Received: 17 April 2019 | Revised: 24 September 2019 | Accepted: 24 September 2019

DOI: 10.1111/epi.16371

FULL-LENGTH ORIGINAL RESEARCH

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Biallelic inherited *SCN8A* variants, a rare cause of *SCN8A*-related developmental and epileptic encephalopathy

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Abstract

Objective: Monoallelic *de novo* gain-of-function variants in the voltage-gated sodium channel *SCN8A* are one of the recurrent causes of severe developmental and epileptic encephalopathy (DEE). In addition, a small number of *de novo* or inherited monoallelic loss-of-function variants have been found in patients with intellectual disability, autism spectrum disorder, or movement disorders. Inherited monoallelic variants causing either gain or loss-of-function are also associated with less severe conditions such as benign familial infantile seizures and isolated movement disorders. In all three categories, the affected individuals are heterozygous for a *SCN8A* variant in combination with a wild-type allele. In the present study, we describe two unusual families with severely affected individuals who inherited biallelic variants of *SCN8A*.

Methods: We identified two families with biallelic *SCN8A* variants by diagnostic gene panel sequencing. Functional analysis of the variants was performed using voltage clamp recordings from transfected ND7/23 cells.

Results: We identified three probands from two unrelated families with DEE due to biallelic *SCN8A* variants. Each parent of an affected individual carried a single heterozygous *SCN8A* variant and exhibited mild cognitive impairment without seizures. In both families, functional analysis demonstrated segregation of one allele with complete loss-of-function, and one allele with altered biophysical properties consistent with partial loss-of-function.

Significance: These studies demonstrate that *SCN8A* DEE may, in rare cases, result from inheritance of two variants, both of which exhibit reduced channel activity. In these families, heterozygosity for the dominant variants results in less severe disease than biallelic inheritance of two variant alleles. The clinical consequences of variants

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Funding information
University of Virginia Robert R. Wagner Fellowship; National Institute of Neurological Disorders and Stroke (NINDS), Grant/Award Number: R01 NS34509 and RO1 NS103090

with partial and complete loss of *SCN8A* function are variable and likely to be influenced by genetic background.

1 | INTRODUCTION

SCN8A, encoding the voltage-gated sodium channel $Na_v1.6$, was first implicated in developmental and epileptic encephalopathy (DEE) in 2012 in a heterozygous individual with a *de novo* variant.[1] The phenotypic spectrum observed in several hundred individuals now includes a range of epilepsies including benign familial infantile seizures with or without paroxysmal dyskinesia,[2] less severe epilepsies with or without comorbid intellectual disability (ID),[3–6] and severe early onset DEEs.[1,7,8] Severe *SCN8A* DEE (Mendelian Inheritance in Man EIEE13) is characterized by intractable seizures with an average age at seizure onset of 4 months, cognitive deterioration, pyramidal/extrapyramidal signs, progressive cerebral atrophy, and visual impairment leading to cortical blindness.[7–9] There are often prolonged focal seizures with prominent hypomotor and vegetative symptoms that may evolve to clonic or bilateral tonic-clonic manifestations. Epileptic myoclonus, spasm-like episodes, and recurrent convulsive status epilepticus are frequently observed.[7–9] All affected individuals described to date are monoallelic (heterozygous) carriers of a gain-of-function (GOF) or loss-of-function (LOF) variant in combination with a wild-type (WT) allele.

Functional studies have demonstrated that *SCN8A*-related epilepsies are typically caused by monoallelic GOF alterations leading to neuronal hyperexcitability.[1,10–12] A small number of heterozygous LOF variants have been found in patients with ID, autism spectrum disorder (ASD), or movement disorders who do not necessarily have epilepsy.[10,13–15] The less severe familial *SCN8A*-related disorders show an autosomal dominant pattern of inheritance, whereas the large majority of EIEE13 cases occur *de novo*.[1–5,7–9,13–15] A similar pattern has been reported for *SCN2A*, in which GOF variants cause early onset seizures, and LOF variants tend to be associated with later onset seizures or with ID or ASD without epilepsy.[16]

In the mouse, homozygosity for partial LOF alleles of *Scn8a* results in movement disorders including ataxia, tremor, and dystonia, whereas complete LOF results in juvenile lethality with loss of ambulation.[17,18] In the present paper, we describe the first two human families with biallelic variants in *SCN8A*. The index patients are born of heterozygous parents who exhibit mild cognitive deficits, whereas the probands suffer from DEE and severe ID. Functional analysis demonstrated a shared mechanism of inheritance of one complete LOF and one partial LOF allele by affected individuals in both families.

