Loss-of-function variants of *SCN8A* in intellectual disability without seizures

OPEN

Jacy L. Wagnon, PhD*
Bryan S. Barker, BA*
Matteo Ottolini, MS
Young Park, PhD
Alicia Volkheimer, MS
Purnima Valdez, MD
Marielle E.M. Swinkels,
MD
Manoj K. Patel, PhD†
Miriam H. Meisler, PhD†

Correspondence to
Dr. Meisler:
meislerm@umich.edu

ABSTRACT

Objective: To determine the functional effect of *SCN8A* missense mutations in 2 children with intellectual disability and developmental delay but no seizures.

Methods: Genomic DNA was analyzed by next-generation sequencing. *SCN8A* variants were introduced into the Na_v1.6 complementary DNA by site-directed mutagenesis. Channel activity was measured electrophysiologically in transfected ND7/23 cells. The stability of the mutant channels was assessed by Western blot.

Results: Both children were heterozygous for novel missense variants that altered conserved residues in transmembrane segments of Na_v1.6, p.Gly964Arg in D2S6 and p.Glu1218Lys in D3S1. Both altered amino acids are evolutionarily conserved in vertebrate and invertebrate channels and are predicted to be deleterious. Neither was observed in the general population. Both variants completely prevented the generation of sodium currents in transfected cells. The abundance of Na_v1.6 protein was reduced by the Glu1218Lys substitution.

Conclusions: Haploinsufficiency of *SCN8A* is associated with cognitive impairment. These observations extend the phenotypic spectrum of *SCN8A* mutations beyond their established role in epileptic encephalopathy (OMIM#614558) and other seizure disorders. *SCN8A* should be considered as a candidate gene for intellectual disability, regardless of seizure status. *Neurol Genet* 2017;3:e170; doi: 10.1212/NXG.0000000000000170

GLOSSARY

cDNA = complementary DNA; HEK = human embryonic kidney.

Whole-exome sequencing has revealed a major role for de novo mutations in the etiology of sporadic intellectual disability.¹ Between one-third and one-half of sporadic cases may be accounted for by de novo mutations in genes required for neuronal development and synaptic transmission. The neuronal sodium channel *SCN8A* (Na_v1.6) is concentrated at the axon initial segment and at nodes of Ranvier of myelinated axons.² Exome analysis for *SCN8A* mutations has thus far focused on children with seizure disorders.³ More than 150 missense mutations of *SCN8A* have been identified, and gain-of-function hyperactivity is the most common pathogenic mechanism for seizures.

By contrast, we previously described a loss-of-function, protein truncation allele of *SCN8A* that cosegregated with cognitive impairment in a family without seizures.⁴ To follow up on that observation, we have now examined the functional effects of 2 *SCN8A* missense mutations identified by exome sequencing in children with intellectual disability who also did not have

*These authors contributed equally to this work as cofirst authors.

†These authors contributed equally to this work as colast authors.

From the Department of Human Genetics (J.L.W., Y.P., M.H.M.), University of Michigan, Ann Arbor; Department of Anesthesiology (B.S.B., M.O., M.K.P.) and Neuroscience Graduate Program (B.S.B., M.K.P.), University of Virginia, Charlottesville; Department of Medicine (A.V.), Veterans Affairs Medical Center (A.V.), and Department of Pediatrics (P.V.), Duke University, Durham, NC; and Department of Medical Genetics (M.E.M.S.), University Medical Centre Utrecht, the Netherlands.

Funding information and disclosures are provided at the end of the article. Go to Neurology.org/ng for full disclosure forms. The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Figure 1 Location and evolutionary conservation of *SCN8A* mutations in individuals with intellectual disability

(A) Four-domain structure of the voltage-gated sodium channel α subunit. p.Gly964Arg (G964R) is located in transmembrane segment 6 of domain II. p.Glu1218Lys (E1218K) is located in transmembrane segment 1 of domain III. (B) Evolutionary conservation of residue G964 in multiple species. (C) Conservation of residue E1218 in multiple species. a = anole; c = chicken; dpara = drosophila "paralytic"; f = fugu; h = human; jscn = jellyfish sodium channel; m = mouse; z = zebrafish. Amino acids are indicated by the single-letter code; dots represent identity to the human amino acid.

seizures. Both mutations caused complete loss of channel activity, confirming the role of loss-of-function mutations of *SCN8A* as a cause of isolated cognitive impairment.

