Homozygous *SCN1B* variants causing early infantile epileptic encephalopathy 52 affect voltage-gated sodium channel function.

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Summary

We identified nine patients from four unrelated families harboring three biallelic variants in *SCN1B* (NM_001037.5: c.136C>T; p.(Arg46Cys), c.178C>T; p.(Arg60Cys), and c.472G>A; p.(Val158Met)). All subjects presented with early infantile epileptic encephalopathy 52 (EIEE52), a rare, severe developmental and epileptic encephalopathy featuring infantile-onset refractory seizures followed by developmental stagnation or regression. Since *SCN1B* influences neuronal excitability through modulation of voltage-gated sodium (Na_V) channel function, we examined the effects of human *SCN1B*^{R46C} (*β1*^{R46C}), *SCN1B*^{R60C} (*β1*^{R60C}), and *SCN1B*^{V158M} (*β1*^{V158M}) on the three predominant brain Na_V channel subtypes $Na_V1.1$, $Na_V1.2$ and $Na_V1.6$. We observed a shift towards more depolarizing potentials of conductance-voltage relationships ($Na_V1.2$/*β1*^{R46C}, $Na_V1.2$/*β1*^{R60C}, $Na_V1.6$/*β1*^{R46C}, $Na_V1.6$/*β1*^{R60C} and $Na_V1.6$/*β1*^{V158M}), channel availability ($Na_V1.1$/*β1*^{R46C}, $Na_V1.1$/*β1*^{V158M}, $Na_V1.2$/*β1*^{R46C}, $Na_V1.2$/*β1*^{R60C} and $Na_V1.6$/*β1*^{V158M}), and detected a slower recovery from fast inactivation for $Na_V1.1$/*β1*^{V158M}. Combined with modeling data indicating perturbation-induced structural changes in *β1*, these results suggest that the *SCN1B* variants reported here can disrupt normal Na_V channel function in the brain which may contribute to EIEE52.

Keywords: *SCN1B*, voltage-gated sodium channel, early infantile epileptic encephalopathy 52, EIEE52, developmental and epileptic encephalopathy

Short summary

We identified nine patients harboring three biallelic variants in *SCN1B*. All subjects presented with early infantile epileptic encephalopathy 52 (EIEE52), a severe epileptic encephalopathy featuring infantile-onset refractory seizures and developmental stagnation. Electrophysiological analysis of these mutants revealed a complex effect on neuronal voltage-gated sodium channel gating that likely contributes to epileptogenesis. Altogether, our work refines the molecular and phenotypic spectrum of a severe encephalopathy caused by bi-allelic *SCN1B* variants. Moreover, our observations are consistent with pre-existing brain dysfunction and support the idea that *SCN1B*-related encephalopathy should be more comprehensively considered as a developmental epileptic encephalopathy.

1. INTRODUCTION

In humans, inherited heterozygous *SCN1B* variants have been associated with mild-to-moderate epileptic disorders within the genetic epilepsy with febrile seizures plus (GEFS+) spectrum. In contrast, biallelic variants cause early infantile epileptic encephalopathy 52 (EIEE52, OMIM #617350), characterized by infantile-onset refractory seizures followed by cognitive decline and neurological features such as hypotonia, spasticity, and ataxia. (1-5) Only a few individuals with EIEE52 have been reported so far and a disease-causing mechanism remains unclear. *SCN1B* encodes for *β1*, an immunoglobulin-like molecule that modulates the function of voltage-gated sodium (Na_V) channels, a family of nine membrane proteins responsible for initiating and propagating action potentials. (5, 6) As such, mutations in *β1*, as well as Na_V channels, have been linked to epilepsy syndromes. (5, 7) We report nine EIEE52 patients from four unrelated Pakistani families that harbor three *SCN1B* variants identified by whole-exome sequencing. We examined the effects of these mutations on the biophysical properties of the ubiquitous brain Na_V channel subtypes $Na_V1.1$, $Na_V1.2$ and $Na_V1.6$. Our results reveal perturbation-induced alterations in multiple channel gating parameters that can be correlated to structural changes in *β1*. Altogether, these data lay the foundation for further studies into a putative relationship between aberrant *SCN1B* function and EIEE52.

