Genotype-phenotype correlations in SCN8A-related disorders reveal prognostic and therapeutic implications

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We report detailed functional analyses and genotype-phenotype correlations in 392 individuals carrying disease-causing variants in *SCN8A*, encoding the voltage-gated Na$^+$ channel Na$_v$1.6, with the aim of describing clinical phenotypes related to functional effects. Six different clinical subgroups were identified: Group 1, benign familial infantile epilepsy ($n = 15$, normal cognition, treatable seizures); Group 2, intermediate epilepsy ($n = 33$, mild intellectual disability, partially pharmaco-responsive); Group 3, developmental and epileptic encephalopathy ($n = 177$, severe intellectual disability, majority pharmaco-resistant); Group 4, generalized epilepsy ($n = 20$, mild to moderate intellectual disability, frequently with absence seizures); Group 5, unclassifiable epilepsy ($n = 127$); and Group 6, neurodevelopmental disorder without epilepsy ($n = 20$, mild to moderate intellectual disability). Those in Groups 1–3 presented with focal or multifocal seizures (median age of onset: 4 months) and focal epileptiform discharges, whereas the onset of seizures in patients with generalized epilepsy was later (median: 42 months) with generalized epileptiform discharges. We performed functional studies expressing missense variants in ND7/23 neuroblastoma cells and primary neuronal cultures using recombinant tetrodotoxin-insensitive human Na$_v$1.6 channels and whole-cell patch-clamping.

Two variants causing developmental and epileptic encephalopathy showed a strong gain-of-function (hyperpolarizing shift of steady-state activation, strongly increased neuronal firing rate) and one variant causing benign familial infantile epilepsy or intermediate epilepsy showed a mild gain-of-function (defective fast inactivation, less increased firing). In contrast, all three variants causing generalized epilepsy induced a loss-of-function (reduced current amplitudes, depolarizing shift of steady-state activation, reduced neuronal firing). Functional effects were known for 170 individuals. All 136 individuals carrying a functionally tested gain-of-function variant had either focal ($n = 97$, Groups 1–3) or unclassifiable ($n = 39$) epilepsy, whereas 34 individuals with a loss-of-function variant had either generalized ($n = 14$), no ($n = 11$) or unclassifiable ($n = 6$) epilepsy; only three had developmental and epileptic encephalopathy. Computational modelling in the gain-of-function group revealed a significant correlation between the severity of the electrophysiological and clinical phenotypes. Gain-of-function variant carriers responded significantly better to sodium channel blockers than to other anti-seizure medications, and the same applied for all individuals in Groups 1–3.

In conclusion, our data reveal clear genotype-phenotype correlations between age at seizure onset, type of epilepsy and gain- or loss-of-function effects of *SCN8A* variants. Generalized epilepsy with absence seizures is the main epilepsy phenotype of loss-of-function variant carriers and the extent of the electrophysiological dysfunction of the gain-of-function variants is a main determinant of the severity of the clinical phenotype in focal epilepsies. Our pharmacological data indicate that sodium channel blockers present a treatment option in *SCN8A*-related focal epilepsy with onset in the first year of life.

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Introduction

Since the first pathogenic SCN8A variant was discovered in an individual with epilepsy,[1] a wide clinical spectrum of neurodevelopmental phenotypes has been reported. The spectrum ranges from benign familial infantile epilepsy (BFIE) with self-limiting seizures and typical cognitive development,[2–4] over an intermediate phenotype with variable seizure onset, treatable seizures and mild to moderate intellectual disability (ID)[5] to developmental and epileptic encephalopathies (DEEs) with onset in the first year of life and moderate to severe ID,[6–10] often with movement disorders, cortical visual impairment, severe gastrointestinal symptoms and increased risk of premature death.[7,10–15] Furthermore, rare clinical presentations with ID, autism spectrum disorder (ASD) and movement disorders without epilepsy have been described.[16–19]

SCN8A encodes $Na_v1.6$, which is one of four voltage-gated sodium channels expressed in the mammalian brain. $Na_v1.6$ is found in the central and peripheral nervous system with predominant expression in excitatory, but also in inhibitory, neurons.[20] Previous studies have revealed that BFIE and DEEs are caused by missense variants with gain-of-function (GOF) effects, whereas truncating variants, deletions and certain missense variants causing loss-of-function (LOF) or both LOF and GOF effects have been associated with ID, ASD and movement disorders with or without seizures.[3,11,17,19,21]

Treatment of SCN8A-related DEEs has resulted in frequent resistance to anti-seizure medications (ASMs), although treatment with sodium channel blockers (SCBs), especially high-dosage phenytoin, is beneficial in some affected individuals.[22]

Here, we combined a detailed clinical analysis of the largest cohort of individuals with SCN8A-related neurodevelopmental disorders investigated to date with functional studies of newly detected variants in mammalian cells and primary neurons and explored the genotype-phenotype correlations in functional studies, computational modelling and treatment response in SCN8A.

Materials and methods

Cohort ascertainment and phenotyping

Affected individuals were recruited through a network of collaborating clinicians, as well as GeneMatcher,[23] by means of a standardized phenotyping sheet to assess clinical characteristics (medical history, seizure and physical characteristics, family history, neurodevelopment and cognition), EEG, neuroimaging and retrospective data on anti-epileptic treatment. Seizures and epilepsy syndromes were classified according to the latest ILAE guidelines.[24,25]

Based on the information available about the presence and severity of the epilepsy, seizure onset and cognitive status, the affected individuals were categorized into the following *a priori* defined subgroups: Group 1: BFIE, infantile onset focal seizures with onset during infancy and normal cognitive development[3]; Group : intermediate epilepsy, individuals with a focal epilepsy (seizure types and EEG) of intermediate severity, reflecting neither BFIE nor DEE[5]; Group 3: DEE,[14] individuals with focal seizure types and EEG; Group 4: generalized epilepsy, individuals with a generalized epilepsy (seizure types and EEG), thus not fitting Groups 1, 2 or 3; Group 5: unclassifiable epilepsy, individuals with insufficient data to be classified into one of the above groups, or, rarely, both focal and generalized seizure types or epileptiform discharges; and Group 6: neurodevelopmental disorder without epilepsy (NDDwoE).

See Supplementary Fig. 2 for a flow chart describing the categorization of SCN8A-related phenotypes.

Treatment response was evaluated by the referring treatment providers as freedom from seizures (at least 6 months without seizures), seizure reduction (affected individuals still on ASMs as the treatment provider and parents consider there to be a beneficial effect), no change (ASMs terminated) or seizure aggravation noted by treatment providers and parents. Phenytoin, carbamazepine, oxcarbazepine, lacosamide, lamotrigine and zonisamide were all classified as SCBs.

Variant pathogenicity was assessed according to the ACMG guidelines.[26] All variants were absent from gnomAD r2.1.1 (https://gnomad.broadinstitute.org/) and BRAVO (https://bravo.sph.umich.edu/freeze8/hg38/). Pathogenic or likely pathogenic variants were included. SCN8A transcript NM_014191.3 was used for coding variant nomenclature.

Previously published cases

A PubMed search for 'SCN8A' was performed, and all publications that included affected individuals were included in the present

study. The latest search was performed on 15 May 2020. Papers not available in English were excluded. Only original cases and only probands were included. Pathogenic or likely pathogenic variants were included, and the variant locations were remapped on NM_014191.3 when necessary to harmonize their presentation in this article. Data on functional studies were also collected. If affected individuals were published more than once, all papers were included in the list. Duplications of affected individuals were avoided by follow-up with the referring clinician/corresponding author.

Ethics

The study was approved by the local ethical committees or followed other local guidelines. Previously unpublished individuals (or parents, in the case of minors) provided informed consent.

Frequency

The Danish Epilepsy Centre is the only tertiary hospital in Denmark that specializes in treating epilepsy, and the majority of affected individuals with non-acquired epilepsy are referred to the centre for genetic testing. Furthermore, inquiries were sent to all national clinical genetics departments. Thus, we were able to estimate the frequency of *SCN8A* variants in the Danish population by using the birth cohort from 2006 to 2017 in Statistics Denmark, the electronic population database of Danish national statistics.

Functional studies

Variants were chosen for functional studies according to the clinical phenotype of the variant carriers and the location of variants in the Na$_v$1.6 channel protein to cover the phenotypic spectrum of *SCN8A*-related disorders (see the 'Results' section for details). All methods have been previously described[19] and are summarized in the Supplementary material.

Neuronal simulations

To investigate how changes in Na$^+$ current properties affect neuronal firing behaviour, we simulated a model neuron with sodium, potassium and leak currents (details provided in the Supplementary material). We simulated firing by injecting step currents of 0–0.75 nA for 2 s and analysed the voltage traces for the firing rate after the neuron had adapted to the injected current. The resulting input-output curve was then analysed for its area under the curve (AUC). We modified the following six parameters of the Na$^+$ current. The half activation voltages $V_{1/2}$ of the activation and inactivation curves were shifted within ±10 mV of the original values. The slopes k of the activation and inactivation curves as well as the maximum conductance g_{Na} were multiplied with a scaling factor ranging from 0.5 to 2. The persistent current z of inactivation ranged from 0 to 5% of the maximum inactivation. Only a single parameter was modified at a time. The effect of alteration i on the AUC was quantified relative to the AUC of the unchanged model AUC_0 by:

$$AUC\ contrast_i = \frac{AUC_i - AUC_0}{AUC_0} \qquad (1)$$

For each altered parameter, the slope m_j of the regression line through the origin between the parameter changes x and its resulting *AUC contrast* was calculated. To compare the magnitudes of effects across different alteration scales, the parameter changes x were set to $\Delta V_{1/2} = V_{1/2,i} - V_{1/2,\ 0}$, $\Delta z = z_i - z_0$ and $log(scaling\ factor_i)$ for k and g_{Na}.