METHODS Molecular diagnostics. Exome sequencing for patient 1 was performed by GeneDX (Gaithersburg, MD). In addition to the *SCN8A* variant, a frameshift mutation in *GJB2* (c.167delT, p.L56RfsX26) was inherited from an unaffected parent. Exome sequencing for patient 2 was performed at the laboratory for DNA Diagnostics in the University Medical Center Utrecht. In addition to the *SCN8A* variant, the *PDH1* variant (c.520G>A, p.Ala174Thr) was present in the child and an unaffected grandfather. Analysis of copy number variation and Fragile X expansion for patient 2 were negative. Procedures were approved by the institutional ethics standard committees.

Standard protocol approvals, registrations, and patient consents. Written consent for research was obtained from the guardians of both patients whose variants were studied.

Site-directed mutagenesis of $\text{Na}_v1.6$ complementary DNA. Mutations were introduced into the tetrodotoxin-resistant mouse complementary DNA (cDNA) $\text{Na}_v1.6\text{R}$ by site-directed

mutagenesis with QuikChange II XL (Agilent Technologies, Santa Clara, CA) as described.⁵ Two independent mutagenesis experiments generated cDNA clones A and B for each mutation. The 6-kb open reading frame was resequenced, and clones lacking other mutations were analyzed.

Electrophysiology. Neuron-derived ND7/23 cells (Sigma Aldrich, St. Louis, MO) were cultured and transfected as described.⁵ Electrophysiologic recordings of fluorescent cells were performed 48 hours after transfection in the presence of 500 nM tetrodotoxin to block endogenous sodium currents. Currents were recorded using the whole-cell configuration of the patch-clamp recording technique.⁵

Western blot. Human embryonic kidney (HEK) 293 cells were cultured at 37°C, transfected with $\text{Na}_v1.6$ cDNA, and lysates were prepared and analyzed 24 hours after transfection as described⁵ using affinity-purified polyclonal rabbit anti-*Scn8a* antibody (Millipore # AB5580, lot 2784259, 1:500 dilution).

RESULTS Identification of novel missense variants of *SCN8A*. Patient 1 is a 7-year-old girl who experienced global developmental delay and hypotonia in early childhood. She walked and spoke her first words at 18 months.

She is receiving special education services at school and is repeating the first grade due to below average academic attainment. Psychoeducational testing revealed receptive-expressive language disorder and borderline intellectual functioning with a diagnosis of social communication disorder. She did not meet criteria for autism spectrum disorder.

Attention-deficit hyperactivity disorder was diagnosed at 6 years and has responded to methylphenidate. Exome sequencing revealed the *SCN8A* variant c.2890G>C (p.Gly964Arg; G964R) which arose de novo and was not present in either parent. Gly 964 is located in transmembrane segment 6 of domain II (D2S6) and is highly conserved through invertebrate and vertebrate evolution (figure 1, A and C).

Patient 2 is a 10-year-old boy who was born after a pregnancy complicated by polyhydramnios. Development was delayed from birth. Early ataxic gait resolved with age. Behavioral problems included temper tantrums. Metabolism and brain MRI were normal. There were no dysmorphic features. Exome sequencing and analysis of 770 genes identified the *SCN8A* variant c. 3652G>A (p.Glu1218Lys; E1218K) located at the distal terminus of transmembrane segment 1 in domain III (D3S1). This residue is highly conserved through evolution (figure 1, A and B). The variant was not present in the maternal genome; the father was not available for testing.

Additional clinical features are detailed in the table. Both mutations were predicted to be deleterious by in silico prediction programs. Neither mutation was previously observed in patients or in the Exome Aggregation Consortium Database.