Key Points

- Biallelic inherited loss-of-function *SCN8A* variants were identified in probands with developmental and epileptic encephalopathy
- Their heterozygous parents exhibited mild cognitive impairment without epilepsy
- Functional analysis demonstrated segregation of one allele with complete loss-of-function and one with partial loss-of-function in both families
- These dominant variants result in partial or complete loss-of-function of the voltage-gated sodium channel $Na_v1.6$ and result in mild cognitive impairment in heterozygous carriers and severe DEE in individuals inheriting two mutant alleles

2 | MATERIALS AND METHODS

2.1 | DNA sequencing

Both families underwent targeted gene panel sequencing as part of their formal diagnostic workup at either the Institute of Human Genetics, University of Leipzig Hospital and Clinics (Family 1) or the Danish Epilepsy Centre (Family 2). The parents or legal guardians of all probands provided written informed consent, and the study was approved by the local ethics committees. In Family 1, targeted sequencing of a custom panel of 131 genes associated with epilepsy (TruSight Rapid Capture Kit) was performed. Genomic DNA was extracted from blood using standard methods, and the library was sequenced on a MiSeq v2 300 sequencer (Illumina). In Family 2, targeted sequencing of 78 epilepsy genes was performed. Genomic DNA was extracted from blood using standard methods, and SureSelect library building was followed by sequencing on the Ion PGM system (Ion PGM 200 Sequencing Kit), as previously described.[19]

Variants with a mutant allele frequency < 1% in the general population (gnomAD, Broad Institute) were

classified according to the ACMG guidelines.[20] In silico evaluation was performed using SIFT Blink (J. Craig Venter Institute), Polyphen2 (Harvard), Combined Annotation-Dependent Depletion (CADD; University of Washington), MutationTaster (Charité), Model Predictive Control (MPC; Harvard), and the following splicing tools: SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and Human Splicing Finder. In addition, database synchronization by ClinVar (National Center for Biotechnology Information) and Human Gene Mutation Database (Biobase) was performed. Sanger sequencing was carried out to confirm all variants and to perform segregation analysis.

2.2 | Electrophysiology

Missense variants were introduced into the tetrodotoxin-resistant mouse cDNA $Na_v1.6R$ by site-directed mutagenesis with QuikChange II XL (Agilent Technologies) and analyzed as previously described.[12] The 6-kb open reading frame of each construct was resequenced to eliminate clones containing extraneous mutations. $Na_v1.6$ variants were expressed by transfection of neuron-derived ND7/23 cells (Sigma-Aldrich).[12] Sodium currents were recorded 48 hours after transfection in the presence of 500 nmol·L^{-1} tetrodotoxin to block endogenous sodium currents, using the whole-cell configuration of the patch-clamp recording technique.[12]

TABLE 1 Clinical features of affected individuals with inherited biallelic *SCN8A* variants

Family ID	Family 1 Patient A	Family 1 Patient B	Family 2
Sex	M	F	M
Current age	4 y	2 y	27 y
Family history	Both parents: mild cognitive deficits. Two maternal uncles and one maternal aunt have ID ± epilepsy. One maternal half-brother has speech delay.	Both parents: mild cognitive deficits. Two maternal uncles and one maternal aunt have ID ± epilepsy. One maternal half-brother has speech delay.	Both parents have mild cognitive deficits.
Development	Severe ID	Severe ID	Profound ID
Age at seizure onset	3 mo	Few hours after birth	7 mo
Seizure type at onset	Focal seizure	Convulsive status epilepticus	Tonic seizure and eye rolling
Seizure types	Clonic, focal, or brief tonic seizures	Brief tonic and automotor seizures	Infantile febrile seizures, tonic seizures, absences
EEG features	12 m: hypsarrhythmia; 3 y: moderate background slowing; mild nonspecific EEG abnormalities	8 d: medium-weight generalized discharges; 4 m: hypsarrhythmia; 17 mo: moderate background slowing; mild nonspecific EEG abnormalities	7 y: ictal regular generalized 3-4–Hz paroxysms of 5-10 s; 26 y: 3-4–Hz spike-waves bilaterally in the posterior quadrants
Movement disorder	No	No	Dyskinesia
Other neurological features	Hypotonia, strabismus; 19 mo: auditory neuropathy	Hypotonia, 8 mo: strabismus	Hypotonia, spastic tetraplegia
Vision impairment	6 mo: VEP/flash-VEP; binocular response pos; hyperopia	11 mo: hyperopia	Myopia
Additional features	Bilateral hip dysplasia, constipation, hemangioma, swallowing difficulties, regurgitation; 28 m: microcephaly (−3.4 SD)	Constipation, swallowing difficulties, apnea, dysphagia; 5 mo: microcephaly (−5 SD); 13 mo: PEG tube	Recurrent pneumonia, constipation; phimosis, reflux, asthma, bilateral hip dysplasia, dysphagia, scoliosis, kyphosis
MRI	4 mo and 26 mo: pineal cyst, otherwise normal	4 mo: delayed myelination	8 y: cerebral atrophy
Allele 1	c.805G>A; p.(Gly269Arg) mat	c.805G>A; p.(Gly269Arg) mat	c.2464G>A; p.(Gly822Arg) pat
Allele 2	c.4079C>A; p.(Thr1360Asn) pat	c.4079C>A; p.(Thr1360Asn) pat	c.4912C>T; p.(Arg1638Cys) mat