These slopes were then used to score the biophysical changes j of ionic current variants to estimate their effect on the firing behaviour and the severity of their neurological impairments with:

$$score_j = x_j \cdot m_j \qquad (2)$$

Where $score_j$ is the scoring for one alteration of a biophysical parameter. This resulted in the following equations for the biophysical scores of *SCN8A* variants:

$$\text{score}_{conductance} = log\ \frac{g_{mut}}{g_{wt}} \cdot 0.091 \qquad (3)$$

$$\text{score}_{activation,V_{1/2}} = \left(V_{1/2,mut} - V_{1/2,wt}\right) \cdot \left(-0.12\ mV^{-1}\right) \qquad (4)$$

$$\text{score}_{activation,k} = log\ \frac{k_{mut}}{k_{wt}} \cdot 1.2 \qquad (5)$$

$$\text{score}_{inactivation,V_{1/2}} = \left(V_{1/2,mut} - V_{1/2,wt}\right) \cdot 0.0016\ mV^{-1} \qquad (6)$$

$$\text{score}_{inactivation,k} = log\ \frac{k_{mut}}{k_{wt}} \cdot 0.011 \qquad (7)$$

$$\text{score}_{inactivation,persistent} = (z_{mut} - z_{wt}) \cdot (-0.004) \qquad (8)$$

Finally, these scores were summed to predict the severity of the respective variants:

$$\text{Electrophysiological score} = \sum \text{score}_j \qquad (9)$$

Classification of phenotype severity

For the correlation analysis between electrophysiological score and severity of the disease in *SCN8A* variant carriers, we classified the individuals into four severity groups: (i) BFIE; (ii) intermediate epilepsy; (iii) DEE with mild or moderate ID; and (iv) DEE with severe ID. We assigned weights to the four grades of severity (BFIE = 1, intermediate epilepsy = 2, DEE with mild or moderate ID = 3 and DEE with severe ID = 4). In case the same variant was associated with different phenotypes, we calculated a weighted average score. As this analysis was performed for the GOF variants only, the generalized epilepsy, unclassifiable epilepsy and NDDwoE groups were not included.

Statistical analysis

Clinical data were analysed using Stata version 15.1 for Mac (StataCorp, College Station, Texas, USA). For categorical data, Fisher's exact test was used, and for continuous data the Kruskal-Wallis test and Mann-Whitney test were used. Significance was evaluated using a two-tailed test of proportions and significance was reached if $P < 0.05$. The do-file used to perform the analyses is available upon request.

Electrophysiological data were analysed using Clampfit software of pClamp 10.6 (Axon Instruments), Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) or Igor Pro (Wavemetrics, Portland, OR, USA). Statistics were performed using one-way ANOVA with Dunnett's *post hoc* test, ANOVA on ranks with Dunn's *post hoc* test or Fisher's exact test in GraphPad prism (GraphPad software, San Diego, CA, USA). For all statistical tests, significance is indicated in the figures (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) or in the text.

Data availability

Data will be made available upon request.

Results

Phenotypes within the whole cohort

We assessed a cohort of 91 unpublished and 301 previously published affected individuals (392 affected individuals in total). One hundred and twenty-seven affected individuals had unclassifiable epilepsy due to a lack of information and have not been included in the analyses below. Age at follow-up/inclusion ranged from 2 months to 44 years (median 4.3 years). We differentiated the following phenotypes:

(i) BFIE (n = 15): median age at seizure onset in this subgroup was 6 months (range: 2 weeks to 1 year). The most prevalent seizure types were focal (40%), focal to bilateral tonic-clonic (27%) and bilateral tonic-clonic without identifiable focal onset (27%). Cognition was normal in all affected individuals. Treatment response was known in 10 patients, all of whom were seizure free. Six patients were treated with SCBs, one with a non-SCB and three with a combination. Seven of 15 (47%) variants were inherited from an affected parent. Two variants were recurrent: p.(Glu1483Lys) and p.(Asn1877Ser).[2,3]

(ii) Intermediate epilepsy (n = 33): intermediate epilepsy was defined as a focal epilepsy fitting neither the BFIE nor the DEE categories (see Supplementary Fig. 2 for a flow chart and distinction criteria). Median age at seizure onset was 5 months (range: 2 months to 7 years). The most prevalent seizure types were focal (64%), tonic (33%) and bilateral tonic-clonic (55%). Cognition was normal in 33% of the patients, while mild ID was present in 52% and moderate ID in 15%. Additional features included speech delay (36%), behavioural disorders (attention-deficit hyperactivity disorder, autistic features, aggression; 19%) or ataxia (11%). Treatment response was known for 31 affected individuals. Fourteen of them were seizure free; eight individuals had been treated with an SCB, three with a non-SCB and three with a combination. Three variants were inherited from an affected parent, while four were unknown and the remainder were de novo. Two variants were recurrent: p.(Gly1475Arg) and p.(Asn1877Ser).[5]

(iii) DEE (n = 177): median age at seizure onset was 3 months (range: first day of life to 36 months). The most frequent seizure types were focal (70%), tonic (76%) and bilateral tonic-clonic (89%). Cognition ranged from moderate (22%) to severe (73%) ID and was unknown in the remainder (5%). Additional features included hypotonia in 50% and cortical vision impairment in 32%. Treatment response was known in 128 affected individuals. Twenty-six affected individuals (20%) were seizure free; 11 of them were treated with SCBs (42%), seven with non-SCBs (27%) and eight with a combination (31%). The vast majority of variants occurred de novo (90%), three were inherited from an affected parent, while inheritance was unknown in 11 individuals. Twenty-five variants were recurrent: p.(Arg850Gln/Gly) and p.(Arg1872Trp/Gln/Leu) were the most common[10,14,27] (Supplementary Table 1).

(iv) Generalized epilepsy (n = 20): the median age at seizure onset was 42 months (range: 9 months to 14 years). The most prevalent seizure types were absence (80%), generalized tonic-clonic (20%) and febrile (15%). Cognition was normal in 20% of individuals and ranged from mild (30%) or moderate (35%) to severe ID (10%). Cognition was unknown in 5% of the patients. Additional features included ataxia (5%), behavioural disorders (autism, aggression, anxiety; 28%) and speech delay (19%). Treatment response was known in 16 affected individuals. Six were seizure free. One was treated with an SCB and five with a non-SCB. Ten variants occurred de novo, five were inherited from an affected parent, and five were of unknown inheritance. None of the variants were recurrent.

(v) NDDwoE (n = 20): individuals with NDDwoE did not have epilepsy at time of inclusion (median age 9 years, range: 3–35 years). Cognition ranged from normal (10%) to mild (45%), moderate (20%) or severe ID (15%) and was unknown in 10%. Additional features included behavioural disorders (ASD, attention-deficit hyperactivity disorder; 43%), delayed speech (24%) and microcephaly (19%). Six variants occurred de novo, whereas nine were inherited from affected or mosaic parents and the remainder were unknown. Three variants were recurrent: p.(Gly384Arg), p.(Arg931Gln) and p.(Ala1622Asp).

Clinical details for all previously unpublished and published individuals (as data were available) are summarized in Supplementary Table 1.

Functional studies

We examined seven SCN8A variants functionally, chosen to represent the most important aspects of the clinical spectrum, particularly the newly identified phenotype with generalized epilepsy, for which functional studies have not been performed to date. Three variants [p.(Leu840Pro), p.(Phe846Ser) and p.(Asn1877Ser)] were seen in affected individuals with onset of seizures in the first year of life; the first two appeared in affected individuals with DEE who were resistant to all ASMs including high-dose SCBs,[7] the third was identified in affected individuals with BFIE or intermediate epilepsy who were responsive to SCBs.[2,5] Three variants [p.(Ile1654Asn), p.(Val1758Ala), p.(Thr1787Pro)] were seen in affected individuals with generalized epilepsy and late onset absence seizures in two cases preceded by febrile seizures.[5,28] The last variant [p.(Asn374Lys)] was found in an affected individual with focal epilepsy and moderate ID with late onset at 7 years of age—an unusual phenotype for which the pathogenicity of the SCN8A variant had to be shown.[5]

The biophysical consequences of these seven variants were first studied in ND7/23 cells. p.(Phe846Ser) and p.(Leu840Pro) variants induced hyperpolarizing shifts of the activation curves, indicating clear GOF effects (Fig. 1B and C and Supplementary Table 2). The p.(Asn1877Ser) variant caused a significant depolarizing shift of steady-state fast inactivation and slightly slowed the time course of fast inactivation (Fig. 1D and F and Supplementary Table 2). In contrast, p.(Ile1654Asn), p.(Val1758Ala) and p.(Thr1787Pro) variants dramatically reduced the peak current density. In particular, cells transfected with p.(Ile1654Asn) exhibited almost no Na^+ current; hence no gating parameters were obtained for this variant (Fig. 2A and B and Supplementary Table 2). Furthermore, the p.(Val1758Ala) and p.(Thr1787Pro) variants shifted the activation curves toward more depolarized potentials, suggesting LOF effects, although the former variant impaired, while the latter enhanced, fast inactivation (Fig. 2B–D and F and Supplementary Table 2). Additionally, these two variants, as well as p.(Asn374Lys) to a minor extent, accelerated recovery from fast inactivation (Fig. 2E and Supplementary Table 2).

Next, intrinsic and firing properties were examined in transfected cultured hippocampal mouse neurons in the absence or presence of tetrodotoxin. In the absence of tetrodotoxin, action potential firing was jointly determined by endogenous and transfected Na^+ channels. Only the p.(Thr1787Pro) variant significantly decreased the action potential firing rate compared to neurons transfected with wild-type channels, as revealed by the area under the curve of input-output relationships (Fig. 2B and C and Supplementary Table 3). The p.(Phe846Ser) variant decreased, whereas the p.(Thr1787Pro) variant increased both the rheobase and action potential threshold compared with the wild-type (Fig. 2D and E and Supplementary Table 3), indicating the former enhanced, whereas the latter impaired, neuronal excitability.