Table Clinical features of patients with intellectual disability and *SCN8A* mutations

	Patient 1	Patient 2
Sex	Female	Male
Age	7 y	10 y
Nucleotide change	c.2890G>C	c. 3652G>A
Inheritance	De novo	Unknown (father unavailable)
Protein change	G964R	E1218K
Channel domain	D2S6	D3S1
In silico predictions	Deleterious	Deleterious
CADD	25	32
PROVEAN	−7.4	−3.8
Polyphen 2	0.9	1.0
Channel activity observed	Inactive	Inactive
Protein	Stable	Unstable
Diagnosis	Language disorder borderline intellectual function	Intellectual disability
IQ	73	56
Social interaction	Social communication disorder; minimal interaction with peers; good eye contact	Temper tantrums
Development	Global delay walk, talk at 18 mo	Global motor delay, severe speech delay
EEG	Normal	Not done
Seizures	None	None
MRI	Not done	Normal
Metabolism	Not done	Normal
Motor development	Hypotonia; motor delay improved with time	Unstable gait, resolved
Other phenotypes	ADHD at 6-y special education, chronic headache, finger chewing	No other phenotypes
Treatment	ADHD responsive to methylphenidate	No medication

Abbreviation: ADHD = attention-deficit hyperactivity disorder.

Electrophysiologic characterization of *SCN8A* mutations G964R and E1218K. ND7/23 cells were transfected with wild-type or mutant cDNAs, and sodium currents were recorded (figure 2, A and B). The macroscopic Na current in nontransfected cells was -10.0 ± 1.8 pA/pF ($n = 8$). Cells transfected with wild-type Na_v1.6 exhibited a robust macroscopic Na current of -102 ± 12 pA/pF (mean $\pm$ SEM) ($n = 30$). The current in cells transfected with mutant cDNA did not differ from nontransfected cells: G964R, -5.2 ± 0.5 pA/pF ($n = 30$) and E1218K, -9.8 ± 1.5 pA/pF ($n = 27$). To confirm the loss of activity, site-directed mutagenesis was repeated and a second independent clone with each mutation was analyzed, with the same result. Both missense variants thus result in a complete loss of channel activity.

Expression of mutant protein. To evaluate the mechanism for loss of channel activity, we examined protein abundance in transfected HEK cells. Wild-type Na_v1.6 and G946R cDNAs generated a protein

with the predicted molecular weight of 250 kDa, which was not present in nontransfected cells (figure 2C). However, repeated transfections of the E1218K mutant cDNA detected only a low level of protein (figure 2, C and D), indicating that this mutation reduces protein stability.

DISCUSSION The patients described exhibited developmental delay and cognitive impairment but no history of seizures. Each carried a pathogenic missense variant of *SCN8A* that altered a highly conserved amino acid residue in a transmembrane segment of the channel, resulting in loss of function. *SCN8A* is one of the most conserved genes in the mammalian genome, with an unusually low rate of coding variation.⁶ The low frequency of frameshift and nonsense mutations among 60,000 individual exomes was used to calculate the probability of 1.0 that *SCN8A* is intolerant to haploinsufficiency.⁷ The population data support our conclusion that haploinsufficiency of *SCN8A* is responsible for cognitive impairment in these patients.

Figure 2 Mutations E1218K and G964R result in loss of Na_v1.6 function

(A) Averaged current-voltage (I-V) relation for cells expressing WT (black) ($n = 30$), E1218K (red) ($n = 27$), and G964R (blue) ($n = 30$) Na_v1.6 channels and nontransfected controls (gray) ($n = 8$). Data represent mean $\pm$ SEM. (B) Representative traces of families of Na currents from ND7/23 cells transfected with the indicated Na_v1.6 complementary DNA (cDNA). (C and D) Western blot of transfected human embryonic kidney 293 cells (30 μ g protein) immunostained with rabbit polyclonal anti-Na_v1.6 antibody. NT = nontransfected; WT = wild-type cDNA. Lanes A and B represent independently generated mutant cDNA clones for each mutation.