2. MATERIALS AND METHODS

2.1 Patient ascertainment

The study was approved by the UCL Institutional Review Board. Written informed consent was obtained from parents. Nine subjects from four unrelated consanguineous families presenting with similar epileptic encephalopathies were investigated using exome sequencing.

2.2 Exome sequencing and analysis

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA blood midi kit as previously described. (8) Priority was given to rare bi-allelic functional variants with an allele frequency <0.001% in public databases (gnomAD, GME Variome, Iranome, and our in-house database of 15,500 exomes), according to a plausible recessive mode of inheritance. Sanger sequencing was performed for variant validation and parental segregation.

2.3 Channel electrophysiology

Human (h) $hNa_V1.1$ (NM_001165963.1), $hNa_V1.2$ (NM_021007.2), $hNa_V1.6$ (NM_014191.3), and h*β1* (NM_001037.5) were expressed and tested as previously described. (9) Significance of all data was analyzed using two-way ANOVA with *post-hoc* Bonferroni correction. Individual time point values were analyzed using two-way Student's *t*-test. Obtained values reflect the mean and error bars reflect standard error of the mean (S.E.M.); $p<0.05$ (*), 0.01 (**) or 0.001 ($^{\#}$). Additional information on data acquisition can be found in supplementary data.

3. RESULTS

3.1 Epileptic phenotype

We identified nine patients from four unrelated consanguineous families of Pakistani ancestry (families A, B, C, and D; Fig. 1A, Suppl. Table 1). Family A consists of two affected siblings, a 9-year-old male (patient 1) and a 5-year-old female (patient 2), born to healthy parents. After normal psychomotor development in the first 6 months after birth, both patients started to suffer from recurrent myoclonic and tonic-clonic seizures (GTCS). Subsequently, they showed developmental stagnation and regression by the age of 3 years, lacking speech and social interaction. Physical examination revealed generalized spasticity and hyperreflexia. Seizures were refractory to antiepileptic drugs (AEDs), including carbamazepine and clonazepam. EEG recordings at age 3 years revealed multifocal epileptic abnormalities within diffusely slowed and dysregulated cerebral activity. Family B consists of three affected children, *i.e.* a 6-year-old female (patient 3) and two males (patients 4, 5) aged 5 and 1.5 years with healthy parents. In addition to developmental delay after birth, patients started to suffer from GTCS by the age of 5 months followed by developmental stagnation. They were nonverbal and microcephaly was present in two subjects (patients 4 and 5, range -2.17 to -3.92 SDs), and they all had hyperreflexia. Brain MRI was unremarkable. Their EEG recordings showed low-voltage cerebral activity intermixed with suppression-burst patterns from age 6 months up to 4 years (Suppl. Fig. 1). In all individuals, seizures were extremely refractory despite the use of several AEDs used alone or in combination. Family C consists of two affected females (patients 6 and 7) deceased at the age of 2 years and 7 months born to healthy parents. After a regular neonatal course, both children were diagnosed with psychomotor delay and started to suffer from seizures. Subsequently, they showed developmental stagnation and regression. Seizures were resistant to AEDs. Both patients prematurely died due to unspecified epilepsy-related complications. Family D (Fig. 1A, Suppl. Table 1) consists of two affected females of 3 years (patient 8) and 9 months (patient 9) with healthy parents. A healthy brother and sister were deceased at 11 months due to unknown causes. Both patients suffered from focal motor seizures and were diagnosed with global developmental delay. Seizures were refractory to multidrug treatment. Neurological examination revealed hyperreflexia in both cases and a brain MRI showed hydrocephalus in patient IV:3. Their EEG at age 12 months revealed globally slowed cerebral activity and theta polymorphic activity intermixed with slow-waves over the posterior regions (Suppl. Fig. 2).