In the presence of tetrodotoxin, action potential firing was dependent on the tetrodotoxin-insensitive transfected Na^+ channels. Under these conditions, p.(Phe846Ser) and p.(Asn1877Ser) variants significantly increased action potential firing, whereas p.(Thr1787Pro) and p.(Ile1654Asn) decreased action potential firing (Fig. 3B and C and Supplementary Table 4). Additionally, the p.(Phe846Ser), p.(Leu840Pro) and p.(Asn1877Ser) variants decreased the rheobase and the former two variants further reduced the threshold for action potential firing, indicating increased neuronal excitability. In contrast, neurons transfected with p.(Val1758Ala), p.(Thr1787Pro) or p.(Ile1654Asn) variants exhibited significantly decreased peak Na^+ currents, resulting in very few action potentials firing in the presence of tetrodotoxin; hence, no parameters of single
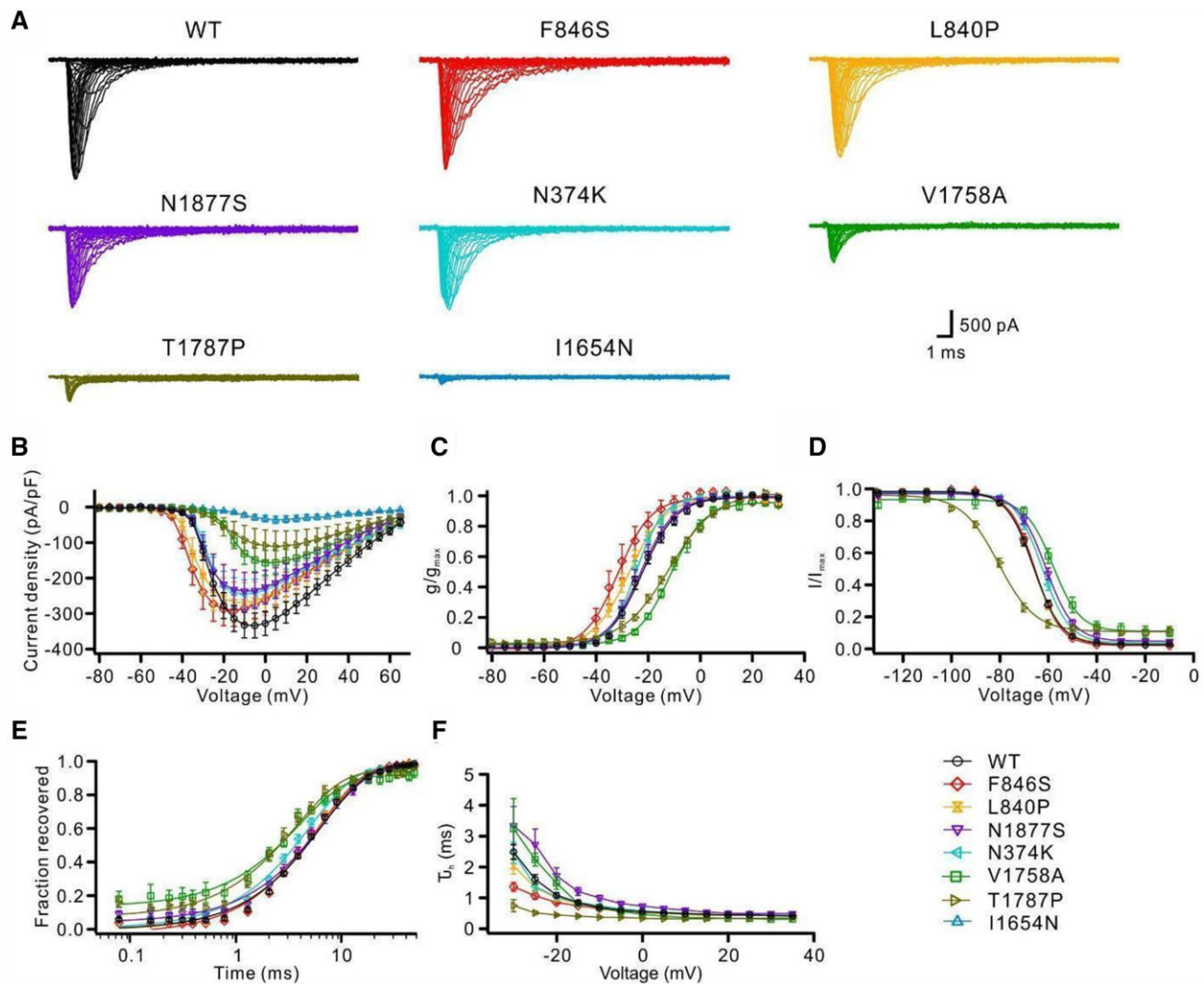
Figure 1 Functional characterizations of *SCN8A* variants in the neuroblastoma cell line ND7/23. Wild-type or mutant Na$_v$1.6 channels were transfected into ND7/23 cells and recorded in the presence of tetrodotoxin to block endogenous Na$^+$ channels. (**A**) Representative Na$^+$ current traces for transfected Na$_v$1.6 wild-type (WT, black) or mutant channels (colour code in the *bottom right*). (**B**) Peak Na$^+$ currents normalized by cell capacitance were plotted versus voltage. Both the p.(Phe846Ser) and p.(Leu840Pro) variants caused a hyperpolarizing shift of the current-voltage relationship, whereas the p.(Val1758Ala) and p.(Thr1787Pro) variants caused a depolarizing shift compared with the wild-type channels. The p.(Ile1654Asn), p.(Val1758Ala) and p.(Thr1787Pro) variants significantly decreased the current density in comparison with the wild-type. Wild-type: n = 30; mutants: n = 14–19. (**C**) Voltage-dependent steady-state activation curves. Lines represent Boltzmann functions fit to the data-points. (**D**) Voltage-dependent steady-state inactivation curves. Lines represent Boltzmann functions fit to the data-points. (**E**) Time course of recovery from fast inactivation at −100 mV. The p.(Val1758Ala), p.(Thr1787Pro) and p.(Asn374Lys) variants accelerated the recovery from fast inactivation compared with the wild-type. (**F**) Voltage-dependence of the major time constant of fast inactivation τ_h. Shown are means ± SEM (**B–F**). Numbers of recorded cells and statistical analysis for all experiments are provided in Supplementary Table 2.

action potentials were obtained. Neurons expressing p.(Asn374Lys) showed comparable Na$^+$ peak currents and action potential firing to wild-type channels (Fig. 3 and Supplementary Table 4).

In summary, p.(Phe846Ser), p.(Leu840Pro) and p.(Asn1877Ser) showed a GOF effect or increased neuronal firing; p.(Asn374Lys) showed mild GOF effects and did not alter neuronal firing; and p.(Val1758Ala), p.(Thr1787Pro) and p.(Ile1654Asn) showed LOF effects or decreased neuronal firing.

Genotype-phenotype correlations in gain- or loss-of-function variant carriers and extrapolation to the cohort

Clinical data combined with functional results of this and earlier studies enabled us to explore genotype-phenotype correlations in 170 affected individuals carrying a known LOF (n = 34) or GOF (n = 136) variant. Detailed phenotypic analysis in those affected individuals and a comparison with the whole cohort may allow the

extrapolation of genotype-phenotype correlations that could be valid for all individuals in which causative *SCN8A* variants have been detected. These phenotypic comparisons are provided below (Fig. 4).

