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Whole exome sequencing and co-expression analysis identify an *SCN1A* variant that modifies pathogenicity in a family with Genetic Epilepsy and Febrile Seizures Plus (GEFS+)

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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.

Summary

Objective: Family members carrying the same *SCN1A* variant often exhibit differences in the clinical severity of epilepsy. This variable expressivity suggests that other factors aside from the primary sodium channel variant influence the clinical manifestation. However, identifying such factors has proven challenging in humans.

Methods: We perform whole exome sequencing in a large family in which an *SCN1A* variant (p.K1372E) is segregating that is associated with a broad spectrum of phenotypes ranging from lack of epilepsy, to febrile seizures and absence seizures, to Dravet Syndrome. We assessed the hypothesis that the severity of SCN1A-related phenotype was affected by alternate alleles at a modifier locus (or loci).

Results: One of our top candidates identified by WES was a second variant in the *SCN1A* gene (p.L375S) that was exclusively shared by unaffected carriers of K1372E allele. To test the hypothesized that L375S nullifies the loss-of-function effect of K1372E, we transiently expressed Nav1.1 carrying the two variants in HEK293T cells and compared their biophysical properties with the wild-type (WT) variant, and then co-expressed WT with K1372E or L375S with K1372E in equal quantity and tested the functional consequence. The data demonstrated that co-expression of the L375S and K1372E alleles reversed the loss-of-function property brought by the K1372E variant, while WT-K1372E co-expression remained partial loss-of-function.

Significance: These results support the hypothesis that L375S counteracts the loss-of-function effect of K1372E such that individuals carrying both alleles in trans do not present epilepsy-related symptoms. We demonstrate that monogenic epilepsies with wide expressivity can be modified by additional variants in the disease gene, providing a novel framework for gene-phenotype relationship in genetic epilepsies.

Keywords

SCN1A-related epilepsy; modifier gene; whole exome sequencing; co-expressed variants; biophysical properties

Introduction

Intragenic complementation (IGC) is a phenomenon whereby particular combinations of mutant alleles at a given locus produce a less severe phenotype than the same alleles do in the homozygous state, or in the presence of noncomplementing alleles [1]. The phenomenon is chiefly known in Drosophila and other organisms with short generation times [2]. In humans, all instances of IGC have been found to involve genes encoding multimeric enzymes, where it contributes to extensive clinical heterogeneity observed among patients suffering from particular diseases associated with these loci [1]. We have discovered a possible case of IGC in an Ashkenazi kindred segregating an SCN1A allele associated with epilepsy [3].

Dravet syndrome and genetic epilepsy with febrile seizures plus (GEFS+) can both arise due to mutations of SCN1A, the gene encoding the alpha 1 pore-forming subunit of the sodium channel [4]. GEFS+ refers to a familial epilepsy syndrome that comprises a range of mild to severe phenotypes varying from classical febrile seizures to Dravet syndrome

(DS) [5]. DS is a severe infantile onset epilepsy syndrome with multiple seizure types, developmental slowing and poor outcome. More than 80% of cases of Dravet Syndrome are caused by loss-of-functions (LOF) variants in *SCN1A*, whereas, only 10% of GEFS+ families have disease-causing *SCN1A* variants. A common feature of GEFS+ epilepsies is variable penetrance and expressivity among individuals with the same mutation, suggesting that genetic modifiers may influence clinical severity [6].

We previously reported on a large Israeli family with GEFS+ [3]. The kindred was composed of 16 unaffected and 14 affected individuals, including the proband who had DS. Upon sequencing all 26 protein-coding exons of SCN1A (including exon/intron boundary 26 exons) of the proband and her brother, we identified a novel SCN1A missense variant in exon 21 (p.K1372E). Genotyping this site in the remaining members of the kindred revealed that K1372E was present in heterozygous form in all affected individuals, as well as 3 unaffected carriers.

We hypothesized that family members carrying the K1372E allele on the opposite end of the phenotypic spectrum possessed alternate alleles at a modifier locus (or loci). Here we ask whether there is evidence for modifier genes protecting against the epilepsy phenotype. Toward this end we performed whole exome sequencing in nine family members representing three generations, including the proband and her brother, their parents and grandparents, as well as the unaffected carriers who are siblings of the father.

Subjects and Methods

DNA was extracted from blood samples collected from a family of Ashkenazi Jewish origin in which there was no consanguinity between the parents as previously described [3]. Written informed consent was obtained from all participants, and detailed neurologic examination and electrophysiological studies were performed [3].

Exome sequencing

Whole exome sequencing was performed as described in Hammer et al. (2017). The mean coverage over the exon target regions ranged from 48 to 119, with 90.3–97.5% of RefSeq exonic base positions covered at least 15-fold. We filtered for variants that were predicted to alter protein function (i.e., nonsynonymous, stop-gain, stop-loss, frameshift, and splice-junction mutations). Because we did not assume that the modifier was pathogenic when not present in combination with the K1372E allele, we allowed candidate variants that were present at intermediate frequency (i.e., ≤50%) in public databases.

In this study we were interested in identifying modifier genes that could explain the lack of epilepsy in the unaffected carriers, as opposed to those that may have led to a more severe outcome in the proband with Dravet Syndrome. We favored the hypothesis that the modifier was acting as a fully protective dominant and therefore initially focused on heterozygous variants that were shared exclusively among the three unaffected aunts and uncles, as well as the grandmother who did not carry the K1372E allele. This produced a list 210 gene variants. To filter out apparent false positive heterozygous calls as judged by a skew in allelic balance, we also applied a lower limit of 1% probability on the ratio of observed reads from the two alleles under a binomial model, leaving 160 variants.