Abbreviations: EEG, electroencephalogram; F, female; ID, intellectual disability; M, male; mat, maternal; MRI, magnetic resonance imaging; pat, paternal; PEG, percutaneous endoscopic gastrostomy; pos, positive; VEP, visually evoked potential.

3 | RESULTS

3.1 | Clinical features of affected children and parents from two families segregating biallelic *SCN8A* variants

The clinical data of the three index patients are summarized in Table 1.

3.1.1 | Family 1

The index patients were a sib pair (3-year-old male and 2-year-old female) born to unrelated parents (Figure 1A). Both parents had mild cognitive impairment/borderline intellectual functioning. They both attended special school, obtained vocational training, and were able to live independent lives. Neuropsychological evaluations were not available. Additional affected family members included a maternal half-brother of the affected sibs and two of the mother's half-brothers and one half-sister, all with mild ID and one with unclassified epilepsy. Unfortunately, none of the maternal relatives were available for genetic testing.

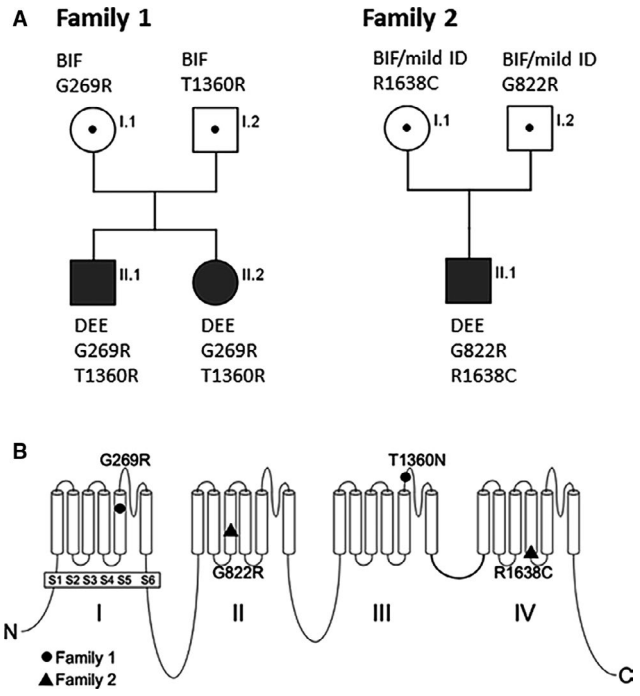

FIGURE 1 Biallelic inheritance of developmental and epileptic encephalopathy (DEE) in two families, and the protein location of the novel variants. A, Individuals affected with DEE (epilepsy plus severe intellectual disability [ID]) are compound heterozygotes for inherited variants of *SCN8A*. Heterozygous parents exhibit mild cognitive deficits. Sanger sequencing of genomic DNA demonstrated inheritance of two mutant alleles by individuals affected with DEE (epilepsy with severe ID) and a single variant in heterozygous carriers with mild cognitive deficits. B, Location of four novel variants in the voltage-gated sodium channel *SCN8A*. BIF, borderline intellectual functioning.

The male sib was born at term after an uncomplicated pregnancy and delivery (Apgar 10/10). Postnatally, he needed treatment of hypoglycemia and was tube fed for several days due to feeding difficulties. He presented with global developmental delay and onset of intractable daily focal seizures at 3 months of age. Treatment with levetiracetam (LEV) was initiated, which led to transient seizure reduction. However, during the course of the disease, he developed clonic and daily brief tonic seizures (lasting a few seconds). His initial electroencephalogram (EEG) showed background slowing and multifocal epileptiform discharges, which progressed to hypsarhythmia. Treatment with sulthiame and prednisolone was tried without any effect. Magnetic resonance imaging (MRI) performed at 4 months of age was normal, except for a small pineal cyst. At follow-up, he presented with muscular hypotonia, severe ID, and microcephaly (−3.4 SD at 28 months). Additional features included strabismus, hyperopia, and bilateral hip dysplasia (type IIa on the right and type Ib on the left side). At 19 months of age, bilateral coxa valga was observed and confirmed by X-ray. Furthermore, he suffered from constipation and swallowing difficulties with regurgitation and vomiting after chunky food. At 19 months of age, he was diagnosed with an auditory neuropathy with almost complete hearing loss. He could turn to both sides at 30 months of age, but was not able to sit, crawl, stand, walk, or communicate. His epilepsy continued to be intractable, and currently he has one to two brief tonic seizures per week despite treatment with LEV, vigabatrin (VGB), and valproic acid (VPA). EEG at follow-up showed background slowing and mild nonspecific abnormalities.