3.2 Exome sequencing results

After exome sequencing, three different missense variants in homozygosity in *SCN1B* were deemed the most plausible causative in the studied families: NM_001037.5: c.136C>T; p.(Arg46Cys) in Family A, NM_001037.5: c.472G>A; p.(Val158Met) in Families B and C, and c.178C>T; p.(Arg60Cys) in Family D. These variants are rare in public databases and affect conserved residues within or close to the immunoglobulin domain (Fig. 1B,

GERP score 3.82-4.09). They are predicted to be pathogenic by *in silico* tools (Suppl. Table 2). Familial segregation analysis confirmed biparental inheritance.

3.3 Effects of *SCN1B* variants on hNa$_V$1.1, hNa$_V$1.2 and hNa$_V$1.6 channels

We examined the effects of all three *β*-subunit mutations on Na$_V$1.1, Na$_V$1.2 and Na$_V$1.6 which are predominantly expressed in the central nervous system. (6, 10) We recorded and compared gating parameters such as the conductance-voltage (G-V) relationship, channel availability, fast inactivation time constants (τ) and recovery from fast inactivation (RFI). We found all *β1*-subunits trafficked to the membrane (Suppl. Fig. 3) and that the G-V relationships (Fig. 2, Suppl. Fig. 4, Suppl. Table 3) of Na$_V$1.2/*β1*^{R46C}, Na$_V$1.2/*β1*^{R60C}, Na$_V$1.6/*β1*^{R46C}, Na$_V$1.6/*β1*^{R60C} and Na$_V$1.6/*β1*^{V158M} are shifted to more depolarized potentials (±9 mV; p<0.01, ±5 mV; p<0.05, ±5 mV; p<0.01, ±10 mV; p<0.001 and ±5.5 mV; p<0.001, respectively) compared to *β1*^{WT}. Also, channel availability of Na$_V$1.1/*β1*^{R46C}, Na$_V$1.1/*β1*^{V158M}, Na$_V$1.2/*β1*^{R46C}, Na$_V$1.2/*β1*^{R60C} and Na$_V$1.6/*β1*^{V158M} is shifted to more positive potentials (±3 mV; p<0.05, ±3 mV; p<0.05, ±6 mV; p<0.01, ±8 mV; p<0.01 and ±3 mV; p<0.05, respectively) compared to *β1*^{WT}. RFI measurements (Fig. 2) of Na$_V$1.1/*β1*^{V158M} show a slower channel recovery (±4 ms; p<0.01). The RFI results of Na$_V$1.2/*β1*^{V158M} show few individual time points that have a significantly faster recovery compared to *β1*^{WT}, but the half-life time of the fit was not significantly different from *β1*^{WT}. For fast inactivation time constants (τ) (Fig. 2), we observed several individual significant data points, although the half-life time of the fit of both *SCN1B* variants was not significantly different from *β1*^{WT}. Surprisingly, τ values for Na$_V$1.2/*β1*^{R60C} showed a consistent U-shaped voltage-dependence with the fastest inactivation observed around -20mV.

We then mapped *SCN1B* R46C/V158M mutations on available Na$_V$ channel cryo-EM structures including Na$_V$1.2 (11) and Na$_V$1.7 (12) of which the latter is in complex with *β1* (Fig. 1B). A superposition of Na$_V$1.2 and Na$_V$1.7 shows that channel residues involved in binding *β1* are conserved, allowing us to make a hybrid model of Na$_V$1.2 with *β1* bound. This shows that Arg46 is a critical residue, making ionic interactions with an Asp in Na$_V$1.2 (D1693, corresponding to D1677 in Na$_V$1.7). The R46C sequence variant is thus predicted to significantly weaken these interactions. Val158 is located at the top of the transmembrane helix of *β1*. As this residue does not directly contact the Na$_V$ channel (Fig.1B), the impact of the V158M sequence variant may involve altered membrane interactions.