Phenotypes of affected individuals with loss-of-function variants

Thirty-four affected individuals (12 previously unpublished) carried clear LOF variants that were deletions, splice-site, frameshift or stop-variants, or missense variants, with an LOF effect confirmed either in this or previous studies.[17–19,21] Twenty-three individuals (68%) had epilepsy. The median age at seizure onset was 24 months (range: 1 month to 14 years). Seizure types included absence (11/23), bilateral tonic-clonic (7/23) and myoclonic (5/23). Additionally, 4/23 had febrile seizures within the first year of life, all with later onset (>2 years of age) of afebrile seizure types. A phenotype of generalized epilepsy was found in 14/34 individuals,
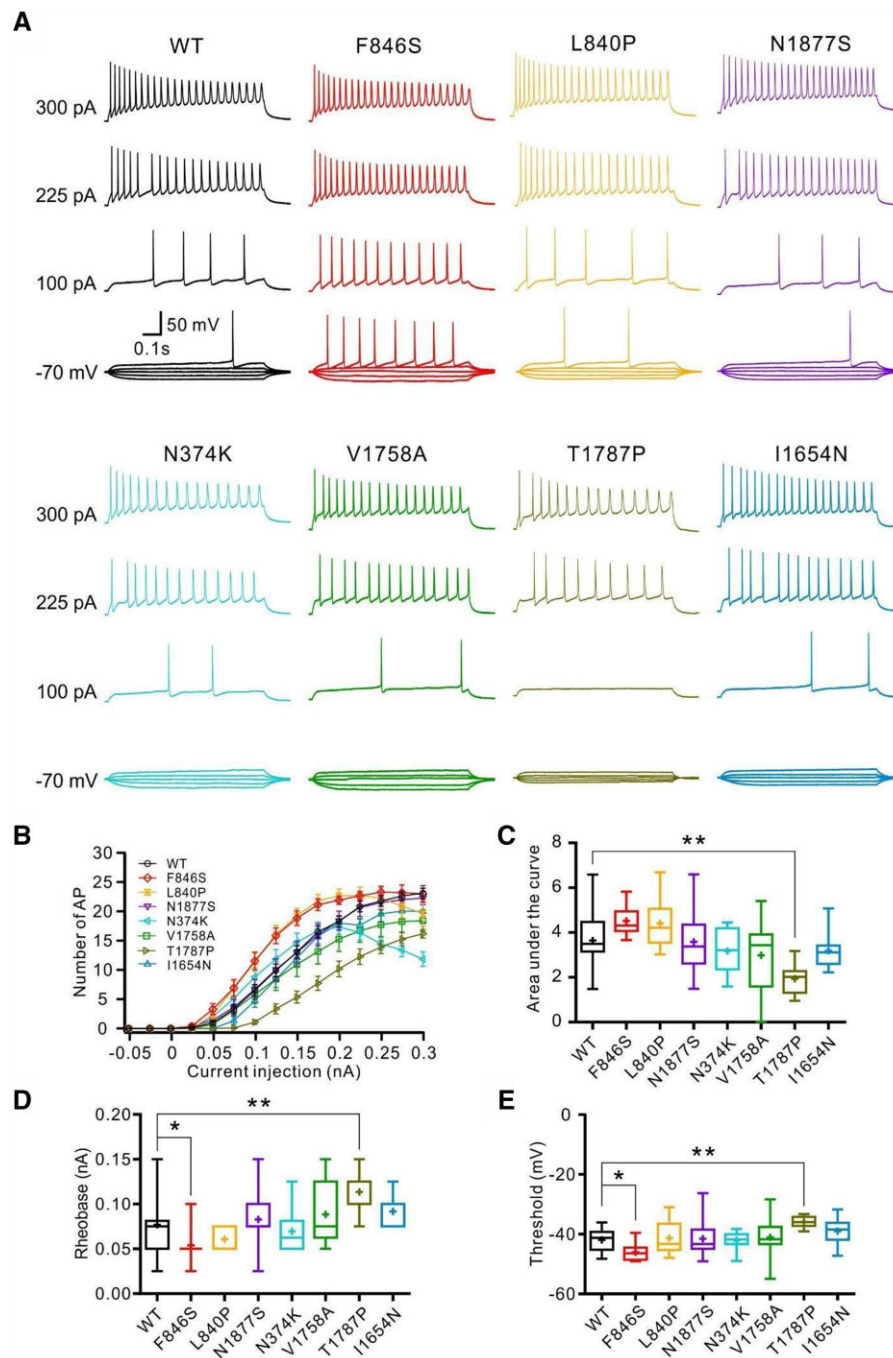
Figure 2 Effects of SCN8A variants in primary cultured hippocampal mouse neurons in the absence of tetrodotoxin. Neurons were transfected with wild-type or mutant Na$_v$1.6 channels and recorded in the absence of tetrodotoxin. (**A**) Representative voltage traces of evoked action potentials (APs) in the absence of tetrodotoxin from neurons transfected with wild-type (WT, black) or mutant neurons (colour code indicated in **B**). (**B**) Numbers of evoked action potentials plotted versus injected current in the absence of tetrodotoxin. Shown are means ± SEM. (**C**) Area under the curve for the input-output relationships. The p.(Thr1787Pro) variant shows a significantly decreased area under the curve compared with the wild-type channels. Rheobase (**D**) and threshold (**E**) of action potentials were decreased for neurons transfected with the p.(Phe846Ser) variant but increased for neurons transfected with the p.(Thr1787Pro) variant compared with the wild-type channels. Box-and-whisker plots (**C–E**) show means (plus sign), the 25th, 50th and 75th percentiles, minima and maxima; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; one-way ANOVA with Dunnett's *post hoc* test or ANOVA on ranks with Dunn's *post hoc* test were performed. Numbers of recorded cells and statistical analysis are provided in Supplementary Table 3.

11/34 had a NDDwoE, 3/34 had DEE and the remaining 6/34 had an unclassifiable epilepsy. EEGs showed background slowing, generalized spike-wave activity, slow rhythmic activity or remained normal.

Of 23 affected individuals with epilepsy, nine were seizure-free: one with a SCB (lamotrigine), seven with non-SCBs (valproate monotherapy in four affected individuals) and one on a combination (lamotrigine and topiramate). Out of four additional individuals who tried SCBs, one experienced a worsening of seizures with zonisamide, two showed no effect and one exhibited a reduction of seizures with lamotrigine.

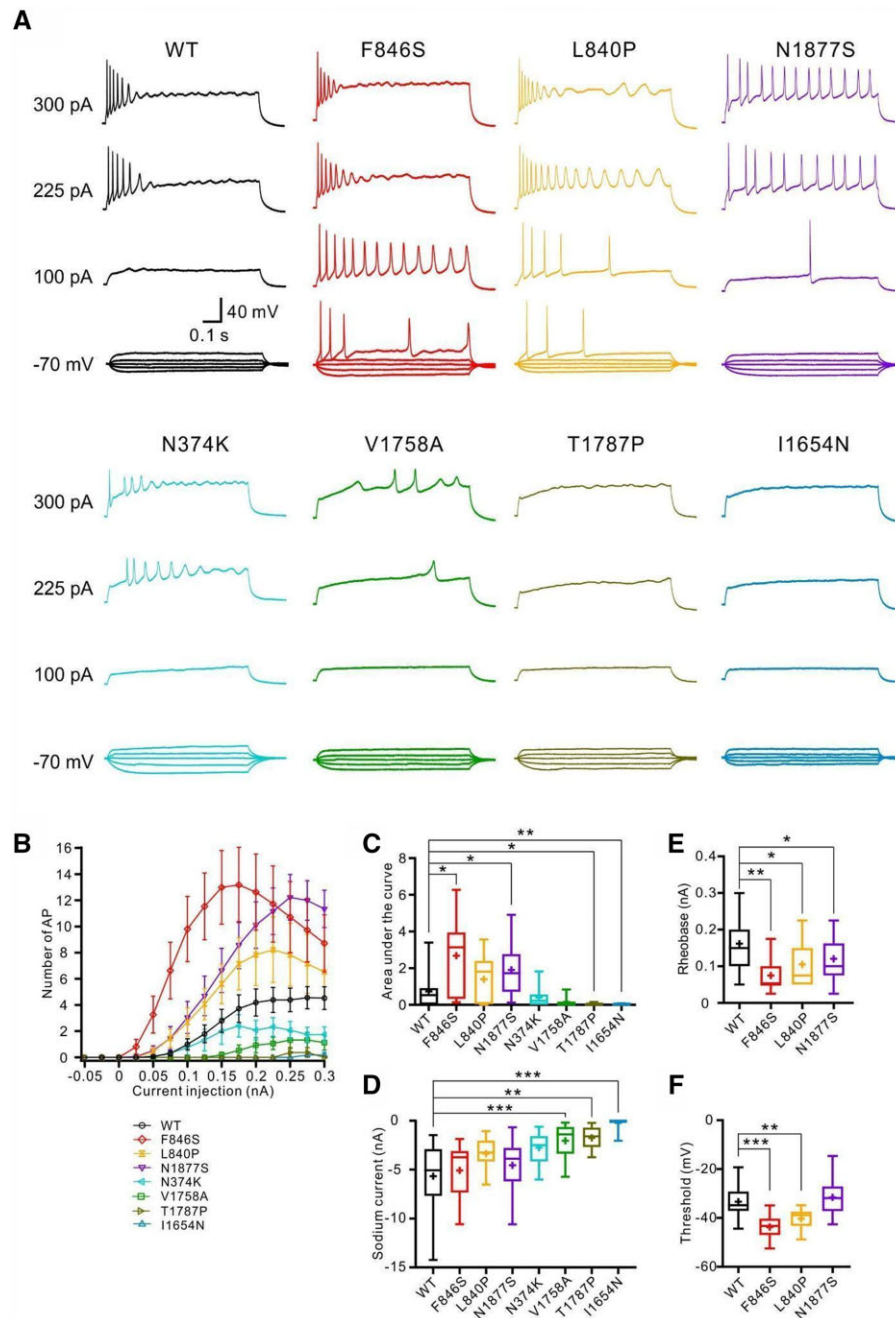None of the individuals with LOF variants died prematurely.

Figure 3 Neuronal properties carried only by transfected wild-type or mutant Na$_v$1.6 channels. Hippocampal neurons transfected with wild-type or mutant Na$_v$1.6 channels were recorded in the presence of tetrodotoxin to block endogenous Na$^+$ channels. (**A**) Representative voltage traces of evoked action potentials (APs) from neurons transfected with wild-type (black) or mutant channels (colour code in the *bottom left*). (**B**) Numbers of evoked action potentials plotted versus injected current in the presence of tetrodotoxin. Shown are means ± SEM. (**C**) Area under the curve for the in-put-output relationships. (**D**) Peak Na$^+$ current amplitudes of neurons transfected with wild-type or mutant Na$_v$1.6 channels in the presence of tetro-dotoxin. Rheobase (**E**) and threshold (**F**) were significantly decreased in neurons transfected with p.(Phe846Ser) or p.(Leu840Pro) variants compared with the wild-type channels. The p.(Asn1877Ser) variant also significantly decreased the rheobase. The rheobase or threshold could not be obtained in neurons transfected with p.(Asn374Lys), p.(Val1758Ala), p.(Thr1787Pro) and p.(Ile 1654Asn) mutant channels due to very few evoked action potentials. Box-and-whisker plots (**C–F**) show means (plus symbol), the 25th, 50th and 75th percentiles, minima and maxima; *P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA with Dunnett's *post hoc* test or ANOVA on ranks with Dunn's *post hoc* test were performed. The numbers of recorded cells and statistical analysis are provided in Supplementary Table 4.

When we compared this presentation of clear LOF variant carriers with the phenotypes of the whole cohort of affected individuals, there was a clear correlation to two groups: one with generalized epilepsy and one with NDDwoE. The only exceptions were three affected individuals with DEE in the LOF group. The main phenotypic features of these groups, including analyses of the age at seizure onset (except febrile seizures) and seizure types, are summarized and contrasted to the groups with GOF variants in Fig. 4 and Table 1.