The female sib was born at term after a risk pregnancy due to maternal hypertension. Birth and postnatal adaptation were complicated by perinatal asphyxia and left cerebral hemorrhage (Apgar 1/1/2). Postnatally, she presented with hypoxic ischemic encephalopathy, and when only a few hours old she developed convulsive status epilepticus. She presented with daily dyscognitive seizures and brief focal motor seizures with a tonic component. She continued to have episodes of convulsive status epilepticus. EEG showed multifocal epileptiform discharges, which evolved to hypsarrhythmia at 4 months of age. At 10 months of age, she suffered from daily brief tonic seizures. She was developmentally delayed from birth, and severe ID was present at follow-up. MRI at 4 months of age showed delayed myelination. At 5 months of age, severe microcephaly was evident (−5 SD). At follow-up, she had adequate head control and was able to grab things, but was unable to turn or sit, and had poor eye contact. Additional features included hyperopia, strabismus, constipation, hypotonia, apneas, and eating difficulties. Tube feeding was initiated at 13 months.

She experienced a transient response to phenobarbital, and her EEG improved during treatment with prednisolone, 5 mg/kg/d for 2 weeks, showing only background slowing and nonspecific EEG abnormalities. VGB and sulthiame treatment resulted in no seizure improvement. Current treatment

includes sulthiame and VPA and is accompanied by 10-15 absence seizures per day.

3.1.2 | Family 2

The proband was a 27-year-old male born to unrelated parents. Both parents had mild cognitive deficits. They were able to live independent lives with guidance and support. Both attended special school, received disability pensions, and worked in a sheltered workshop. Neuropsychological evaluations were not available, and both parents were lost for follow-up.

The patient was born at term after an uncomplicated pregnancy and delivery. He was readmitted to the hospital within the first week of life due to cyanosis, hypotonia, and apathetic behavior. At 6 months of age, delayed psychomotor development became evident (no eye contact, lack of head control, hypotonia), and at 7 months of age, he experienced his first seizure, characterized by eye rolling and stiffness of the whole body. His EEG was reported as slightly abnormal, but no antiepileptic treatment was initiated. A computed tomography scan showed atrophy of the frontal lobes. At this approximate age, he was removed from his parents and put in foster care. In the following years, he was diagnosed with severe ID, spastic tetraplegia, myopia, and bilateral hip dysplasia. He never gained the ability to walk or communicate, and was admitted several times due to febrile seizures, asthmatic bronchitis, gastroesophageal reflux, pneumonia, and constipation. At the age of 7 years, brief episodes with staring were noticed. Ictal EEG recordings showed regular diffuse 3-4–Hz epileptic discharges lasting for 5-10 seconds. Oxcarbazepine was introduced but was administered irregularly for a few days by the foster mother, only during febrile episodes. At 8.5 years, it was stopped. MRI performed at the age of 8 years did not show abnormalities. When he was 9 years old, he was moved to a residential care institution, where he now lives. Tonic-clonic seizures, occasional dystonic/dyskinetic episodes, and eating difficulties were reported. At the age of 10 years, topiramate (TPM) and VPA were initiated, achieving seizure control for some years. He was also treated with risperidone for behavioral problems (agitation and screaming). At age 15 years, TPM was stopped. In the following years, the seizure frequency progressively increased, and at 20 years of age, he was referred to the Danish Epilepsy Center because of drug-resistant epilepsy with weekly tonic seizures and staring episodes. His interictal EEG showed subcontinuous theta-delta activity and high-amplitude spike and slow waves, bilaterally in the posterior quadrants, as well as less prominent focal slowing and infrequent spike and slow waves in the frontotemporal regions. The staring episodes were recorded on video-EEG and did not have an EEG correlate. LEV was added to VPA, with improvement of seizure duration and frequency from weekly to monthly. At latest examination, he was profoundly intellectually disabled, nonverbal, and wheelchair bound and had spastic tetraplegia, dyskinesia, dysphagia, scoliosis, kyphosis, and severe myopia.