These were further filtered based on the following criteria: 1) the gene is expressed in the brain as assessed by signal intensity score (Log2) ≥ 6 in the Human Brain Transcriptome (https://hbatlas.org/pages/hbtd), and 2) the gene is associated with epilepsy or synaptic function as assessed by presence of the gene on the Prevention Genetics Autism Spectrum Disorder, Intellectual Disability (ASDID) & Epilepsy Comprehensive Sequencing Panel (https://www.preventiongenetics.com). This resulted in 16 variants, as shown in Table 1. We also considered PolyPhen-2[7] scores for missense variants, prioritizing those that were predicted to be possibly or probably damaging.

DNA constructs

Our optimized human cDNA construct for wild-type (WT) Nav1.1 was designed in-house [8], encoding for the amino acid sequence corresponding to the accession number NP_055006.1 in the NCBI database. Mutations L375S and K1372E were introduced individually into the WT construct using QuikChange® II XL site-directed mutagenesis kit from Agilent Technologies according to the manufacturer's instructions. Mutant channel constructs were fully sequenced (ACGT, Inc.) to confirm the presence of the correct mutation and the absence of additional mutations.

Cell culture and transfection

The use of human embryonic kidney 293T (HEK293T) cells was approved by the Institutional Biosafety Committee and followed the ethical guidelines for the National Institutes of Health for the use of human-derived cell lines. The cells were grown under standard tissue culture conditions and transiently transfected using Invitrogen Lipofectamine 2000 Transfection Reagent according to the manufacturer's instructions. Briefly, lipid-DNA mixture [5μg channel construct and 0.5μg enhanced green fluorescent protein (EGFP) construct] in Opti-MEM medium was added to cells for 3h, after which transfected cells were split onto 35mm dish with fresh medium. For co-expression experiments, 2.5μg of each channel variant construct were used, totaling 5μg channel constructs. 24–32h after transfection, whole-cell voltage-clamp recordings were performed. Transfected cells were identified by EGFP expression under a fluorescent microscope.

Whole-cell patch clamp recordings

All recordings were obtained at room temperature (~22ºC) using a HEKA EPC-10 amplifier and the PatchMaster program (v2×73.2, HEKA Electronic) as previously described [9, 10]. For voltage-clamp recordings, electrodes were fabricated from 1.7mm capillary glass and fire-polished to a resistance of 0.8–1.0 MΩ using a Sutter P-1000 Micropipette puller (Sutter Instrument Company). The series of recording protocols was started 3 min after break-in for each cell, which controlled for time-dependent shifts in channel properties. Cells were not considered for analysis if the initial seal resistance was < 1 GΩ or if they had a series resistance > 3 MΩ. Voltage errors were minimized using >80% series resistance compensation and passive leak currents were cancelled by subtraction. The extracellular solution contained (in mM): 140 NaCl, 20 TEA-Cl, 3 KCl, 1 $MgCl_2$, 1 $CaCl_2$, and 10 HEPES, adjusted to a pH of 7.30 with NaOH. The intracellular solution contained (in mM): 140 CsF, 10 NaCl, 1.1 EGTA, and 10 HEPES, adjusted to a pH of 7.30 with CsOH. Osmolarity of all solutions was adjusted to 300 mOsm.

Patch clamp protocols and data analysis

Activation protocol—Transient sodium current (I_{NaT}) was measured during a 50ms depolarizing step (–80mV to +45mV; 5mV increment) from a holding potential of –100mV. The current density was calculated by dividing the measured I_{NaT} by the capacitance of the cell. Sodium current conductance (G_{Na}) was converted from I_{NaT} using the equation

$$G_{Na} = I_{NaT}/(V - V_{rex}),$$

where V_{rev} is the reversal potential of Na^+ obtained in FitMaster (v2×73.5, HEKA Electronic) for each cell. Activation curves were generated by plotting normalized G_{Na} against depolarizing potentials and fitting it with the Boltzmann function in the form of

$$G_{Na}/G_{max} = 1/(1 + \exp[(V_{50,act} - V)/k_{act}])$$

where G_{max} is the maximal G_{Na}, $V_{50,act}$ is the potential at which activation is half-maximal, V is the depolarizing potential, and k_{act} is the slope factor.

Steady-state inactivation—Availability of sodium channels was measured by the peak sodium current during a 20ms test pulse at 0mV following a 500ms prepulse (–120mV to +30mV; 10mV increment) that allows channels to enter equilibrium states. Steady-state inactivation curves were generated by plotting normalized sodium current against prepulse potentials and fitting it with the Boltzmann function in the form of

$$I/I_{max} = 1/(1 + \exp[(V_{50,\ inact} - V)/\ k_{inact}\ )$$

where I_{max} is the maximal sodium current obtained in this protocol, $V_{50,\ inact}$ is the potential at which half of the sodium channels are available for activation, V is the prepulse potential, and k_{inact} is the slope factor.

Recovery from inactivation—A 20ms depolarization prepulse at 0mV was applied to allow channel activation and subsequent inactivation, which was followed by a repolarizing step to –80mV for durations ranging from 0ms to 50ms with 2ms increment. The non-inactivated sodium currents were measured during a subsequent 20ms test pulse at 0mV and normalized to the maximum current obtained in this protocol. The normalized non-inactivated sodium current was plotted against the duration of repolarizing step and fitted with a single exponential function.