4. DISCUSSION

While the role of heterozygous *SCN1B* variants in temporal epilepsy and GEFS+ is well-established, the scenario in subjects harboring bi-allelic variants is more complex. In rare cases, Dravet syndrome has been reported in these individuals (2, 3, 8); however, a distinct association between homozygous *SCN1B* variants and a severe epileptic encephalopathy is now emerging. Nine individuals from seven unrelated families have been reported (Suppl. Table 1), including a subject whose Dravet syndrome was reclassified to early infantile epileptic encephalopathy given the severe epileptic phenotype, cognitive decline, and premature death. (2-4, 8) Recurrent clinical features are early infantile-onset seizures followed by psychomotor stagnation or regression, microcephaly, axial hypotonia, appendicular spasticity, and nonspecific brain atrophy. Seizure semiology and EEG features in EIEE52 are variable (Suppl. Table 1). The response to AEDs is generally poor and epilepsy remains refractory in most cases. (2-4, 8) Similar to Aeby et al., (1) developmental delay was present before seizure onset in families B, C, and D. Together with the identification of neuronal pathfinding deficits in *SCN1b* null mice before epilepsy onset, (13) these observations are consistent with pre-existing brain dysfunction and support the idea that *SCN1B*-related encephalopathy should be more comprehensively considered as a developmental epileptic encephalopathy. Hence, effective seizure control might exert a less dramatic impact on the overall cognitive performances in comparison to other, similar conditions.

Electrophysiological analysis of *SCN1B*^{R46C}, *SCN1B*^{R60C}, and *SCN1B*^{V158M} showed a complex effect on neuronal Na_V channel gating (Fig. 2). Although there is still much to unravel about the underlying causal relationship, most *SCN1B* variants leading to changes in Na_V channel gating or kinetics are predicted to increase neuron excitability (see (1, 3, 14) and Supplementary references 4-10). In concert, previous studies on Na_V channel variants linked to epilepsy syndromes assumed hyperexcitability as the underlying mechanism. (15, 16) However, recent work uncovered epilepsy-linked Na_V channel mutants causing a depolarizing shift in voltage-dependence of channel activation and inactivation, with or without delayed recovery from inactivation. (15, 17, 18) Assuming that the effects of *SCN1B*^{R46C} , *SCN1B*^{R60C}, *and SCN1B*^{V158M} remain the same *in vivo*, it is not unreasonable to postulate that action potentials are also modulated by these *SCN1B* perturbations and contribute to EIEE52. (5, 7, 14, 17) Although the identification of possible genotype-phenotype correlations would be extremely intriguing, postulating whether a specific variant and its effect on Nav1.1 v/s Nav1.2 or Nav1.6 function might be directly responsible for the phenotypic differences observed in affected individuals is difficult based on the available data. Further experiments, likely entailing the use of dedicated mutant mouse lines, will likely play a pivotal role in the investigation of the exact underlying mechanisms.

In conclusion, we show an abnormal gating of human Na_V channels as a possible result of *β1* perturbations, supporting the role of *SCN1B* variants in epileptogenesis. Furthermore, we refined the molecular and phenotypic spectrum of the severe encephalopathy caused by bi-allelic *SCN1B* variants. The results of this study also suggest that the response to AEDs in *SCN1B*-related disorders is challenging to

predict due to the complex effects of the subunit on Na_V channel function. Additional cases will help to establish the best therapeutic approaches in *SCN1B* mutant patients.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest. 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.

AUTHOR CONTRIBUTIONS

MS and FB designed and performed the research, analyzed the data, and wrote and revised the paper. MS, MP, SE, and RM collected and analyzed genetic data. MS, SE, and PS reviewed and characterized the electro-clinical phenotype of the studied families. JDW made channel constructs, performed two-electrode voltage-clamp recording and analysis of channel activity and statistical analysis. PS, HH, and FB supervised the research and revised the paper.

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FIGURE LEGENDS

FIGURE 1. Pedigrees of the studied families and *SCN1B* variants modeling.