3.2 | Identification and inheritance of *SCN8A* mutations

3.2.1 | Family 1

Gene panel testing revealed that the two affected sibs were compound heterozygotes for the *SCN8A* missense variants p.Gly269Arg (c.805G>A) located in the pore loop of domain I and p.Thr1360Asn (c.4079C>A) in the pore loop of domain 3 (Figure 1B). Sanger sequencing demonstrated that both variants were inherited (Figure 1B).

3.2.2 | Family 2

Gene panel sequencing revealed that the index patient was compound heterozygous for the *SCN8A* missense variants p.Gly822Arg (c.2464G>A) located in the middle of transmembrane segment D2S3 and p.Arg1638Cys (c.4912C>T) at the cytoplasmic end of transmembrane segment D4S4. Sanger sequencing demonstrated that both variants were inherited (Figure 1B).

SCN8A is highly intolerant of variation in the general population, with a probability of LOF intolerance of 1.00 and a missense *z* score of 7.94 in the gnomAD database. There are only four protein truncations and 384 missense variants in the gnomAD database,[21] compared to the prediction of 80 truncations and 1114 missense variants. The four variants detected in the present study are absent from the gnomAD database and are predicted to be deleterious by two or more prediction programs including CADD score, MPC score, and PolyPhen.

3.3 | Functional analysis of *SCN8A* variants

ND7/23 cells were transfected with $Na_v1.6$ cDNA, and sodium currents were recorded. Cells transfected with WT $Na_v1.6$ generated current density of -90 ± 11 pA/pF ($n = 37$; Figure 2A). Transfection of variant G269R from Family 1 (allele 1) did not generate any detectable current (-6 ± 1.4 pA/pF, $n = 5$; Figure 2B) and was not significantly different ($P > .05$) from nontransfected cells (-4 ± 0.8 pA/pF, $n = 5$). In contrast, the variant T1360N (Family 1, allele 2) generated current density similar in magnitude to the WT channel (-82 ± 17 pA/pF, $n = 7$; Figure 2C). The voltage dependence of activation of T1360N channels did not differ from WT (Figure 2D,E; Table 2). However, analysis of voltage-dependent steady-state inactivation demonstrated a hyperpolarizing shift of −7.5 mV in the half maximal voltage of inactivation ($V_{1/2}$) of T1360N compared to WT ($P < .01$; Figure 3F-H, Table 2). The predicted effect of this mutation is to reduce channel availability.

Family 2 allele 1 (G822R) did not generate detectable sodium current (-7 ± 1 pA/pF, $n = 12$; Figure 3B,3D).