Statistics

GraphPad Prism (v 6.00, GraphPad Software) was used for statistical analysis and curve fitting with the nonlinear least-squares minimization method. Data are presented as mean ± standard error of mean (SEM) or mean ± standard deviation (SD) of the indicated number of cells (n). One-way ANOVA followed by Tukey multiple comparisons test was performed.

Results

Whole exome sequencing of sibship

To identify possible genetic modifiers, we performed Whole Exome Sequencing (WES) on a subset of the 27-member Ashkenazi kindred shown in Figure 1 of Goldberg-Stern et al. [3]. Here Fig. 1A depicts a sibship within the larger pedigree illustrating variable expressivity among six carriers of the K1372E variant. While the proband suffers from DS (black fill), her brother, father, and grandfather (gray hatched fill) all have much milder epilepsy (FS/FS+ and absence seizures), and three of her father's siblings are unaffected carriers (dotted).

Candidate protective variants

Upon filtering variants that fit the segregation pattern shown in Figure 1, a total of 220 variants passed our allelic quality filters for all 9 subjects. A total of 50 (Laurel will check the 220 and 50 numbers) of these variants also had read depths ≥20 for both alleles. Loci with multiple variants were considered less likely to be correctly mapped and upon removal there only remained a total of 16 (check this number) brain-expressed variants (Table 1). Only four of these variants are predicted to be damaging either by polyphen-2 for the three missense variants or by MutationTaster [28] for the frameshift variant (Table 1).

One of our top candidates exclusively shared between the grandmother of the proband and her unaffected aunt and uncles was a second variant in the SCN1A gene: L375S (chr2:166904183 A>G). The inheritance pattern informs us that K1372E and L375S variants are in trans configuration in the unaffected carriers, while *in silico* analysis predicts that both amino acid substitutions are deleterious and/or function altering (i.e., high PhyloP, CADD, and Polyphen scores). Neither variant is present in public databases of healthy individuals, and each variant maps to regions of the protein that are known to be hotspots for pathogenic variation linked to DS (Fig. 1B). Interestingly, the individual that introduced this variant into the family—the grandmother of the proband—is not known to have epilepsy or a family history of epilepsy.

We also utilized a separate pipeline developed at CHOP in which non-population variants ($p \leq 0.01$) were filtered in all nine individuals and sorted by the probability of being LoF intolerant (pLI) score [11]. The pLI score was developed on the premise that genes vary in the extent to which biallelic LoF is tolerated by natural selection [12]. Two of the same variants as listed in Table 1. were identified, including the SCN1A- L375S variant and the UBR3-N1807L variant (data not shown).

Functional studies

To examine how K1372E and L375S mutations affect the electrophysiological properties of the Nav1.1 channel, we transiently expressed Nav1.1 carrying one of the two variants in HEK293T cells and compared their biophysical properties with the wild-type (WT) variant. As shown in Fig. 2, the K1372E channel conducts extremely small sodium current (−27.48 ± 12.77 pA/pF) compared to the WT channel (−310.8 ± 148.6 pA/pF, $P < 0.0017$ in one-way ANOVA) (Table 2), revealing a nearly complete loss-of-function effect on

Nav1.1. This suggests that K1372E is a pathogenic variant and it aligns with the notion that loss-of-function mutations in Nav1.1 are likely to cause epilepsy syndromes. On the other hand, the L375S mutation does not incur significant alteration of Nav1.1 current density compared to the WT channel (−412.0 ± 309.8 pA/pF, P = 0.3697 in one-way ANOVA).

Because individuals carrying both L375S and K1372E mutations are unaffected, while WT-K1372E heterozygous carriers suffer from various degrees of epilepsy, we hypothesized that L375S nullifies the loss-of-function effect of K1372E. To simulate such heterozygous conditions, we co-expressed WT with K1372E (WT- K1372E) or L375S with K1372E (L375S-K1372E) in equal quantity and tested the functional consequence. We observed that the WT-KE co-expressing cells generated about 50% of sodium current (−216.2 ± 162.1 pA/pF) as the WT-expressing cells, (429.1 ± 226.1 pA/pF, P = 0.0370 in one-way ANOVA), while LS-KE produced current at the same level as WT (−354.1 ± 223.1 pA/pF, P = 0.4856 versus WT, P = 0.0057 versus WT-KE in one-way ANOVA) (**Table 3**). These data demonstrated that co-expression of LS-KE reversed the loss-of-function property brought by the K1372E variant, while WT-KE co-expression remained partial loss-of-function. This explains the remarkable phenomena that individuals carrying both K1372E and L375S do not present epilepsy-related symptoms.

We also studied the voltage dependence of the different Nav1.1 channel variants expressed individually and jointly in HEK293T cells. L375S plus K1372E displays a 4–5mV depolarizing shifts in both voltage-dependent activation and inactivation (Fig. 3 and Table 2). L375S expressed alone also presents similar shifts (Fig. 3 and Table 2), while WT plus K1372E co-expression does not alter the voltage dependence of Nav1.1 compared to WT (Fig. 3 and Table 2). Generally, a depolarizing shift of steady-state inactivation is considered a gain-of-function effect, while a depolarizing shift of activation is considered loss-of-function. Therefore, we could not draw a definite conclusion about the likely impact of a L375S variant on neuronal excitability or its potential impact on a complicated neural network based on its voltage dependence alterations.