A, multi-generation pedigrees of families A-D showing parental consanguinity and history of recurrent family A miscarriages. B, I. 3D model of $Na_V1.7$ channel encompassing *β1* (brown) and *β2* (green) subunits. The location of the R46C and V158M mutations in *SCN1B* (NM_001037.5) is indicated. II. Superposition of $Na_V1.2$ (dark) and $Na_V1.7$ (light) at the contact area for Arg46 in *β1*, showing that the interface is conserved. Arg46 makes multiple contacts with the channel. III. Dotted line represents an ionic interaction between Arg46 in *β1* and Asp1677 in $Na_V1.7$ (corresponding to Asp1693 in $Na_V1.2$). IV. Structural details around the *β1* residue V158 are shown.

FIGURE 2. Functional characterization of *SCN1B* missense variants.

A-C, Normalized conductance-voltage (G-V, open circles) and channel availability relationship (I-V, filled circles) of $\beta1^{WT}$ (black), $\beta1^{R46C}$ (red), $\beta1^{R60C}$ (blue) or $\beta1^{V158M}$ (green) co-expressed with $Na_V1.1$, $Na_V1.2$ or $Na_V1.6$. G-V and I-V $V_{1/2}$ values are reported in Supplementary Table 3. D-F, Normalized RFI using the same color scheme measured over a 40ms timeframe using a double-pulse protocol to the maximum current of the I-V in A-C. Half-life recovery time points (ms) are presented in Supplementary Table 3. Error bars are S.E.M. with n = 5-11; p<.05 (*), 0.01 (**) or 0.001 (#). G-I, Rate (τ) of channel fast inactivation. Half-life time points (ms) are presented in Supplementary Table 3. Error bars reflect S.E.M. with n = 5-6; p<0.05 (*), 0.01 (**) or 0.001 (#).