Impaired cognition in the patients is also consistent with the evidence that heterozygous loss-of-function mutations in mouse Na_v1.6 also result in cognitive and behavioral deficits without spontaneous seizures. Impaired learning in water maze and eye-blink conditioning tests^{8,9} and elevated anxiety in the open-field test¹⁰ have been described.

At the cellular level, complete inactivation of mouse *Scn8a* reduces repetitive firing, resurgent current and persistent current in cerebellar Purkinje cells, prefrontal cortical pyramidal cells, and hippocampal CA1 cells.² These changes decrease the frequency of action potentials. Reduced neuronal activity is a likely consequence of the loss-of-function mutations of *SCN2A* and receptors involved in excitatory neurotransmission that have also been associated with

intellectual disability. Chronic reduction of neuronal activity may alter the dynamics of synaptic plasticity during maturation and lead to aberrant cerebral circuitry and intellectual disability.

In contrast to the loss-of-function variants described here, we previously identified 8 gain-of-function variants resulting in channel hyperactivity resulting in epileptic encephalopathy (reviewed in reference 3). We also found loss-of-function missense variants in 2 patients with seizures,^{11,12} indicating that genetic background influences clinical outcome. Both gain-of-function and loss-of-function variants of the related sodium channel *SCN2A* have also been associated with seizures.¹³ A recent analysis of de novo mutations in more than 7,000 individuals with developmental disorders identified 5 missense variants of *SCN8A* in patients with seizures and 2 missense variants of *SCN8A* in patients with cognitive impairment but no seizures.¹⁴ We would suggest that the former variants are likely to cause channel hyperactivity and the latter to cause loss of function. Protein truncation variants of *SCN8A* are underrepresented in all populations studied to date, including controls, patients with intellectual disability, and patients with seizures. It seems likely that these protein truncations are associated with distinct disorders not yet subjected to large-scale exome sequencing, such as neuromuscular and movement disorders.

The de novo mutation of *SCN8A* in patient 1 is consistent with the growing recognition of the role of de novo mutations in sporadic intellectual disability. In 3 earlier studies examining 142 individuals with intellectual disability, 1 de novo missense mutation of *SCN8A* was detected.^{14–17} Better estimates of the quantitative contribution of *SCN8A* mutations to sporadic and inherited forms of isolated cognitive impairment will emerge from additional large-scale screening of patient populations.

AUTHOR CONTRIBUTIONS

Jacy L. Wagnon provided molecular data and contributed to writing the manuscript. Bryan S. Barker provided electrophysiologic data and manuscript editing. Matteo Ottolini provided electrophysiologic data. Young Park provided molecular data. Alicia Volkheimer provided clinical information for patient 1. Purnima Valdez provided clinical information for patient 1. Marielle E.M. Swinkels provided clinical information for patient 2. Manoj K. Patel provided experimental data and edited the manuscript. Miriam H. Meisler initiated the study and drafted the manuscript.

STUDY FUNDING

Supported by NIH (R01 NS 34509 [M.H.M.] and R01 NS 75157 [M.K.P.]).

DISCLOSURE

J.L. Wagnon has received research funding from the NIH and the Dravet Syndrome Foundation. B.S. Barker, M. Ottolini, Y. Park, A. Volkheimer, P. Valdez, M.E.M. Swinkels, and M.K. Patel report no disclosures. M.H. Meisler has served on scientific advisory boards for the Dravet Syndrome Foundation and the Cate Syndrome Foundation and

Received March 10, 2017. Accepted in final form May 11, 2017.