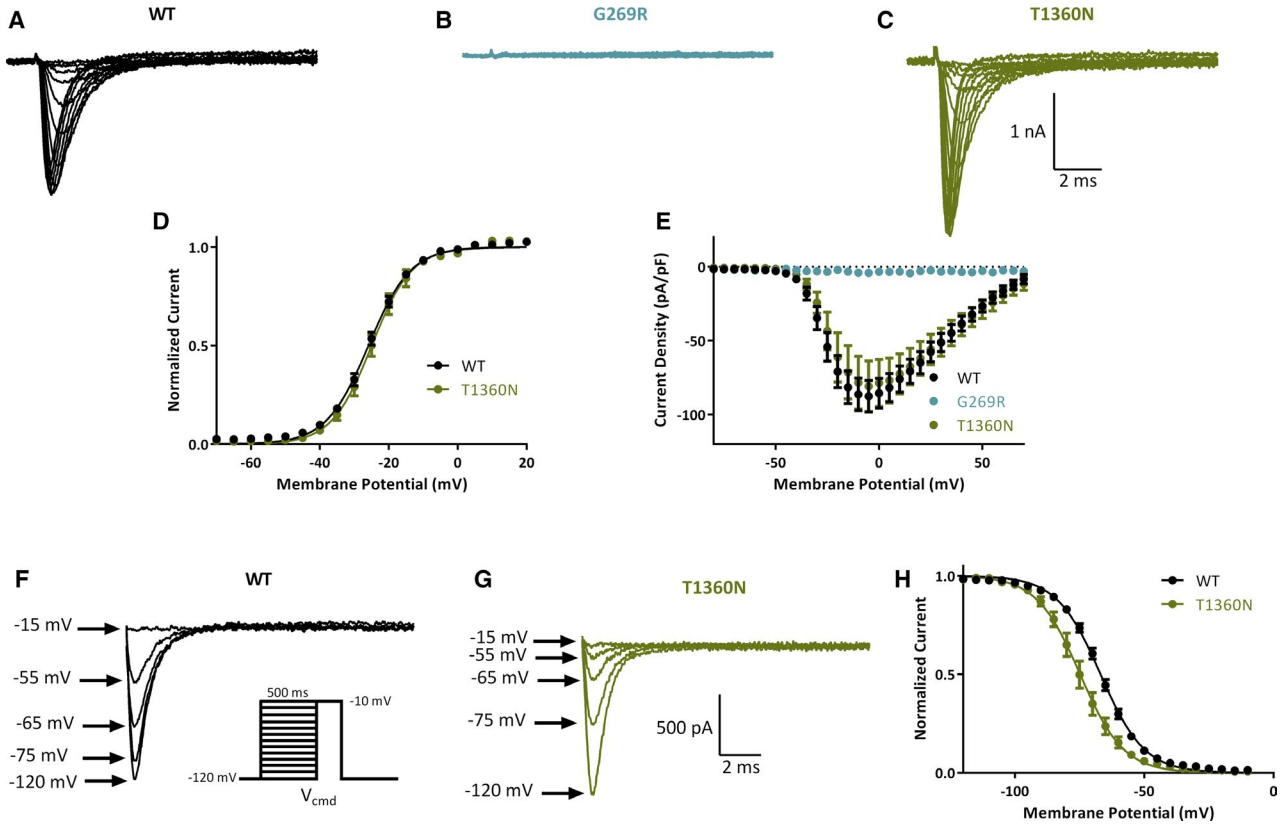

FIGURE 2 Biophysical characterization of sodium channel variants in Family 1. A-C, Representative traces of families of sodium channel currents recorded from ND7/23 cells expressing wild type (WT; A), G269R (B), and T1360N (C). D, Averaged current-voltage curves for WT (n = 37) and T1360N (n = 7). E, Voltage dependence of channel activation. F, G, Example traces of steady-state inactivation curves for WT (F) and T1360N (G). Inset shows voltage protocol for steady-state inactivation (Vcmd: voltage command). H, Voltage dependence of steady-state inactivation for WT (n = 37) and T1360N (n = 7). Data represent mean ± SEM. Smooth lines in D and H correspond to single Boltzmann equation fits to average data

Family 2 allele 2 (R1638C) generated current density of −63 ± 8.7 pA/pF (n = 12), which did not differ from WT ($P > .05$; Figure 3C,3D). The $V_{1/2}$ for activation of R1638C was shifted in a depolarizing direction by +3.8 mV in comparison with the WT channels ($P < .05$; Figure 3F-H, Table 2). This is predicted to reduce the number of channel openings at a given voltage. The $V_{1/2}$ for steady-state inactivation between R1638C and WT channels did not differ (Figure 3E, Table 2). The depolarizing shift in voltage-dependent activation exhibited by R1638C is consistent with reduced neuronal activity.

4 | DISCUSSION

We describe two pedigrees (Figure 1) in which probands with inherited biallelic variants of *SCN8A* are severely affected with DEE. The monoallelic (heterozygous) parents have only mild cognitive deficits. In each family, one parent had a more severe allele with complete LOF and one parent had a partial LOF variant, with the probands inheriting one of each type of variant. These variants exhibit dominant expression in heterozygous carriers with mild phenotypes and a much more severe phenotype in compound heterozygous carriers with two variant alleles. None of the four variants had been previously described in patients, but all are predicted to likely be pathogenic and are absent from the control population in the gnomAD database.[22] Functional tests demonstrated reduced or complete LOF of the variant channels. In the only previous report of a patient with two variants of *SCN8A*, the missense variant (p.Ile1583Thr) was probably not deleterious, and the deletion was a somatic mosaic.[23] In two families with biallelic variants of *CACNA1A*, the probands with biallelic variants suffered from DEE whereas the heterozygous parents and siblings had milder symptoms,[24,25] similar to our observations.

In addition to demonstrating a previously unreported inheritance pattern for *SCN8A*-related disorders, these families suggest an explanation for the observation that the frequency of *SCN8A* variants previously observed is lower than that for *SCN1A* (Johannesen et al, personal communication).[26] In contrast to *SCN1A* heterozygotes, who are often affected with Dravet syndrome, the *SCN8A*

TABLE 2 Activation and inactivation parameters of wild-type and variant $Na_v1.6$ currents

	Activation		Inactivation	
	$V_{1/2}$, mV	k	$V_{1/2}$, mV	k
WT, n = 37	−25.8 ± 0.8	5.37 ± 0.22	−66.9 ± 0.9	7.75 ± 0.14
Family 1, allele 2 T1360N, n = 7	−26.0 ± 1.6	5.29 ± 0.47	−74.4 ± 1.6*	7.79 ± 0.35
Family 2, allele 2 R1638C, n = 12	−22.0 ± 0.8**	6.65 ± 0.29*	−66.7 ± 2.0	10.4 ± 0.88***

Abbreviations: WT, wild type; $V_{1/2}$, half maximal voltage of inactivation.
*$P < .01$, **$P < .05$, ***$P < .001$ compared to WT by unpaired *t* test.