Phenotypes of affected individuals with gain-of-function variants

One hundred and thirty-six affected individuals from the total cohort (29 unpublished) carried missense variants that were shown to cause GOF.[1,6,17,19,29,30] All 136 patients suffered from epilepsy. The most prevalent seizure types were focal (32%), focal tonic (31%) or bilateral tonic-clonic seizures (43%). Febrile seizures were seen in 4% of the individuals. The median age at seizure onset was 4 months (range: first day of life to 45 months). Half of the affected individuals (54%) were diagnosed with DEE, followed by unclassifiable epilepsy in 29%, intermediate epilepsy in 10% and BFIE in 7%. Individuals with BFIE had either no interictal epileptiform abnormalities or rare diffuse spike and wave complexes. Individuals with intermediate epilepsy had heterogeneous EEG features with trains of beta and delta activity and focal spike and slow waves, bilaterally in the parieto-occipital regions, with or without diffuse spreading. Most individuals with severe DEE had background slowing, polymorphic delta and beta activity and multifocal spike and slow waves, predominant in the posterior quadrants.

Treatment data were available for 84 affected individuals. Twenty-six of the 84 affected individuals were seizure-free; 16 with SCBs. Seizure reduction was seen in 47 affected individuals, 18 with SCBs. Two affected individuals showed worsening of seizures with SCBs; both affected individuals were resistant to several ASMs and had increased seizure frequency with oxcarbazepine and levetiracetam.[31] Eight affected individuals did not try SCB treatment: two had a mild phenotype and were seizure free with valproate or valproate + levetiracetam,[5,32] and one affected individual also had a mild phenotype and seizure reduction with valproate.[33] The remaining five affected individuals all had pharmacoresistant seizures; it is unknown why SCBs were not tried.

Twelve affected individuals were prematurely deceased (9%), all with a DEE phenotype; 10 deaths were due to a general worsening in the patients' overall and neurological condition followed by organ failure, and two were due to definite or probable sudden unexpected death in epilepsy (SUDEP) (1.5%).

As in the LOF group, there was a clear correlation in the GOF group to specific phenotypes in the whole cohort, namely BFIE, intermediate epilepsy and DEE. None of the GOF variant carriers had generalized epilepsy or NDDwoE.

A summary of these data is provided in Fig. 4, Table 1 and Supplementary Table 5. The obvious difference in the age of onset was statistically significant between the GOF and LOF variant carriers (Mann-Whitney test, $P < 0.001$) and between BFIE/intermediate epilepsy/DEE and generalized epilepsy (Kruskal-Wallis test, $P < 0.001$). Statistical analysis also confirmed that affected individuals with LOF variants mostly had a generalized epilepsy, whereas affected individuals with GOF had one of the focal epilepsy phenotypes (BFIE, intermediate epilepsy or DEE; Fisher's exact test, $P < 0.001$).

Correlation of clinical and electrophysiological phenotypes in the gain-of-function group

We scored all GOF variants according to their functional characterizations from this and previous studies, including a total of 16 variants. The LOF variants were not included because of a large proportion of deleterious variants, which lead to non-functional proteins that cannot be well differentiated. The electrophysiological score was determined by the effect of biophysical changes of variants on neuronal firing simulated by a single-compartment conductance-based model (see the 'Materials and methods' section and Supplementary material). This analysis revealed that (i)

alterations of activation properties severely affect neuronal firing; (ii) changes in the sodium conductivity have a moderate effect; and (iii) changes of inactivation properties and the persistent current affect neuronal firing mildly (Fig. 5A–F and Supplementary Table 5).

The electrophysiological scores of GOF variants significantly correlated with the clinical severity of affected individuals carrying these variants (Fig. 5G; $P = 0.0079$, $r = 0.64$), which were previously classified into BFIE, intermediate epilepsy, DEE with mild/moderate ID and DEE with severe ID (scored as 1, 2, 3 and 4, respectively). Variants causing different phenotypes were averaged over this score (see the 'Materials and methods' section). In a separate analysis, we also compared the individual severity of each affected individual with the electrophysiological score of the variants. The scores in the groups with DEE are significantly higher than the ones in BFIE and intermediate epilepsy, but there is no significant difference between BFIE and intermediate epilepsy or DEE with mild/moderate ID and DEE with severe ID (Fig. 5H).

Genetic landscape of SCN8A variants

We analysed 256 different variants in 392 individuals. Missense variants ($n = 233$) accounted for the majority of disease-causing variants (91%). Nineteen variants were deleterious frameshift, stop-gain or canonical splice-site variants. Seven were inframe indels or deletions. Three patients had biallelic missense variants.[34] Sixty-one recurring missense variants were found in 244 affected individuals. Two hundred and ninety-seven variants occurred *de novo*, 31 were inherited from an affected or unaffected mosaic parent, and for 64 segregation was unknown.

The distribution of variants across the Na$_v$1.6 channel protein is shown in Fig. 6. Most GOF variants and also variants causing focal epilepsy (BFIE/intermediate epilepsy/DEE) without functional data are concentrated at the cytoplasmic side and in the voltage sensors (protein regions given as codon positions: 212–263, 398–418, 838–883, 966–986, 1283–1336, 1450–1523, 1602–1660 and 1753–1775), whereas most LOF variants were found in the pore region (protein regions: 274–408, 893–976, 1347–1460 and 1669–1765). The region-specific distribution of GOF and BFIE/intermediate epilepsy/DEE variants in comparison to the distribution of LOF and generalized epilepsy/NDDwoE variants is highly significant (Fisher's exact P-value < 0.00001). This is also illustrated in a 3D structural model which additionally reveals that population variants taken from the gnomAD database are located in different regions. Most disease-causing variants were located in functionally important and evolutionary conserved regions of the channel, whereas variants from the gnomAD database are in less conserved regions [Supplementary Fig. 1 and Supplementary Video 1 (the video is available to view at Figshare: https://doi.org/10.6084/m9.figshare.15141018)].

Frequency of SCN8A-related disorders in the Danish population

From the year 2006 to 2017, an average of 60 934 children were born per year. During the same period, 13 affected individuals were diagnosed with an SCN8A-related disorder in Denmark, yielding an estimated frequency of 1/56 247.

Evaluation of treatment effects

Treatment responses to specific ASMs were grouped into four categories—seizure-free, seizure reduction, no change and worsening—as described in the methods section. We differentiated between SCBs and non-SCBs, in LOF and GOF variant carriers,
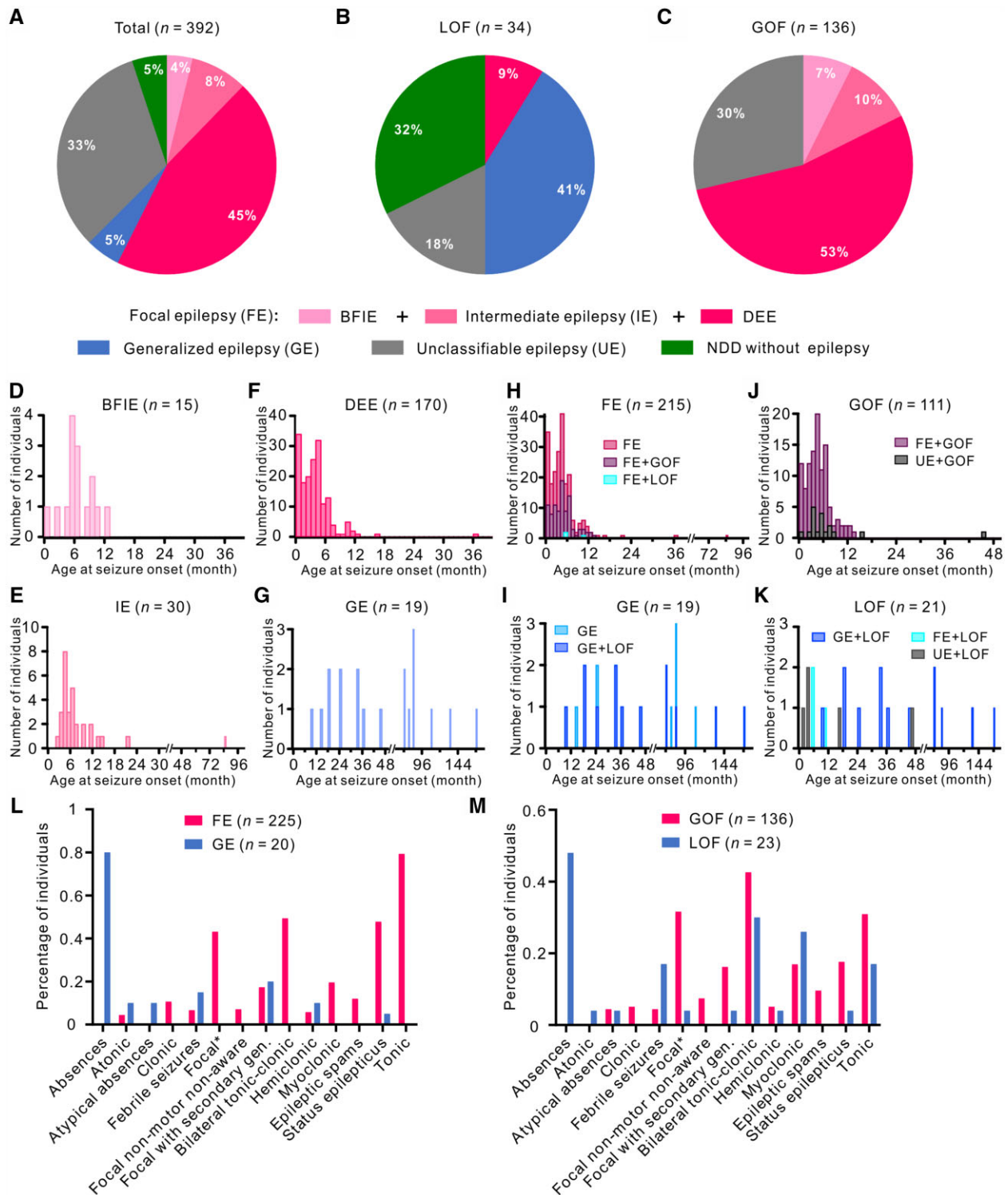
Figure 4 Distribution of affected individuals carrying SCN8A variants according to phenotype and age of seizure onset. Phenotypic subgroups of the total cohort (**A**) and individuals carrying LOF (**B**) or GOF (**C**) variants. (**A**) In the total cohort, 225 affected individuals (57.4%) had BFIE, intermediate epilepsy or DEE; 20 individuals (5.1%) had generalized epilepsy; and 20 individuals (5.1%) had a NDDwoE. (**B**) Twenty-five affected individuals had generalized epilepsy or an NDDwoE, accounting for 73.5% of LOF variant carriers, whereas 71.6% of GOF variant carriers had BFIE, intermediate epilepsy or DEE (**C**). Histogram of the age at seizure onset in affected individuals with BFIE (**D**), intermediate epilepsy (**E**), DEE (**F**) and generalized epilepsy (**G**). (**H**) Stacked histogram of age at seizure onset in affected individuals with focal epilepsy (FE) carrying GOF (FE + GOF), LOF (FE + LOF) or *SCN8A* variants which are not functionally characterized. (**I**) Stacked histogram of age at seizure onset in affected individuals with generalized epilepsy (GE) carrying LOF (GE + LOF) or *SCN8A* variants which are not functionally characterized. (**J**) Stacked histogram of age at seizure onset in affected individuals carrying GOF variants with FE (FE + GOF) or unclassifiable epilepsy (UE + GOF). (**K**) Stacked histogram of age at seizure onset in affected individuals carrying LOF variants with generalized epilepsy (GE + LOF), FE (FE + LOF) or unclassifiable epilepsy (UE + GOF). Affected individuals with BNIE, intermediate epilepsy or DEE exhibited a significant earlier age at seizure onset than individuals with generalized epilepsy (Kruskal-Wallis test, $P < 0.001$), which is also observed for GOF versus LOF variant carriers (Mann-Whitney test, $P < 0.001$). Histogram bin size = 1 month. Seizure type distributions of affected individuals with FE versus generalized epilepsy (**L**) and GOF versus LOF variant carriers (**M**).