FIGURES

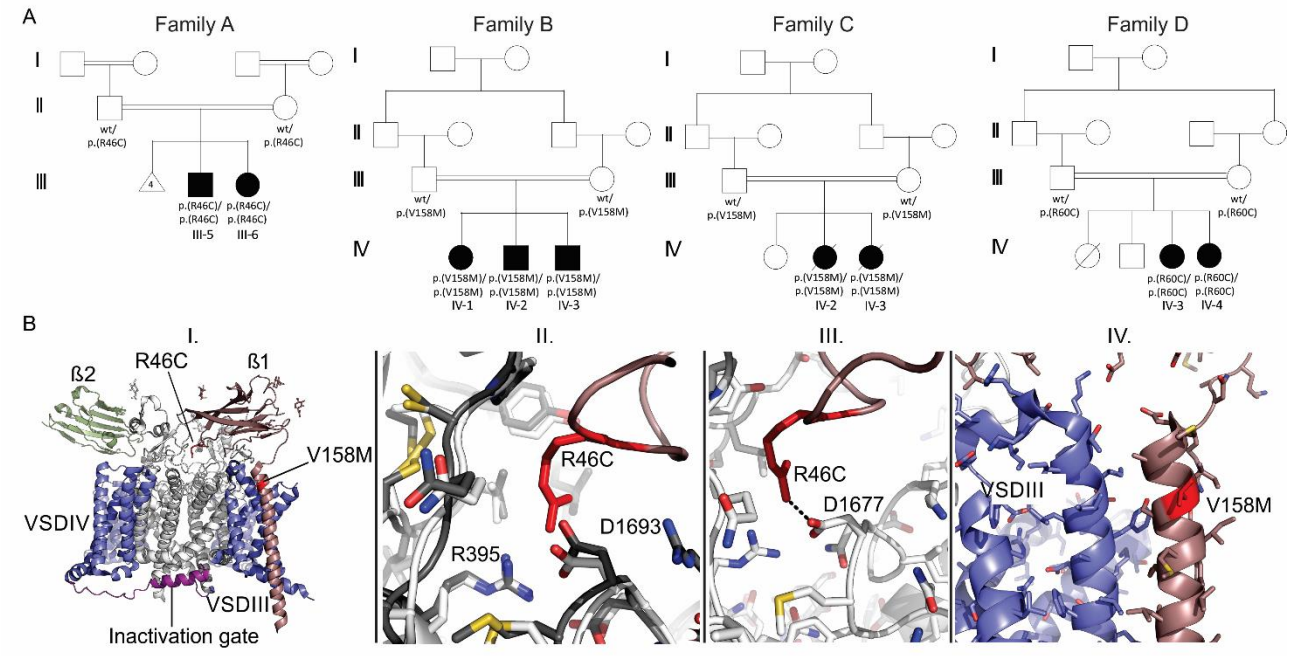

Figure 1

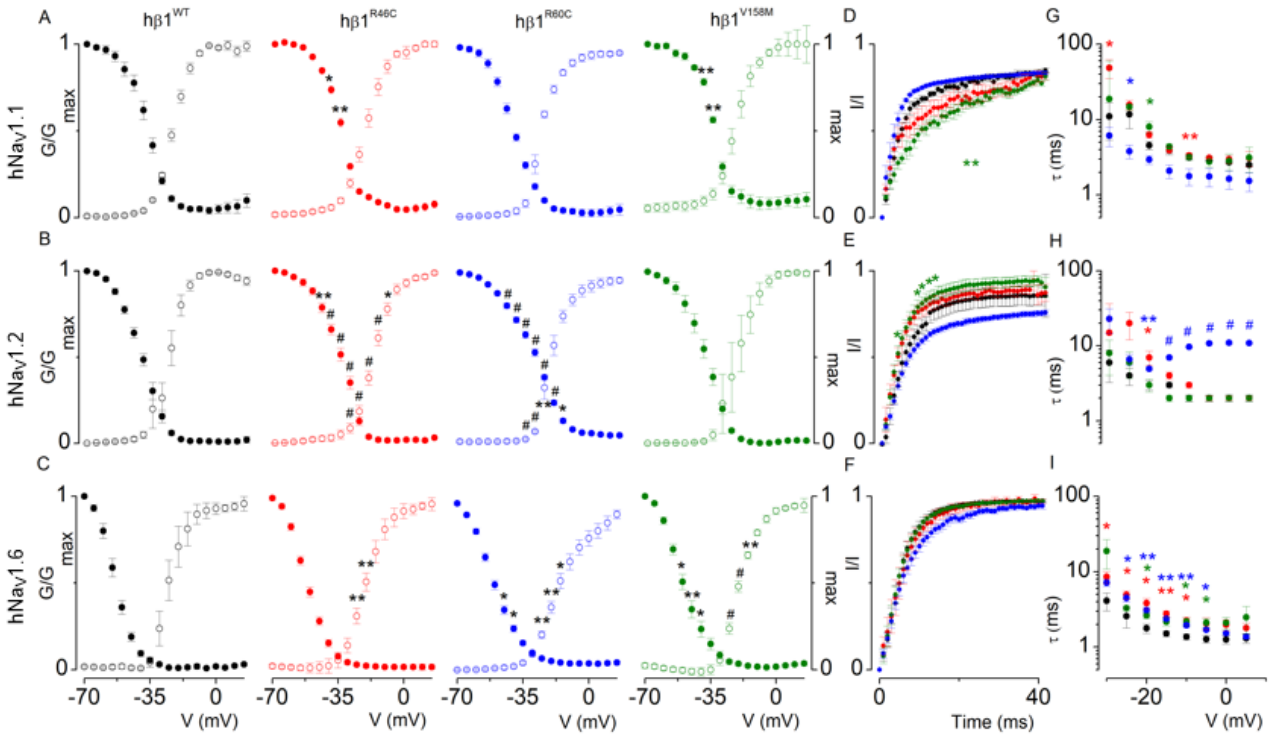
A
hβ1WT
hβ1R46C
hβ1R60C
hβ1V158M
D
G
hNaV1.1
B
E
H
hNaV1.2
C
F
I
hNaV1.6
G/Gmax
I/Imax
τ (ms)
V (mV)
Time (ms)

Figure 2