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

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## 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

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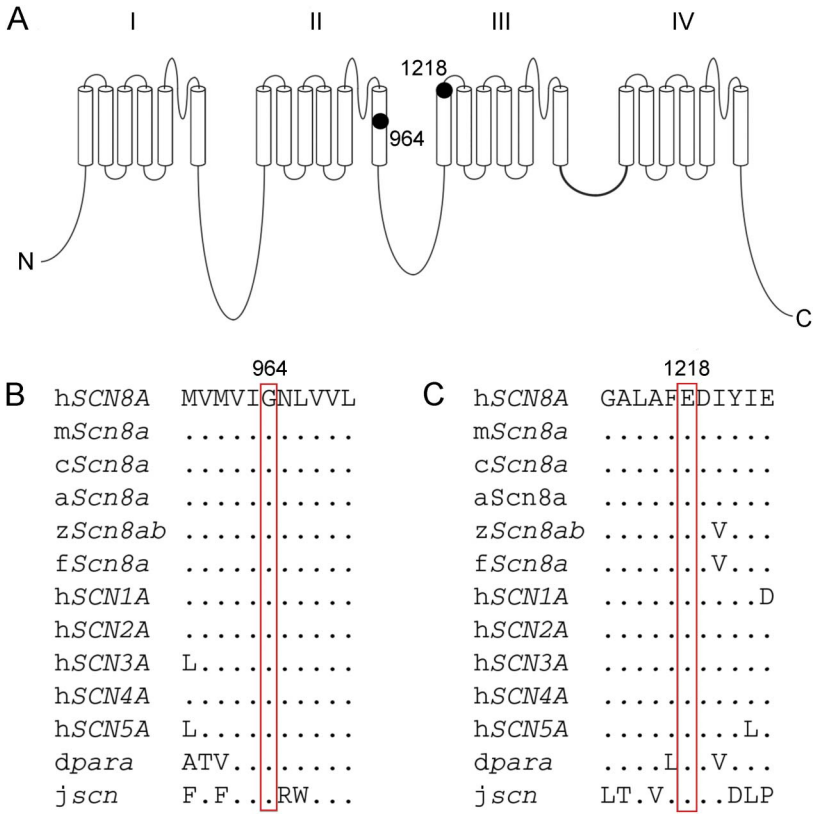
**Figure 1** **Location and evolutionary conservation of *SCN8A* mutations in individuals with intellectual disability**




(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 *PDHA1* 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 $Na_v1.6$ complementary DNA.** Mutations were introduced into the tetrodotoxin-resistant mouse complementary DNA (cDNA) $Na_v1.6R$ 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 $Na_v1.6$ cDNA, and lysates were prepared and analyzed 24 hours after transfection as described[5] 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 |

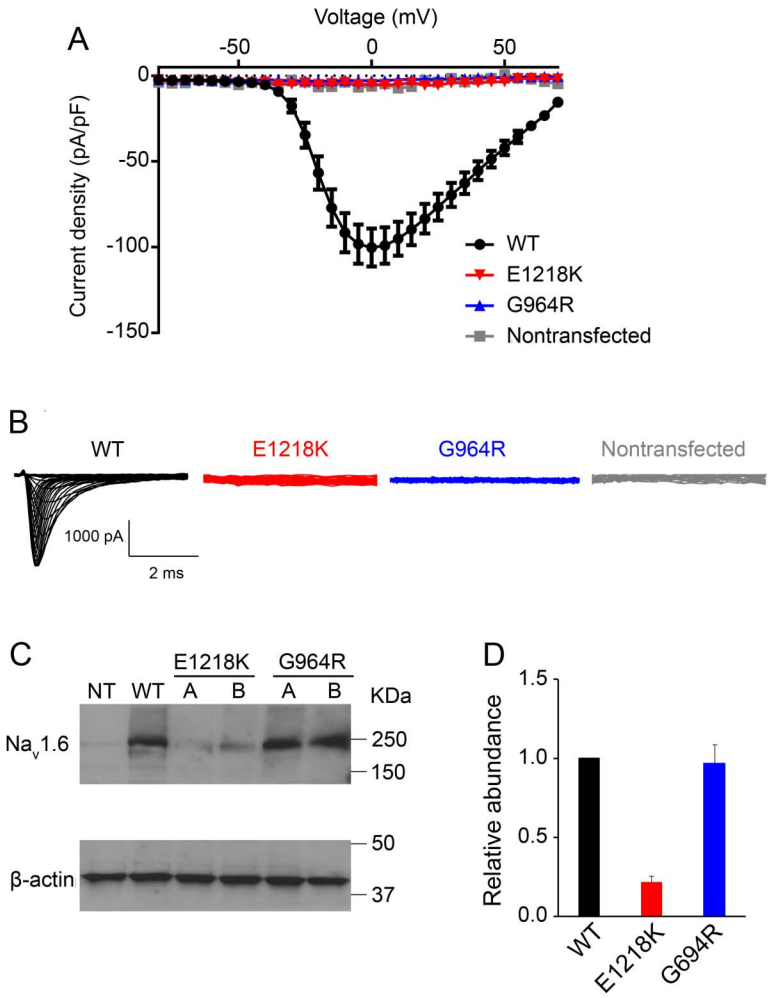
Abbreviation: ADHD = attention-deficit hyperactivity disorder.

**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 ± 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 ± 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 μg 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 eyeblink conditioning tests[8,9] and elevated anxiety in the open-field test[10] 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.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