Fig. 4 shows that L375S-K1372E currents, measured when HEK293T cells co-expressed both channel constructs, also recover faster from inactivation compared to either WT or WT-K1372E currents (Table 2). This indicates a slight gain-of-function effect of the L375S variant. Similar to the change in voltage dependence, the outcome of this alteration on neuronal excitability and neural network properties requires further assessment.

Discussion

Pathogenic variants in the voltage-gated sodium channel SCN1A are associated with a wide range of phenotypes with varying disease severity. On the more severe end of this phenotypic spectrum is Dravet Syndrome, a developmental and epileptic encephalopathy characterized by intractable seizures and mild to severe intellectual disability (ID) [13, 14]. The opposite end of the spectrum includes GEFS+ syndrome and febrile seizures, in which seizures show a milder course and no cognitive comorbidities [15].

Part of the explanation for such a wide range of phenotypes is that different pathogenic variants in the α-subunit of a neuronal sodium channel Nav1.1 can have different effects on channel function. Patients with complete loss of function (truncating) variants will often present on the more severe end of the disease spectrum (e.g., DS), whereas missense variants associated with variable disturbances in channel function are typically associated with milder clinical phenotypes. However, even individuals with DS carrying equivalent truncating variants show a range of phenotypic severity [16, 17], and family members carrying the exact same pathogenic *SCN1A* variant often exhibit variability in phenotype severity [18–20]. Indeed genetic background has been shown to influence disease severity in DS [17], while evidence for specific modifier genes has emerged [16]. In some cases variants in other ion channels have been shown to be associated intra-familial variability in phenotype severity, for example, in SCN9A [21], CACNA1A [22], CACNB4 [23], and KCNQ2 [17].

Here we study a four-generation family kindred in which a single pathogenic variant is segregating among multiple members with a range of severities ranging from unaffected carriers, to febrile seizures to DS [3]. We set out to discover genetic factors co-segregating with severity phenotype by sequencing the exomes of a subset of 9 closely related family members that were unaffected, or that presented with mild epilepsy or DS (Figure 1A). WES analysis revealed four variants at ≤0.01 frequency in the Ashkenazi population and with an inheritance pattern consistent with transmission of a fully protective, dominant allele inherited from the grandmother of the proband. While all four of these loci are brain expressed (Table 1.) and exhibit functional features that could make them candidates as potential modifiers of *SCN1A*, only *SCN1A* and *UBR3* have been previously implicated in epilepsy or synaptic function. We reasoned it most prudent to first rule out L375S (i.e., as a variant with properties that could counteract the effect of K1372E) before designing studies to assess the functional effects of putative modifiers at other loci. There is good reason to suspect that L375S alters $Na_V1.1$ function; however, given the grandmother's medical history, we hypothesized that this variant leads to a benign gain-of-function that counterbalances the effect of K1372E and does not lead to epilepsy by itself.

To test this hypothesis, we constructed both mutants in a cellular system to study their electrophysiological properties individually and jointly in a cellular model. Overall, K1372E presents a nearly complete loss of sodium current when expressed on its own, while it causes a 40% loss of current in the presence of a WT variant. Moreover, the biophysical features of voltage dependence and recovery from inactivation of the WT-K1372E channel combination remains the same as the WT channel. This suggests that K1372E is not a dominant-negative variant, and it likely does not interfere with the trafficking and gating of the WT channel. However, it remains to be tested whether K1372E is a trafficking- or gating-defective variant. Intriguingly, co-expressing L375S-K1372E generates the same level of sodium current as the WT channel, effectively abolishing the loss-of-function effect exerted by the K1372E variant. The rescue of sodium current amplitude is particularly interesting, although the exact mechanism is not clear. It is possible that the L375S variant assumes higher expression level to compensate for the loss of K1372E current. It is also possible that L375S facilitates trafficking and surface expression of K1372E. There is evidence that the cardiac sodium channel Nav1.5 can assemble and gate as dimers, and evidence suggests that the neuronal isoforms Nav1.1 and Nav1.2 are also subject to dimerization [24]. We speculate

that the L375S channel may have formed dimers with K1372E, preventing the loss of sodium current, while the WT channel fails to do the same. It remains to be investigated whether Nav1.1 dimerization occurs physiologically and whether it is the L375S variant that facilitates and/or it is the K1372E variant that disrupts the dimerization process. It is critical to note that while co-segregation of the p.L375S variant might explain the unaffected status of individuals 5, 6 and 7, there is still considerable clinical variability between individuals carrying the p.K1372E. The reason for this variability is currently unknown, but in line with previous data [25, 26], we would expect at least some effect of other, as yet unidentified genetic factors, including common variants.

Caveats/Limitations

Because our cohort has only a few members that are unaffected carriers, the number of variants that fit the inheritance pattern consistent with linkage to the variable phenotype is large. We chose particular criteria to limit the number of these gene variants. On our final list were a few variants that are important to note. SCN2A Arg19Lys is the most common polymorphism in SCN2A with numerous homozygote individuals reported in gNomAD. While we cannot rule out the possibility of Nav1.1/Nav1.2 dimer interactions, it seems unlikely that this particular SCN2A variant has significant physiological impact. Interestingly, UBR3 has been reported to modify Nav1.5 cardiac sodium channels [27]. While we cannot rule out the possibility that other variants observed might impact the phenotype associated with the SCN1A-K1372E variant, based on our data L375S remains the most likely candidate. However, it is quite possible that variants at other genes are segregating in this family kindred that play a role in influencing the severity of epilepsy, whether with small or moderate effect sizes [17]. Future studies of the mechanism by which the L375S variant nullifies the pathogenic effect of the K1372E variant may provide clues on gene therapies that can rescue other alleles associated with *SCN1A* haploinsufficiency.