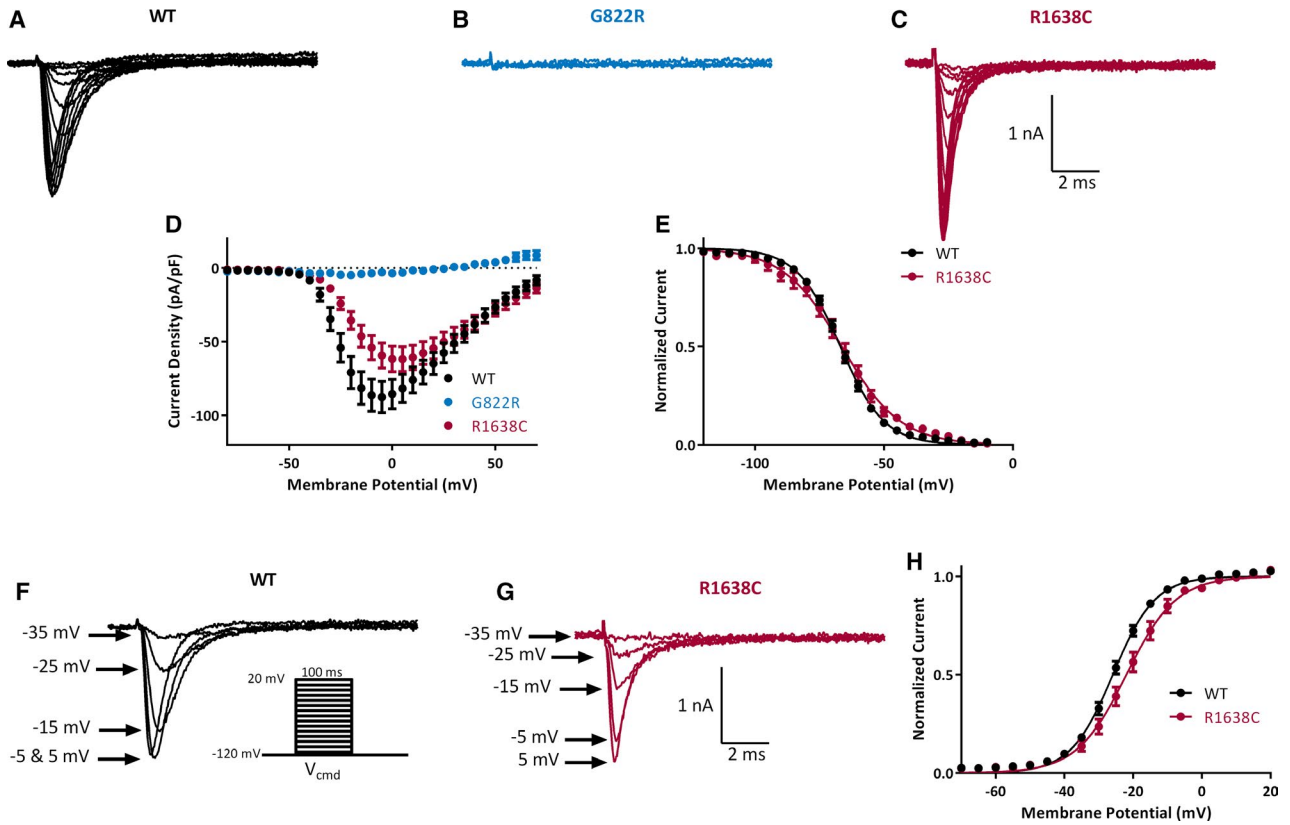

FIGURE 3 Biophysical characterization of sodium channel variants in Family 2. A-C, Representative traces of families of sodium channel currents recorded from ND7/23 cells expressing wild type (WT; A), G822R (B), and R1648C (C). D, Averaged current-voltage curves for WT (n = 37), G822R (n = 9), and R1638C (n = 12). E, Voltage dependence of steady-state inactivation for WT (n = 37) and R1638C (n = 12). F, G, Example traces of sodium currents recorded for WT (F) and R1638C (G) showing a reduction in current amplitude in R1648C cells compared with WT at a given activation voltage. Inset shows activation voltage protocol. H, Voltage dependence of activation for WT (n = 37) and R1638C (n = 12). Data represent mean ± SEM. Smooth lines in E and H correspond to single Boltzmann equation fits to average data

heterozygotes presented in this study were not sufficiently ill to be candidates for genetic testing. There are likely to be many more individuals with mild *SCN8A*-related phenotypes who will not be discovered until genetic testing becomes more widespread.

