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GAIN OF FUNCTION *SCN1A* DISEASE-CAUSING VARIANTS: EXPANDING THE PHENOTYPIC SPECTRUM AND FUNCTIONAL STUDIES GUIDING THE CHOICE OF EFFECTIVE ANTISEIZURE MEDICATION

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ABSTRACT

Objectives: To refine the spectrum of *SCN1A*-epileptic disorders other than Dravet syndrome (DS) and genetic epilepsy with febrile seizures plus (GEFS+) and optimize anti-seizure management by correlating phenotype-genotype relationship and functional consequences of *SCN1A* variants in a cohort of patients. We also performed a literature review including individuals with *SCN1A* variants causing non-DS and non-GEFS+ phenotypes and compared the features of the two cohorts.

Results: Sixteen probands were ascertained via a national collaborative network, nine (56%) with de novo pathogenic variants causing developmental and epileptic encephalopathy (DEE) with seizure onset at a median age of 2 months and severe intellectual disability. Seven subjects (54%), five with inherited and two with de novo variants, manifested focal epilepsies (FE) with mild or no intellectual disability. Sodium-channel-blockers never worsened seizures, and 50% of patients experienced long periods of seizure freedom. We found 13 *SCN1A* missense variants, eight of them were novel and never reported. Functional studies of three representative variants showed a gain of channel function. The literature review led to the identification of 44 individuals with *SCN1A* variants and non-DS, non-GEFS+ phenotypes. The comparison with our cohort highlighted that DEE phenotypes are a common feature.

Significance: The boundaries of *SCN1A*-disorders are wide and still expanding. In our cohort, more than 50% of patients manifested focal epilepsies, which are thus a frequent feature of *SCN1A* pathogenic variants beyond DS and GEFS+. *SCN1A* testing should therefore be included in the diagnostic workup of pediatric, familial and non-familial, focal epilepsies. Alternative, non-DS/non-GEFS+ phenotypes might be associated with gain of channel function, and sodium-channel-blockers could control seizures by counteracting excessive channel function. Functional analysis evaluating the consequences of pathogenic *SCN1A* variants is thus relevant to tailor the appropriate ASM.

Keywords: *SCN1A* gene, Gain of Function, Focal Epilepsy, Sodium channel Blockers

Key Points:

- The boundaries of *SCN1A*-disorders are wide and still expanding
- Focal epilepsies and other DEEs should be included in the spectrum of *SCN1A*-disorders
- Functional studies of *SCN1A* missense variants associated with these alternative phenotypes show a moderate gain of channel function
- Sodium-channel-blockers could control seizures by counteracting excessive channel function
- Functional studies for evaluating the consequences of *SCN1A* pathogenic variants are relevant to tailor the appropriate ASM

1. INTRODUCTION

The *SCN1A* gene encoding the voltage-gated sodium channel Nav1.1 is the target of thousands of pathogenic variants responsible for a broad spectrum of neurological phenotypes.[1-5] Dravet syndrome (DS) remains the prototypic developmental and epileptic encephalopathy (DEE) linked to de novo *SCN1A* pathogenic variants. Less than 10% of variants are inherited from unaffected or mildly affected parents whose phenotypes fall within the genetic epilepsy with febrile seizures plus (GEFS+) spectrum.[6] Rare patients carrying *SCN1A* pathogenic variants have been classified as early-onset DEE with movement disorders and, more recently, neonatal presentation with arthrogryposis.[7-10] In addition to epilepsy, *SCN1A* variants have also been linked to paroxysmal non-epileptic attacks, including hemiplegic migraine and neurodevelopmental disorders.[5,11]

The most common underlying epileptogenic mechanism is related to loss-of-function (LoF) of $Na_V1.1$, leading to hypoexcitability of GABAergic neurons and reduced inhibition.[1,2] This S*CN1A* haploinsufficiency[12] makes sodium-channel blocker (SCB) drugs contraindicated in these patients.[13] The clinical evidence that some DS patients have increased seizure frequency when treated with SCBs supports the experimental hypothesis.[14,15]