In summary, we report a possible case of intragenic complementation in a large family with Genetic Epilepsy with Febrile Seizures Plus (GEFS+), describing a situation where a second variant in *SCN1A* in *trans* counteracts the disease-causing variant in the family, including functional studies. This example highlights the possibility that clinical presentations in monogenic epilepsies are influenced by modifier genes, a mechanism previously only reported in animal models. Identifying modifier genes including variants in the same gene resulting in intragenic complementation provides a novel framework for pathogenicity in genetic epilepsies, including new avenues for treatment.

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Key Points Box

- Identifying genes that modify the clinical severity of epilepsy may lead to the discovery of novel therapeutic targets
- Whole exome sequencing was performed on family members carrying the same SCN1A variant associated with Dravet Syndrome and milder/no epilepsy
- A second SCN1A variant was identified that was segregating only in unaffected carriers of the pathogenic loss of function variant
- Heterologous co-expression studies revealed that the modifier variant reversed the loss-of-function property of the pathogenic variant
- This represents the first case of an SCN1A variant nullifying the pathogenic effects of an SCN1A variant associated with Dravet Syndrome

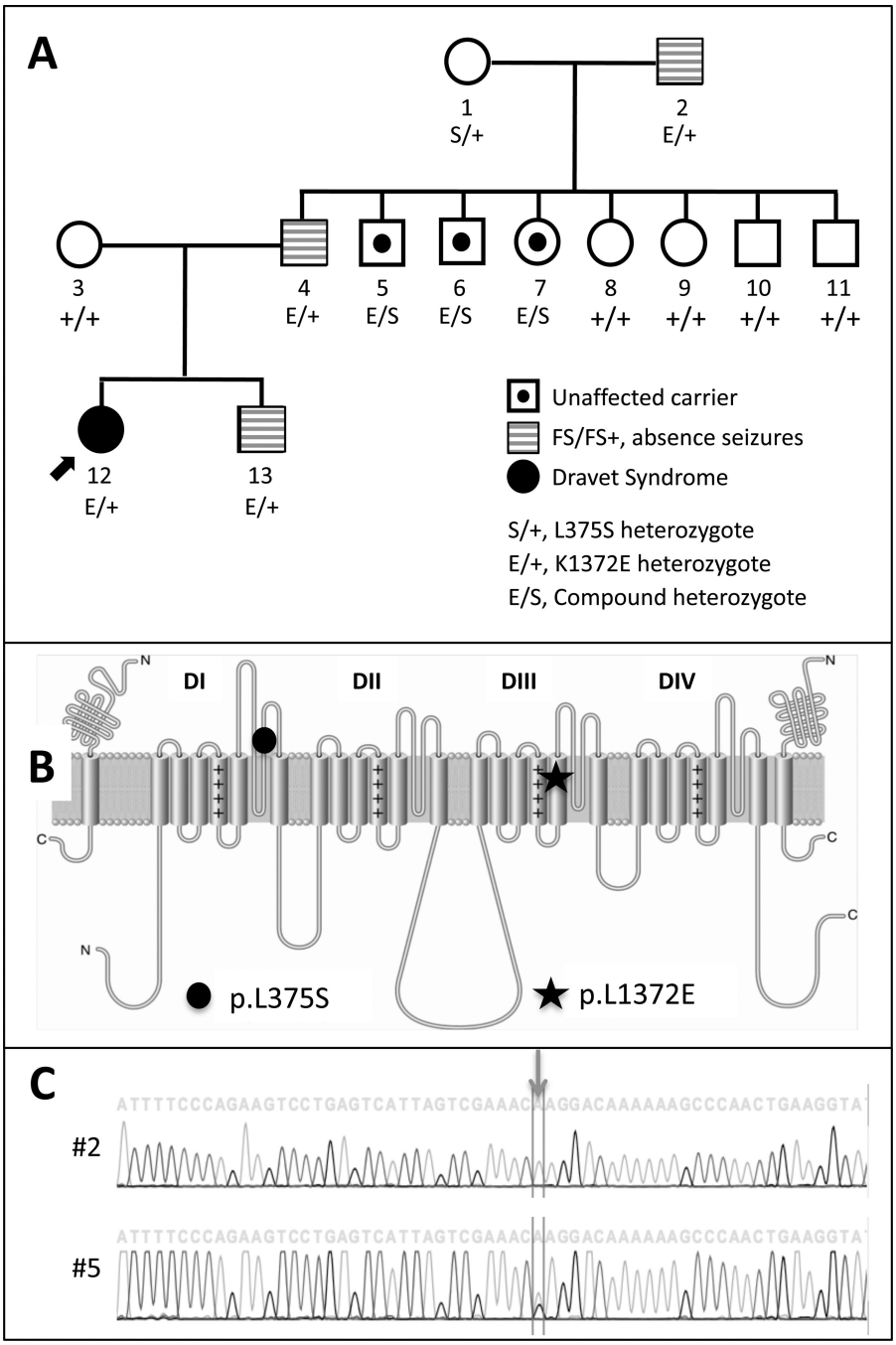

Figure 1.

A. Pedigree and SCN1A genotypes of individuals examined by whole exome sequencing. **B**. Position of variants of interest in the $Na_V1.1$ channel. **C**. Sanger sequencing confirmation of L375S variant. The homozygous (A/A) and heterozygous (A/G) states at position 166904183 (blue arrow) of the SCN1A gene sequencing is shown for representative family members #2 and #5.