Table 1 Clinical characteristics of LOF and GOF variants and phenotypic subgroups

	BFIE	IE	DEE	GE	NDDwoE	LOF	GOF
n	15	33	177	20	20	34	136
Percentage with epilepsy	100%	100%	100%	100%	0%	68%	100%
Median age at seizure onset	6 months	5 months	3 months	42 months	–	24 months	4 months
Most common seizure types	Focal, focal to bilateral TC and bilateral TC	Focal, bilateral TC and tonic	Bilateral TC, focal and tonic	Absences, bilateral TC and febrile seizures	–	Absence seizures, bilateral TC and myoclonic seizures	Bilateral TC, tonic and focal seizures
Phenotype subgroups	–	–	–	–	–	GE 41% NDDwoE 32% DEE 9% UE 18%	BFIE 7% IE 10% DEE 53% UE 30%
Cognition	Normal 100%	Normal 33% Mild ID 52% Moderate ID 15%	Moderate ID 22% Severe ID 73% Unknown 5%	Normal 20% Mild ID 30% Moderate ID 35% Severe ID 10% Unknown 5%	Normal 10% Mild ID 45% Moderate ID 20% Severe ID 15% Unknown 10%	Normal 15% Mild ID 36% Moderate ID 15% Severe ID 21% Unknown 15%	Normal 11% Mild ID 9% Moderate ID 15% Severe ID 39% Unknown 26%
Comorbidities	Paroxysmal kinesigenic dyskinesia	Language delay, behavioural issues	Hypotonia, CVI, ataxia	Language delay, behavioural issues	Behavioural disorders (ASD, ADHD), delayed speech, microcephaly	Language delay, ASD, behavioural issues, ataxia	Hypotonia, CVI, dyskinesia, ataxia
Mortality	0%	0%	10.2%	0%	0%	0%	9.0%
Precision medicine	SCBs	SCBs	SCBs				SCBs

ADHD = attention-deficit hyperactivity disorder; bilateral TCs = bilateral tonic-clonic seizures; CVI = cortical vision impairment; GE = generalized epilepsy; IE = intermediate epilepsy; UE = unclassifiable epilepsy.

generalized epilepsy and focal epilepsies (BFIE/intermediate epilepsy/DEE). The results are shown in Fig. 7. GOF variant carriers responded better to SCBs than to non-SCBs (Fisher's exact test, $P < 0.001$), whereas there was no difference between treatment with SCBs and non-SCBs for LOF variant carriers (numbers very small, Fisher's exact test, $P = 0.48$). Similar results were obtained for the treatment with SCBs versus non-SCBs in affected individuals with focal epilepsy versus those with generalized epilepsy.

Discussion

Our study provides a detailed analysis of the correlation between clinical phenotypes, genotypes and electrophysiological characterizations, suggesting the following four main conclusions:

(i) There are five main phenotypes in SCN8A-related disorders in this largest series of 392 individuals collected to date: BFIE, intermediate epilepsy, DEE, generalized epilepsy and NDDwoE.

(ii) There is a significant correlation between GOF variants causing focal epilepsy of different/increasing severity (BFIE, intermediate epilepsy, DEE) and LOF variants causing generalized epilepsy or NDDwoE, allowing an extrapolation from the clinical phenotypes of clear GOF and LOF variant carriers to the whole cohort. There was only one exception from this rule: three DEE affected individuals with onset between 5 and 10 months carrying stop-gain or canonical splice-site (hence, predicted LOF) variants (see below for exact phenotypes).

(iii) The clinical severity of epilepsies associated with GOF variants is at least partially determined by the degree of the electrophysiological dysfunction, as there was a significant correlation between the clinical and electrophysiological phenotypes using a new system to estimate the influence of different gating parameters on neuronal firing.

(iv) As previously suggested in smaller studies, seizures in individuals with disease-causing GOF variants respond better to SCBs than to other ASMs.

Functional studies

Altogether, we examined 14 variants (this study and previously[19]) covering the whole clinical spectrum of SCN8A-related phenotypes. Using both neuroblastoma cells to study the effects of the mutant channels on gating properties and murine primary neuronal cultures to study the effects on intrinsic neuronal properties and firing, we found mild GOF effects or an increase in neuronal firing to be associated with BFIE or intermediate epilepsy [p.(Gly1475Arg), p.(Glu1483Lys) and p.(Asn1877Ser)], whereas more severe gating defects, particularly strong hyperpolarizing shifts of steady-state activation that also on average led to a stronger increase in neuronal firing, were associated with DEE [p.(Arg223Gly), p.(Leu840Pro), p.(Phe846Ser), p.(Met1760Ile) and p.(Arg1872Trp)]. One previously published variant, p.(Arg223Gly), was initially suggested to cause a predominant LOF[11] and had a phenotype of spasms with onset at 6 months, severe ID and an epileptic encephalopathy. However, there were also GOF features identified, and when we retested this variant in our system, GOF effects predominated and it increased neuronal firing (Liu et al., unpublished results). When we used a one-compartment neuronal model system, we were able to weight the gating defects of all GOF variants that were functionally investigated to date according to their effect on neuronal firing, revealing a significant correlation between the severity of the clinical and electrophysiological phenotypes. Such a correlation was not observed when we used a different scoring system (data not shown) based only on the degree of the electrophysiological dysfunction (i.e. without weighting the effects on neuronal firing), which, however, worked well previously to correlate clinical and electrophysiological phenotypic severity in SCN2A-related epilepsy.[35]
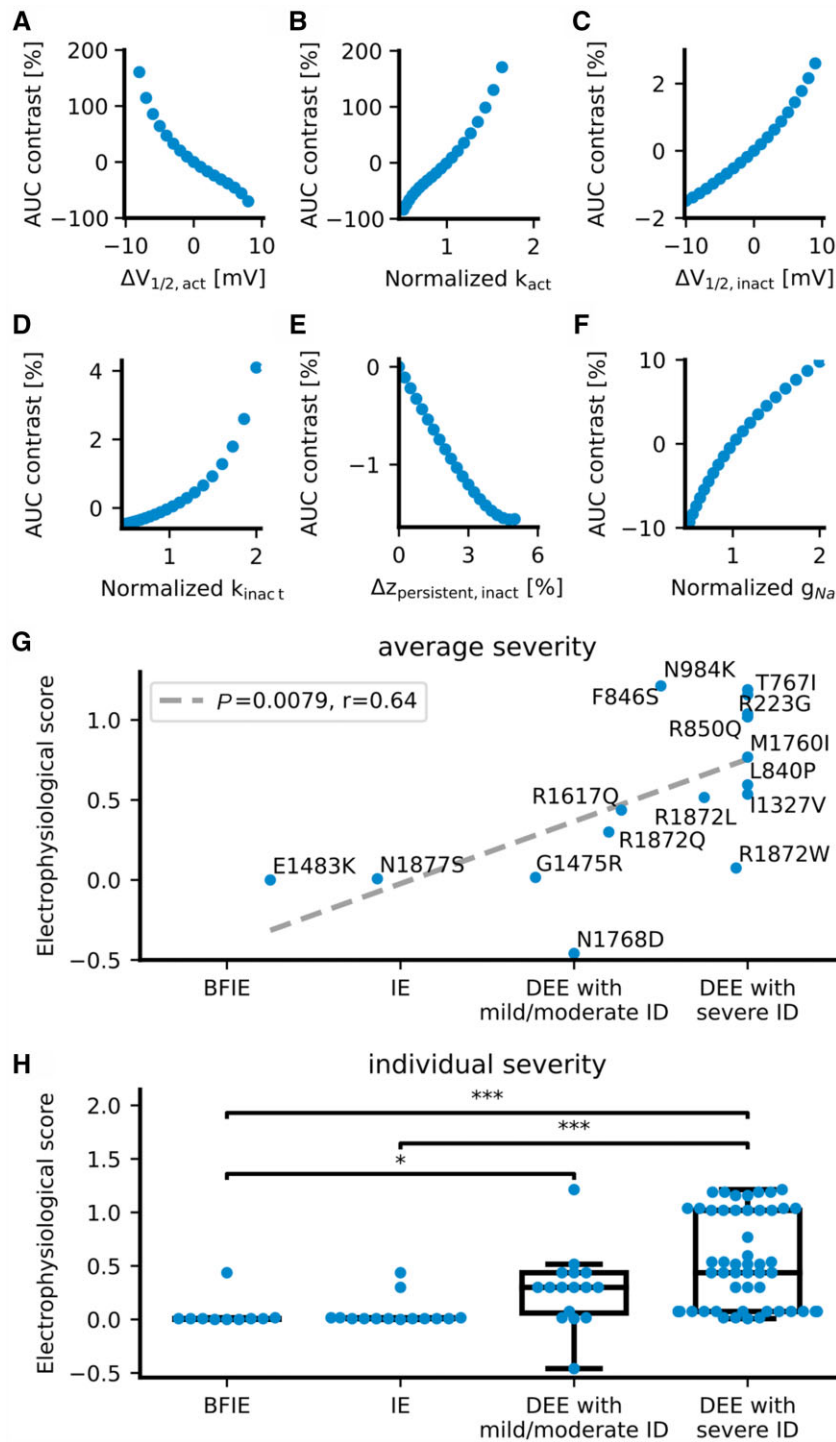
Figure 5 Correlation of a computed electrophysiological score with the clinical severity of *SCN8A* GOF variants. Electrophysiological scores of *SCN8A* GOF variants were obtained according to the effect of variants on action potential firing simulated by a single-compartment conductance-based model. (**A–F**) The contrast of the simulated area under the input-output curve (AUC, Equation 1) as a function of the changes in single Na$^+$ current gating parameters, such as: (**A**) the $V_{1/2}$ of the activation curve; (**B**) the slope factor k of the activation curve; (**C**) the $V_{1/2}$ of the fast inactivation curve; (**D**) the slope factor k of the fast inactivation curve; (**E**) the persistent Na$^+$ current; and (**F**) the Na$^+$ conductivity (or current density). Changes in the $V_{1/2}$ and the slope of the activation curve had a much stronger effect on the AUC than other parameters. (**G**) Correlation (dashed grey line) of the simulation-based score (Equation 9) with the severity of each *SCN8A* GOF variant averaged over affected individuals (blue dots with the respective one-amino acid code). (**H**) Distributions of simulation-based scores of each patient (blue dots) for each of the four categories of clinical severities. $^*P < 0.05$; $^{***}P < 0.001$ (ANOVA on ranks with Dunn's *post hoc* test).