SCN1A pathogenic variants have also been found to cause $Na_V1.1$ gain-of-function (GoF) in patients with hemiplegic migraine and in several patients with neonatal and early-onset DEE.[10,16-19] Overall, current knowledge suggests that when the pathogenic variants cause $Na_V1.1$ GoF, the phenotype is a 'non-DS and non-GEFS+ disorder', and SCBs could be effective medications in these patients, counteracting excess function of $Na_V1.1$ and possible neuronal hyperexcitability.[10]

Our study aims to assess the phenotypes associated with *SCN1A* pathogenic variants alternative to DS and GEFS+ and to analyze the effect of treatments with antiseizure medications (ASMs), including SCBs, in 16 newly diagnosed and 44 previously reported patients. To gain insight into pathological mechanisms, we also performed functional analysis of three variants representative of the phenotypic spectrum of this cohort.

2. MATERIALS & METHODS

2.1 Study design and data collection.

A national multicenter retrospective observational cohort study was performed by selecting patients carrying *SCN1A* pathogenic variants presenting with phenotypes other than DS or GEFS+. Patients with a medical history of prolonged, focal clonic (hemiclonic) or myoclonic seizures triggered by fever/illness in the first year of life supposed to have a DS phenotype were excluded. Furthermore, patients with typical febrile seizures that continue beyond the age of 6 years, evolving into afebrile seizures, supposed to have a GEFS+ phenotype were excluded.[20]

Patients were collected by contacting tertiary epilepsy centers in Italy. Clinical and genetic data were collected from medical records using a specific form. Data were stored in a dataset sheet for analysis. EEG recordings and neuroimaging were reviewed by the expert epileptologist and neuroradiologist of the referral center.

We assessed: age, gender, family history, age at seizure onset, seizure type and frequency, episodes of status epilepticus, triggering factors, seizure off-set, duration of follow-up from seizure onset, EEG and MRI findings, treatment with ASMs and response to treatment, neurological findings, psychomotor development, and additional features.

Seizure types were classified according to the 2017 Operational Classification of the International League Against Epilepsy,[21] and seizure frequency was reported as daily, weekly, monthly, or sporadic. Seizure classification for each patient was based on a combined description of events from parents and caregivers, home video, and ictal video-EEG. Parents and caregivers compiled seizure diaries allowing determination of seizure frequency, worsening, and improvements.

We compared the median number of seizures in the 3 months preceding the introduction of SCBs with the median number of seizures following the drug introduction and the achievement of the therapeutic dose. Treatment with ASMs was classified as “effective” when leading to seizure freedom or to greater than 75% seizure reduction, “mildly effective” between 50 to 75%, “no

significant changes" with a reduction between 0-50%, and "non-effective" when seizures persisted at the same frequency or worsened.

Psychomotor development was defined as normal or delayed with mild, moderate, or severe impairment according to neurological examination and evaluation of main motor and cognitive milestones, including independent walking, speech development, and interactive skills.

2.2 Genetic analysis.

SCN1A disease-causing variants were identified through NGS epilepsy gene panels from different laboratories. We included in the study only patients whose variants were classified as "pathogenic" (Class-5) and "likely pathogenic" (Class-4), according to criteria detailed by Antoniadi et al., [22] and based on the Classification following the Association of Clinical Genetics Science Practice Guidelines.[23]

2.3 Functional study.

We used the cDNA of the shorter splice variant isoform (-11aa) of the human $Na_v1.1$ channel alpha subunit (GenBank database accession no. NM_006920.4). which we subcloned into the pCDM8 vector to minimize rearrangements[27] and that we used in numerous other studies[18,19,24-26]. The variants p.T162I, p.T1501A, and p.R1892L were introduced by site-directed with the Quick Change Lightning Kit (Stratagene). The entire open reading frame was sequenced after each amplification to avoid spurious mutations. We expressed wild-type (WT) or mutant $hNa_v1.1$ in the cell line tsA-201 (Sigma-Aldrich 96121229), which was maintained and transiently transfected with $CaPO_4$.[25] Cells were co-transfected with the pCDM8-$hNa_v1.1$ vector and a reporter vector expressing Yellow Fluorescent Protein (pEYFP-N1; Clontech) to identify the transfected cells for electrophysiological recordings. For some experiments, we co-transfected the human clones of β1 and β2 accessory subunits at equimolar ratio with the wild-type or mutant hNav1.1, using the bicistronic plasmids

pIRES-YFP-hβ1 and pIRES-CFP-hβ2, to express with the same plasmid both the protein of interest and the fluorescent protein as reported[18,25,26].