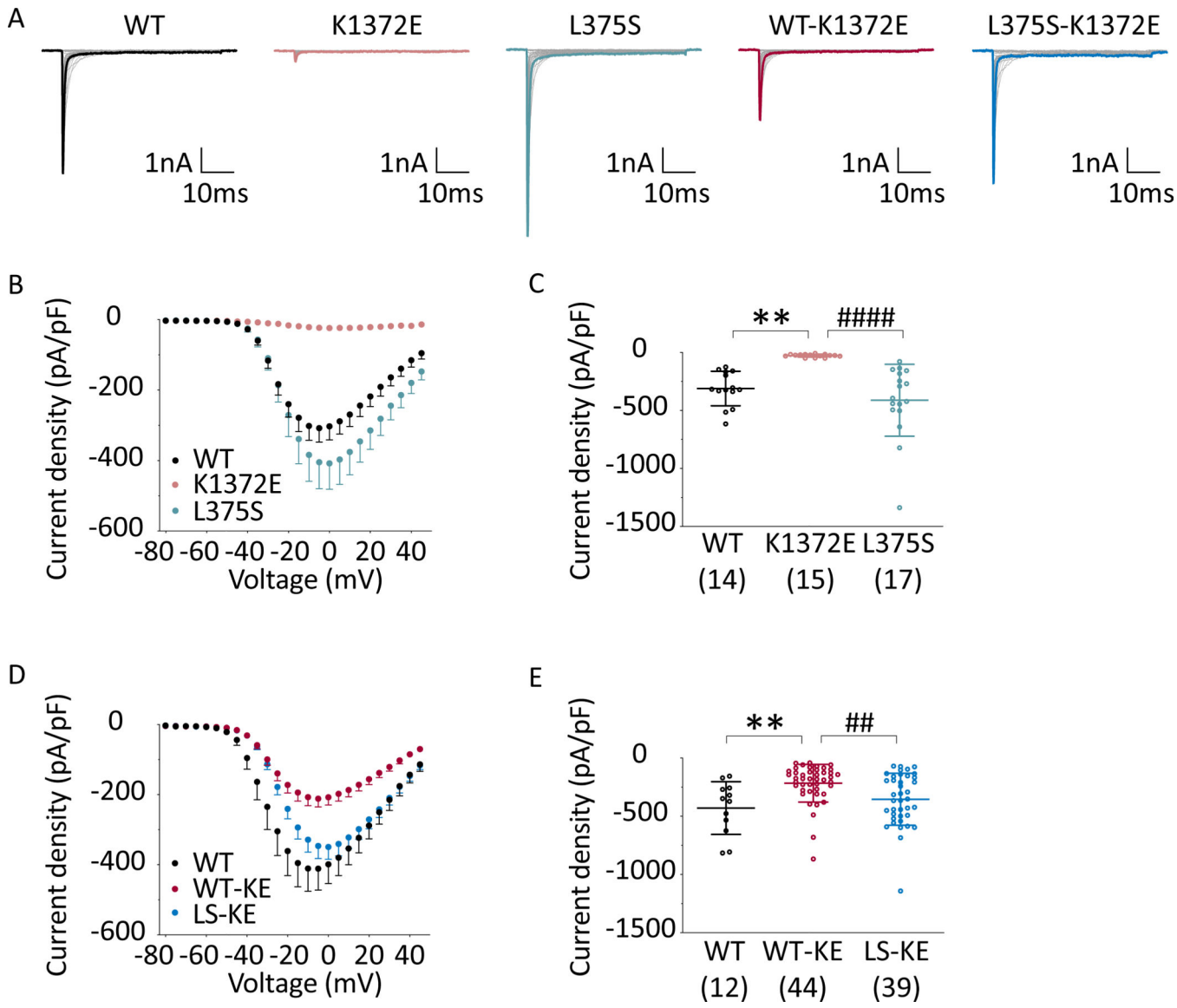

Figure 2.

Loss-of-function mutation K1372E can be rescued by co-expressing L375S. **A**. Representative current traces from HEK 293 cells transiently transfected with Nav1.1 variants on their own or in combination. The maximum current traces are highlighted with black, pink, light blue, red and dark blue, respectively. **B,D**. Current density-voltage plots. Data presented as mean ± SEM. **C,E**. Maximum current density of Nav1.1 variants elicited with the activation protocol. Data presented as mean ± SD. Values are reported in Table 2. ** P < 0.01, **** P < 0.0001 in one-way ANOVA with Tukey's multiple comparisons test.