In contrast, the p.(Ile1654Asn), p.(Thr1787Pro) and p.(Val1758Ala) variants, all causing generalized epilepsy with absence seizures as a new recurring phenotype, showed an LOF in neuroblastoma cells or decreased neuronal firing. Also, variants not causing epilepsy either caused a clear LOF and decreased neuronal firing [p.(Gly964Arg) and p.(Arg1620Leu)] or a strong GOF on channel gating leading to a depolarization block, i.e. LOF on a neuronal level [p.(Ala1622Asp)].[19] Additional variants characterized by other groups confirm these
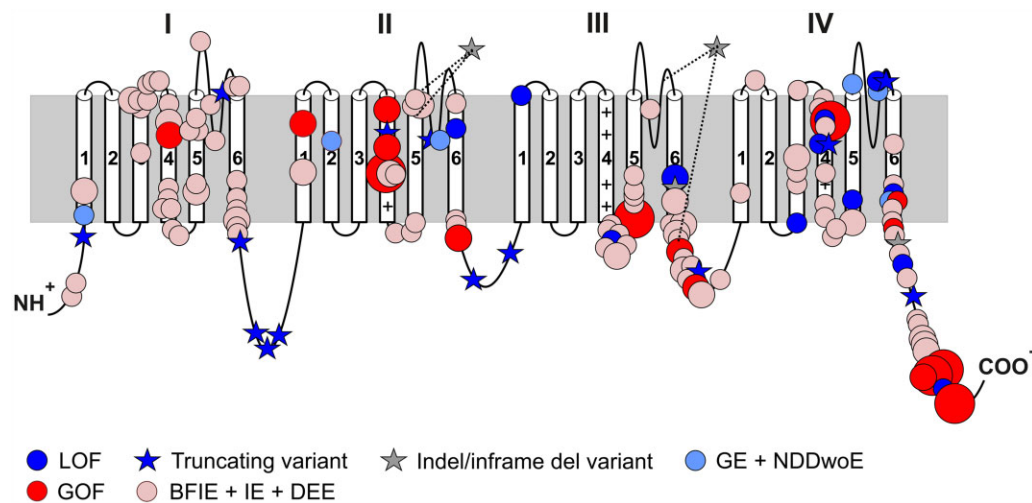
Figure 6 Location of SCN8A variants associated with neurodevelopmental disorders. Schematic 2D representation of the Na$_v$1.6 channel displaying the location of pathogenic variants. A comparison of the location of missense variants with proven GOF or a BFIE/intermediate epilepsy/DEE phenotype (without functional analysis) on one hand, and variants with proven LOF or generalized epilepsy/NDDwoE phenotypes (without functional analysis) on the other, revealed a significant difference in the distribution of variants (see main text and Supplementary material). Recurring variants are indicated with larger symbol size relative to the number of patients and in frame indels or deletions are indicated showing the whole affected regions [p.(Ile888_Val892delinsMet), p.(Glu1774_Ala1777del) and p.(Pro1428_Lys1473del)].

results. Many other variants causing GOF effects mainly on channel gating (only few studies with limited results on neuronal firing) were clearly associated with focal epilepsies (Supplementary Table 1).

The p.(Ala1319Thr) variant caused a depolarizing shift of the activation curve of Na$^+$ current in *X. laevis* oocytes[36,37] and decreased firing of cerebellar Purkinje cells,[38,39] indicating an LOF. The *Scn8a* Med^{jo} mouse carrying the corresponding variant in the mouse channel exhibited absence epilepsy revealed by slow-wave discharges in EEG recordings associated with absence-like seizures,[40] reproducing the clinical phenotype found in the affected individual carrying the p.(Ala1319Thr) variant. Other epilepsy-related LOF variants were also associated with absence epilepsy, such as p.(Asn544fs*39) and p.(Glu587*).[5,31] In another conditional mouse model, a complete *Scn8a* LOF restricted to inhibitory neurons of the thalamic reticular nucleus also caused absence-like epilepsy with generalized slow-wave discharges.[40,41] It is therefore intriguing to speculate that *SCN8A* variants cause absences and other generalized seizures due to an LOF in inhibitory neurons, whereas the GOF effects are more important in excitatory neurons, as discussed in more detail below.

The p.(Asn374Lys) variant showed only very mild GOF gating defects but did not change parameters in neurons. The phenotype with late onset focal epilepsy at 7 years without other neuropsychiatric symptoms also does not fit the five main categories. Thus, while we cannot exclude a mild contribution of this variant to seizures in this affected individual, we consider it to be a variant of unknown significance.

Phenotype correlations in gain- versus loss-of-function variant carriers

When we compared the clinical phenotypes of the 136 confirmed GOF and 34 confirmed LOF variant carriers with the whole group of 392 individuals, there were strikingly congruent phenotypes, which strongly suggested that BFIE, intermediate epilepsy and DEE are generally caused by GOF, while generalized epilepsy and NDDwoE are caused by LOF variants. Not only did the seizure types resemble each other in those groups, but also an analysis of the age of onset of seizures strongly confirmed this hypothesis (Table 1 and Fig. 4). Only three cases with presumed DEE and an LOF variant did not fit into this pattern. One patient carried a p.(Met1481Ilefs*12) variant with onset of pharmaco-resistant tonic and bilateral tonic-clonic seizures and episodes of status epilepticus at 5 months of age, severe ID, no speech and poor eye contact. Two patients with seizure onset at 5 or 10 months carried a splice-site variant predicting exon skipping to cause a large in-frame deletion cutting out the whole pore region of domain III, p.(Pro1428_Lys1473del; Fig. 6). They had severe ID, no speech, poor eye contact and hypotonia. EEG showed multifocal epileptiform discharges. Thus, these single cases could not be differentiated from the DEE cohort caused by GOF variants. The epilepsy phenotype of two further patients with similar splice-site variants was unclassifiable. Alternative consequences on the Na$_v$1.6 protein other than those predicted here [p.(Pro1428_Lys1473del) and p.(Met148Ilefs*12)], e.g. resulting from activation of cryptic splice sites, cannot be ruled out completely.

