[13] we showed, by using three independent antibodies and by using the KI mice as negative controls, that the Nav1.1 protein is expressed dominantly in axons and moderately in somata of parvalbumin-positive inhibitory interneurons (PV cells). In the neocortex, periphery of somata of pyramidal cells also gave signals. Most of these signals would be derived from the Nav1.1 located at the axon termini of the basket cells which are clinging onto the somata of pyramidal cells, and would not be derived from the pyramidal cell itself. Our data suggest that Nav1.1 is dominantly expressed in PV-positive inhibitory interneurons and plays critical roles in the spike output from these cells, and further suggest that the impaired function of PV-interneurons caused by SCN1A mutation is the molecular and cellular basis of Dravet syndrome. Based on our results, we propose that PV interneurons and Nav1.1 in the cells are critical targets for antiepileptic drugs of Dravet syndrome and its related epilepsies.

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