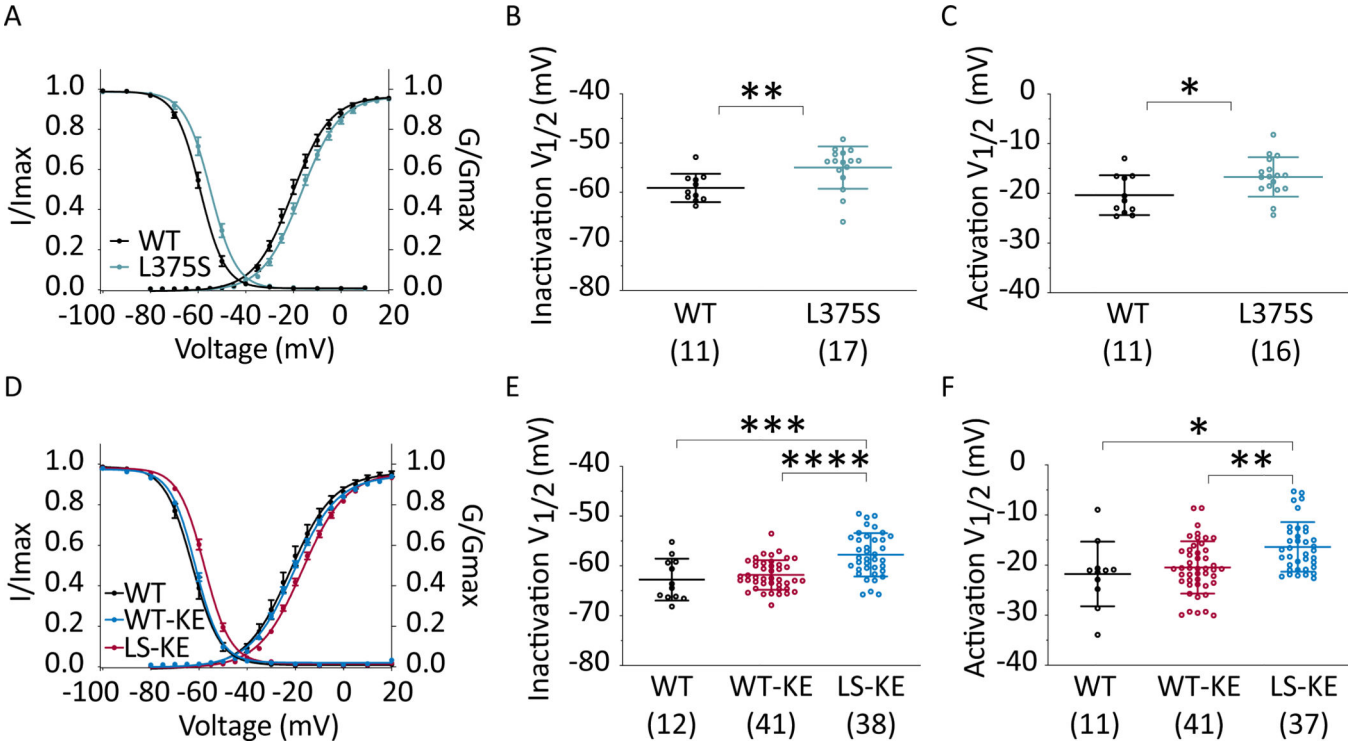

Figure 3.

L375S shifts the voltage dependence of Nav1.1 by itself and in combination with K1372E. **A,D**. Steady-state inactivation and activation curves fitted with Boltzmann functions. Data presented as mean ± SEM. **B,C,E,F**. Mid-point voltage of inactivation (B,E) and activation (C,F) extrapolated from individual voltage-dependent curves. Data presented as mean ± SD. Values are reported in Table 2. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, in t-test (B,C) or in one-way ANOVA with Tukey's multiple comparisons test (E,F).