Electrophysiological analysis of the variants G269R and G822R identified complete absence of channel activity in transfected ND7/23 cells. G269R is located in segment 5 of domain I, and G822R is located in segment 3 of domain 2, a region that is relatively lacking in variants causing dominant DEE.[11] Both variants completely eliminate channel activity, indicating that the affected residues are essential for channel function. In contrast, the variants R1638C and T1360N retained channel activity with altered biophysical properties that are consistent with partial LOF. Depolarizing shifts of voltage-dependent activation in the R1638C mutation reduce the number of channels available to open at any membrane voltage and thereby reduce neuronal excitability. The hyperpolarized shift in channel inactivation in the T1360N channel reduces channel availability due to premature entry into inactivated channel states. The predicted result in affected compound heterozygotes is altered excitability of both excitatory

and inhibitory neurons, modulating overall neuronal network function.

Both families segregate one allele with complete LOF and one allele with altered biophysical properties, both of which are predicted to result in reduced neuronal activity. In contrast, most of the *de novo* variants associated with DEE result in elevated neuronal activity.[11] The most common biophysical effect of previously described *SCN8A* variants in DEE is impaired channel inactivation and elevated neuronal activity, for example, the recurrent variant R1872W.[12,27] Even in the presence of a WT allele, variants with elevated channel activity produce a severe dominant phenotype. However, for the LOF variants, the WT allele in heterozygous carriers provides compensating channel activity and heterozygous parents exhibit only mild cognitive deficits. In the individuals affected with DEE, who are compound heterozygotes for reduced function alleles, the total channel activity is predicted to be <50% of normal, resulting in the severe phenotype of *SCN8A* encephalopathy. However, it is not clear why reduced activity of Nav1.6 results in seizures. The biallelic LOF individuals in our families have phenotypes that are indistinguishable from monoallelic GOF variants, including early onset DEE with multiple seizure types (focal, clonic, and tonic), severe ID with absent speech, axial hypotonia, central visual impairment, microcephaly, and gastrointestinal symptoms, features that are all described in patients with elevated channel activity.[7] It cannot be excluded that other rare genetic variants may contribute to the observed phenotypes.

A small number of heterozygous carriers of partial or complete LOF of *SCN8A* have been described previously, with variable consequences. Myoclonus in the absence of seizures was observed in one family with four affected family members.[15] Mild to moderate ID or ASD in the absence of seizures was observed in four families.[10,14] On the other hand, absence epilepsy was observed in a family with the heterozygous protein truncating variant p.Asn544fs*39,[6] and focal epilepsy with mild ID was observed for the protein truncation variant p.Arg1820*0.[28] It is likely that variations in other genes in the genetic background contribute to this variation; the effects of modifier genes on seizure phenotypes have been well established in the mouse.[18,29] It is also possible that missense variants that appear to cause complete LOF in vitro may actually retain some activity in vivo that contributes to the relatively mild phenotype in the monoallelic (heterozygous) parents. Recent evidence suggests that the sodium channel alpha subunits may function as dimers.[30] In this case, LOF alleles that produce full length protein could have a dominant negative effect in heterozygotes, resulting in <50% residual activity and a more severe phenotype than that of LOF alleles encoding truncated or unstable proteins.

SCN8A is widely expressed in both excitatory and inhibitory neurons of the central nervous system and peripheral nervous system. LOF variants of *Scn8a* in the mouse result in severe movement disorders without seizures; loss of 90% of normal activity results in tremor, ataxia, and dystonias,[18] and loss of 100% of activity results in hind limb paralysis and juvenile lethality.[17] Homozygosity for complete LOF alleles may also be incompatible with human life. Reduced activity of Nav1.6 in colonic mesenteric neurons may contribute to the gastrointestinal disturbances in patients with *SCN8A* encephalopathy.[31] Future studies of inhibitory neuron populations in *SCN8A* encephalopathy could be helpful to reconcile the existence of both GOF and LOF variants in patients with developmental and epileptic encephalopathy.

ACKNOWLEDGMENTS

We thank the patients and their families for participating in this study.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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How to cite this article: Wengert ER, Tronhjem CE, Wagnon JL, et al. Biallelic inherited *SCN8A* variants, a rare cause of *SCN8A*-related developmental and epileptic encephalopathy. *Epilepsia*. 2019;60:2277–2285. https://doi.org/10.1111/epi.16371