In a previous study on *SCN2A*,[42] we demonstrated that different neonatal and infantile epilepsy syndromes were caused by GOF variants, whereas later onset seizures were due to an LOF of Na$_v$1.2. In *SCN2A*, the boundary for the onset of seizures between GOF and LOF was very early—approximately 3 months of age.[42] In the present study, the onset in both groups was later (median age at onset of 4 months in the GOF subgroup and 24 months in the LOF subgroup), and the ranges were more overlapping. This difference may reflect the differential developmental expression of *SCN2A* versus *SCN8A*, as *SCN2A* is expressed neonatally and earlier than *SCN8A*.[43,44] An additional difference is that *SCN2A* variant carriers have better defined electroclinical epilepsy syndromes. Both groups share a phenotype of BFNIE/BFIE, with a later onset in *SCN8A*-related epilepsy. Some individuals carrying *SCN8A* variants develop paroxysmal kinesigenic dyskinesia,[3] whereas *SCN2A* variant carriers may develop a form of later-onset episodic ataxia.[45] There were also differences regarding the syndromes of LOF variant carriers in both genes. Generalized epilepsy was not generally observed in *SCN2A*, although absence epilepsy has been reported in mice with complete LOF of *Scn2a* when it has been knocked out in excitatory neurons.[46] Instead, West syndrome was a common phenotype in human *SCN2A*, but phenotypes with generalized seizures and EEG abnormalities were also found in the LOF group
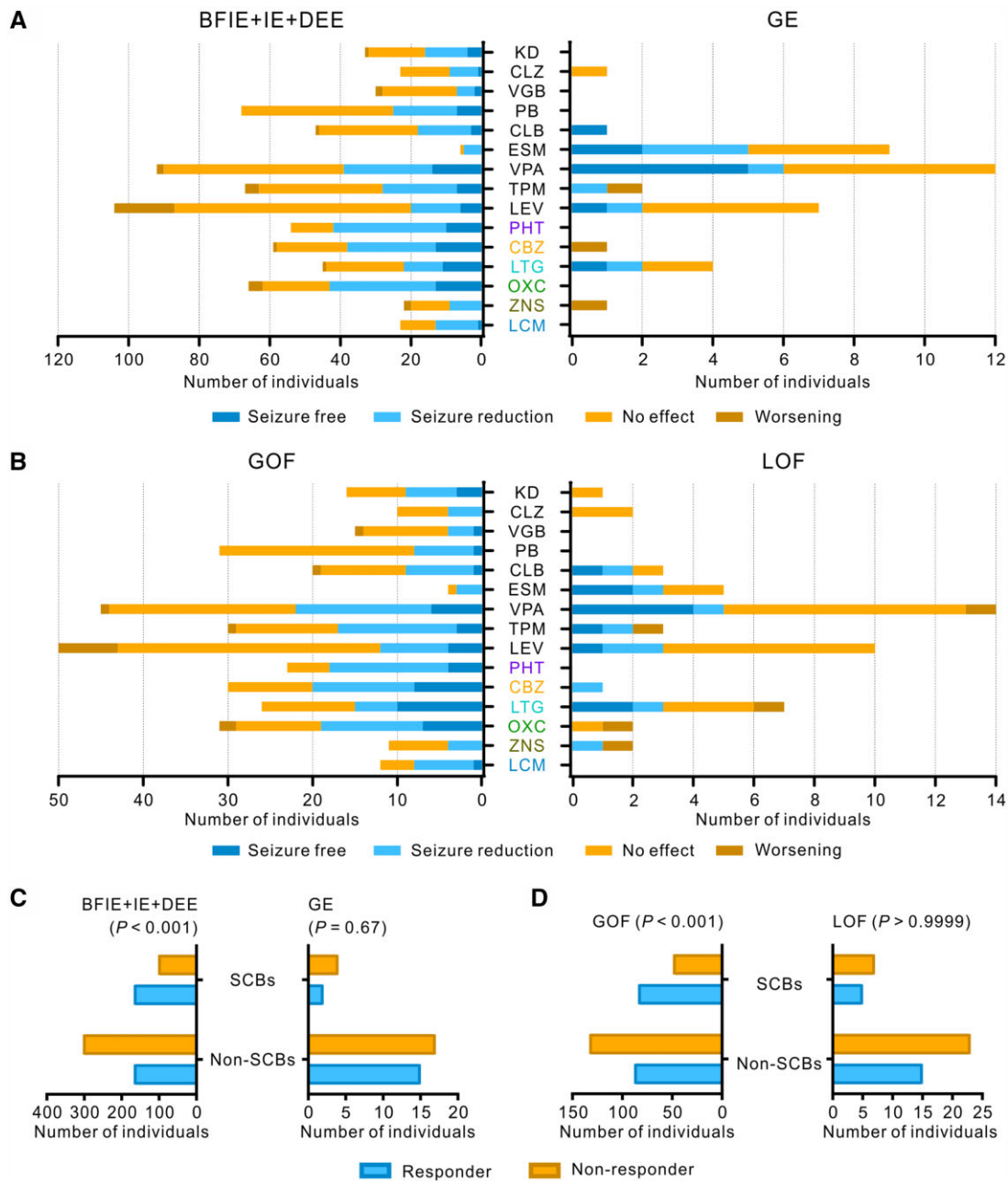
Figure 7 Treatment responses to anti-seizure medications in affected individuals carrying *SCN8A* variants. Treatment effects of ASMs on seizures in affected individuals with BFIE, intermediate epilepsy or DEE versus those with generalized epilepsy (**A**) and those carrying *SCN8A* GOF versus LOF variants (**B**). Phenytoin, carbamazepine, lamotrigine, oxcarbazepine, zonisamide and lacosamide are SCBs. (**C** and **D**) Individuals with BFIE, intermediate epilepsy or DEE and those carrying *SCN8A* GOF variants responded significantly better to SCBs than non-SCBs, whereas treatment with SCBs or non-SCBs did not cause a different effect in individuals with generalized epilepsy and those carrying *SCN8A* LOF variants (please consider the very small numbers in these latter categories). *P* values derived from Fisher's exact test are provided in the figure. Responders were defined as those who became seizure-free or experienced a seizure reduction while staying on the drug; non-responders were defined as those experiencing no effect or seizure worsening. CBZ = carbamazepine; CLB = clobazam; CLZ = clonazepam; ESM = ethosuximide; KD = ketogenic diet; LCM = lacosamide; LEV = levetiracetam; LTG = lamotrigine; OXC= oxcarbazepine; PB = phenobarbital; PHT = phenytoin; TPM = topiramate; VGB = vigabatrin; VPA = valproate; ZNS = zonisamide.

(epilepsy with myoclonic-atonic seizures or Lennox-Gastaut syndrome). For *SCN8A*, the phenotypes were rather unspecific generalized epilepsy, mainly with absence seizures. However, we found that an LOF subgroup had a phenotype resembling *SCN1A* LOF, with febrile seizures preceding additional seizure types, resembling a generalized/genetic epilepsy with febrile seizure plus (GEFS +) phenotype. *SCN8A* is expressed in both excitatory and inhibitory neurons,[47] which may well explain the phenotypic

similarity between some of the *SCN1A*- and *SCN8A*-related phenotypes, since *SCN1A* is considered to be the main sodium channel in inhibitory GABAergic neurons.[48,49] Additionally, it has been found that knockout of *Scn8a* in mice decreases cortical excitability, resulting in convulsive seizure protection.[40] Furthermore, mice with global reduction of *Scn8a* have fewer seizures compared with mice that exhibit *Scn8a* deletion only in inhibitory neurons.[41] In contrast, mice carrying a GOF *Scn8a* variant [p.(Arg1872Trp)]

exhibited convulsive seizures and premature death; however, activation of this variant only in inhibitory neurons did not induce seizures or overt neurological dysfunctions.[20] Altogether, *SCN8A* GOF variants may mainly cause hyperexcitation in excitatory neurons leading to tonic-clonic and focal seizures, whereas *SCN8A* LOF variants mainly induce hypoexcitation in inhibitory neurons, causing generalized and, particularly, absence seizures. Further studies may elucidate the distinct roles of *SCN8A* and the other sodium channel genes in different neuronal circuits.

Genetic landscape

The vast majority of the detected variants were missense, and we found several recurrent variants. Those detected in more than 10 affected individuals included: p.(Arg850Gln/Gly), seen in 12 affected individuals; p.(Gly1475Arg), seen in 19 affected individuals; p.(Arg1617Trp/Gln/Leu), seen in 18 affected individuals; p.(Arg1872Trp/Gln/Leu), seen in 41 affected individuals; and p.(Asn1877Ser), seen in 23 affected individuals. All of these variants cause a clear GOF. Why some of these variants, especially p.(Gly1475Arg) and p.(Arg1617Trp/Gln/Leu), have a high phenotypic variability and others not, remains to be elucidated. Other genetic or environmental factors might play an important role. Additionally, we found families with intra-familial variability, such as the family of Proband 377, whose mother and two older siblings had BFIE and were seizure-free with normal intellect, whereas the proband suffered from pharmacoresistant DEE.

Estimated frequency

We found the frequency of *SCN8A*-related disorders in the Danish population to be 1/56 247, which is higher than for *SCN2A* (1/78 608),[42] but lower than for *SCN1A* (1/22 000).[50] Since these three genes are of similar size and homology, we would assume a similar variant frequency. Large-scale genetic testing in isolated ASD cohorts have failed to detect large numbers of *SCN8A*-affected individuals,[51] suggesting that a large number of undiagnosed *SCN8A*-ASD affected individuals does not exist and that this is not the cause behind the discrepancy in numbers. It could be that some individuals with truncating/LOF variants are too mildly affected (learning disabilities, mild ID etc.) to be candidates for genetic testing but are also not healthy enough to be part of control populations (such as blood donors), as reflected in the almost complete absence of truncating variants in gnomAD (pLI score: 1). Intrauterine death in *SCN8A*-related disease could be a third cause, which has not been investigated to date.

Treatment implications

In general, and as expected, affected individuals with GOF effects showed an overall positive response to treatment with SCBs, as has been hypothesized previously.[22] However, many of the affected individuals with DEE still have pharmacoresistant epilepsy (81%), underlining the severity of *SCN8A*-related epilepsy. A recent study evaluated the effect of a novel SCB: GS967, which primarily targets the elevated persistent currents without affecting peak currents, as well as having increased potency compared with phenytoin.[52] Long-term treatment with GS967 was shown to protect *Scn8a*[N1768/+] mice against premature death and also alleviated seizure burden.[52] GS967/PRAX-330 (https://adisinsight.springer.com/drugs/800050600) and another new drug—XEN901 (https://clinicaltrials.gov/ct2/show/NCT03467100) specifically targeting *SCN8A* with the goal of rectifying the effects of GOF variants—are currently in phase I clinical trials. Furthermore, antisense oligonucleotide therapy was recently shown to prolong survival in *Scn8a*[R1872W/+] (GOF) mice.[53] These novel compounds provide promising advances for precision medicine in *SCN8A*-affected individuals. As

functional testing is not yet readily available for all affected individuals, it will be important to have clinical markers that may predict the underlying functional effect, when specific blockers of the Na$_v$1.6 channel become available on the market. Our study provides an important contribution in this direction, as genotype-phenotype correlations revealed a clearly differential pattern for both GOF and LOF variants with very few exceptions.

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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