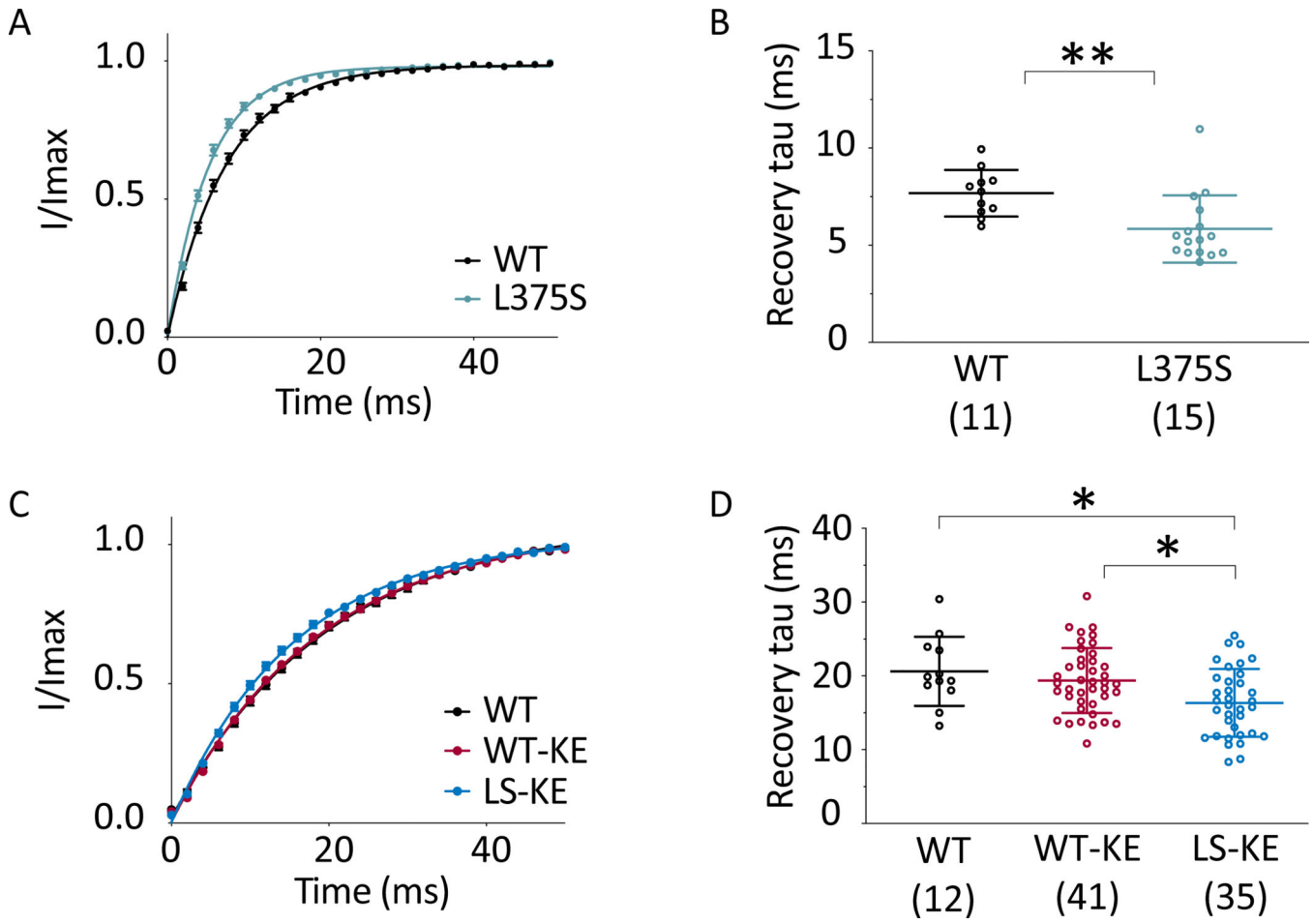

Figure 4.

L375S recovers faster from inactivation by itself and in combination with K1372E.

A,C. The fraction of channel recovery from inactivation with recovery duration from 0 to 50ms. Curves are fitted with a single exponential function. Data presented as mean ± SEM.

B,D, recovery time constant **τ** extrapolated from individual single exponential curves. Data presented as mean ± SD. Values are reported in Table 2. ** P < 0.01 in t-test (B), * P < 0.05 in one-way ANOVA with Tukey's multiple comparisons test (D).

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Table 1.

Top candidate variants

Gene name	Amino acid change	Ensembl Isoform	Expressed in Brain[1]	CNS Disorder link[2]	hvar pph2 prob[3]	hvar pph2 prediction	Ashkenazi Genome Frequency
SCN1A	p.Leu375Ser	ENST00000423058	+++	yes	0.995	probably damaging	0.00
ATP8A2	p.Leu289Val	ENST00000255283	+++	yes	0.732	possibly damaging	0.00
UBR3	p.Asn1807Lys	ENST00000272793	+++	yes	0.202	possibly damaging	0.00
BIN1	p.Pro422Argfs*79	ENST00000316724	+++	yes	NA	NA[4]	0.00
SACS	p.Ala3661Val	ENST00000382298	+++	yes	0.284	benign	0.20
CHSY1	p.Pro359Ser	ENST00000254190	+++	yes	0.012	benign	0.07
SCN2A	p.Arg19Lys	ENST00000357398	+++	yes	0.007	benign	0.08
BBS9	p.Ala455Thr	ENST00000242067	+++	yes	0.005	benign	0.00
VPS13B	p.Ser3142Gly	ENST00000358544	+++	yes	0.003	benign	0.03
ZFPM2	p.Ala403Gly	ENST00000407775	+++	yes	0.000	benign	0.12
POLR1D	p.His93Arg	ENST00000399697	+++	yes	0.000	benign	0.00
BAG3	p.Cys151Arg	ENST00000369085	++	yes	0.317	benign	0.20
KIAA1109	p.Ile978Thr	ENST00000388738	++	yes	0.01	benign	0.15
GIGYF1	p.Ala66Thr	ENST00000275732	+	yes	0.122	benign	0.04
C2orf69	p.Asp64Glu	ENST00000319974	+	yes	0.019	benign	0.22
EZH2	p.Asp185His	ENST00000460911	in utero (+++)	yes	0.299	benign	0.09

[1] Human Brain Transcriptome

[2] CNS, Central Nervous System; gene included on Prevention Genetics Autism Spectrum Disorder, Intellectual Disability (ASDID) & Epilepsy Comprehensive Panel Sequencing Panel

[3] Polyphen-2 score (http://genetics.bwh.harvard.edu/pph2/dokuwiki/overview)

[4] Predicted loss of function via nonsense-mediated RNA decay [1]

Table 2.

Biophysical properties of Nav1.1 variants individually (top panel) and jointly (bottom panel)

	Max current density (pA/pF)		n	Inactivation V1/2 (mV)		n	Activation V1/2 (mV)		n	Recovery tau (ms)		n
WT	−310.8 ± 148.6		14	−59.12 ± 2.89		11	−20.35 ± 3.99		11	7.68 ± 1.20		11
L375S	−412.0 ± 309.8	####	17	−54.97 ± 4.30	**	16	−16.68 ± 4.00	*	17	5.84 ± 1.73	**	16
K1372E	−27.45 ± 12.8	**	15	NA			NA			NA		
WT	−429.1 ± 226.1		12	−62.75 ± 4.21		12	−21.77 ± 6.45		11	20.62 ± 4.68		12
WT-KE	−216.2 ± 162.1	**	44	−61.68 ± 3.00		41	−20.47 ± 5.23		41	19.38 ± 4.40		41
LS-KE	−354.1 ± 223.1	$$	39	−57.75 ± 4.35	***$$$$	38	−16.37 ± 4.95	*$$	37	16.36 ± 4.59	*$	35

Values are presented as mean ± SD.

*compared to WT, #compared to K375E, $compared to WT-KE

*,$ P < 0.05;

**,##,$$ P < 0.01;

*** P < 0.001;

****,####,$$$$ P < 0.0001