REFERENCES

1. Vissers LE, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet* 2016;17:9–18.
2. O'Brien JE, Meisler MH. Sodium channel *SCN8A* (Nav1.6): properties and de novo mutations in epileptic encephalopathy and intellectual disability. *Front Genet* 2013;4:213.
3. Meisler MH, Helman G, Hammer MF, et al. *SCN8A* encephalopathy: research progress and prospects. *Epilepsia* 2016;57:1027–1035.
4. Trudeau MM, Dalton JC, Day JW, Ranum LPW, Meisler MH. Heterozygosity for a protein truncation mutation of sodium channel *SCN8A* in a patient with cerebellar atrophy, ataxia and mental retardation. *J Med Genet* 2006;43:527–530.
5. Wagnon JL, Barker BS, Hounshell JA, et al. Pathogenic mechanisms of recurrent epileptogenic mutations of *SCN8A* in epileptic encephalopathy. *Ann Clin Transl Neurol* 2015;3:114–123.
6. Petrovski S, Wang Q, Heinzen EL, Allen AS, Goldstein DB. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet* 2013;9:e1003709.
7. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–291.
8. Woodruff-Pak DS, Green JT, Levin SI, Meisler MH. Inactivation of sodium channel *Scn8a* (Nav 1.6) in Purkinje neurons impairs learning in Morris water maze and delay but not trace eyeblink classical conditioning. *Behav Neurosci* 2006;120:229–240.
9. McKinney BC, Chow CY, Meisler MH, Murphy GG. Exaggerated emotional behavior in mice heterozygous null for the sodium channel *Scn8a* (Nav1.6). *Genes Brain Behav* 2008;7:629–638.
10. Levin SI, Khaliq ZM, Aman TK, et al. Impaired motor function in mice with cell-specific knockout of sodium channel *Scn8a* (Nav1.6) in cerebellar purkinje neurons and granule cells. *J Neurophysiol* 2006;96:785–793.
11. de Kovel CGF, Meisler MH, Brilstra EH, et al. Characterization of an *SCN8A* mutation in a patient with epileptic encephalopathy. *Epilepsy Res* 2014;108:1511–1521.
12. Blanchard MG, Willemsen MH, Walker JB, et al. De novo gain of function and loss of function mutations of *SCN8A* in patients with intellectual disabilities and epilepsies. *J Med Genet* 2015;52:330–337.
13. Wolff M, Johannesen KM, Hedrich UBS, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in *SCN2A*-related disorders. *Brain* Epub 2017 Mar 4.
14. Deciphering Developmental Disorders Study. Prevalence and architecture of de novo mutations in developmental disorders. *Nature* 2017;542:433–438.
15. Rauch A, Wieczorek D, Graf E, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 2012;380:1674–1682.
16. Hamdan FF, Srour M, Capo-Chichi JM, et al. De novo mutations in moderate or severe intellectual disability. *PLoS Genet* 2014;10:e1004772.
17. Gilissen C, Hehir-Kwa JY, Thung GT, et al. Genome sequencing identifies major causes of severe intellectual disability. *Nature* 2014;511:344–347.

Neurology[®] Genetics

Loss-of-function variants of *SCN8A* in intellectual disability without seizures

Jacy L. Wagnon, Bryan S. Barker, Matteo Ottolini, et al.

Neurol Genet 2017;3;

DOI 10.1212/NXG.0000000000000170

This information is current as of July 6, 2017

Updated Information & Services	including high resolution figures, can be found at: http://ng.neurology.org/content/3/4/e170.full.html
References	This article cites 16 articles, 2 of which you can access for free at: http://ng.neurology.org/content/3/4/e170.full.html##ref-list-1
Citations	This article has been cited by 5 HighWire-hosted articles: http://ng.neurology.org/content/3/4/e170.full.html##otherarticles
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): All Genetics http://ng.neurology.org/cgi/collection/all_genetics Ion channel gene defects http://ng.neurology.org/cgi/collection/ion_channel_gene_defects Mental retardation http://ng.neurology.org/cgi/collection/mental_retardation
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://ng.neurology.org/misc/about.xhtml#permissions
Reprints	Information about ordering reprints can be found online: http://ng.neurology.org/misc/addir.xhtml#reprintsus

Neurol Genet is an official journal of the American Academy of Neurology. Published since April 2015, it is an open-access, online-only, continuous publication journal. Copyright © 2017 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2376-7839.