Sodium currents were recorded using the whole-cell configuration of the patch-clamp technique, as previously reported.[18,19,24-26] We started the recordings 5 min after establishing the whole-cell configuration (t_0), to allow a complete dialysis of the cytoplasm and stabilize the initial time-dependent shift of the voltage dependences; the different protocols were run in all the conditions at the same time points from t_0. Cells were recorded at room temperature (20-24°C) using a MultiClamp 700A amplifier and pClamp 10.2 software (Axon Instruments/Molecular devices). Signals were filtered at 10 kHz and sampled at 50 kHz. Electrode capacitance and series resistance were compensated during the experiment. Pipette resistance was 2-2.5 MΩ and maximal accepted voltage-clamp error was less than 2.5 mV. The remaining transient and leakage currents were eliminated using a P/4 subtraction paradigm. Recording solutions were (in mM): external solution 150 NaCl, 1 $MgCl_2$, 1.5 $CaCl_2$ and 10 HEPES (pH 7.4 with NaOH); internal pipette solution 105 CsF, 35 NaCl, 10 EGTA, 10 HEPES and (pH 7.4 with CsOH). Recordings were not corrected for junction potentials. Voltage dependence of activation was studied by applying test pulses of 100-ms from -110 to +60 mV from a holding potential at -120 mV. Voltage dependence of inactivation was studied with a 100-ms prepulse at different potentials followed by a test pulse at -10 mV. Conductance-voltage curves were derived from current-voltage (I-V) curves according to $G=I/(V-V_r)$, where I is the peak current, V is the test voltage, and V_r is the apparent observed reversal potential for tsA-201. The voltage dependence of activation and of inactivation were fit to Boltzmann relationships in the form $y=1/(1+\exp((V_{½}-V)/k)$, where y is normalized G_{Na} or I_{Na}, $V_{½}$ is the voltage of half-maximal activation (V_a) or inactivation (V_h) and k is a slope factor; for the inactivation curve, we included a baseline for taking into account incomplete inactivation.[1] The kinetics of current decay was quantified at -10mV, fitting the first 10 ms of the decay with a single exponential function. Recovery from fast inactivation was studied using a test pulse at 0 mV followed by repolarization at -80 mV of different duration and a test pulse to 0 mV. The persistent sodium current (I_{NaP}) was quantified about 5 minutes after

obtaining the whole-cell configuration as the mean current between 40 and 50 ms after the beginning of the voltage step and expressed as a percentage of the maximal transient current (I_{NaT}). Action potential clamp recordings were performed using as voltage stimulus a GABA-ergic neuronal discharge recorded from fast spiking basked cell injecting a 1s-long depolarizing current step as in Chever et al.[28]; the instantaneous firing frequency of the discharge was between 106 and 82Hz. The intersweep interval was 8s for all the protocols. Data were analyzed with pClamp 10.2 (Axon Instruments/Molecular devices) and Origin2021 (OriginLab).

For statistical analysis, normality was tested with the Kolmogorov-Smirnov test, homogeneity of variance with the Levene's Test, and statistical comparisons were performed with one-way ANOVA followed by Tukey post-hoc test. $P < 0.05$ was considered significant.

2.4 Literature review: PubMed search using the term "*SCN1A* gene mutations or pathogenic variants" and included in the analysis only studies reporting patient-relevant information and genetic findings, including epileptic phenotypes other than DS and GEFS+. Additional patients with phenotypes other than DS and GEFS+ reported in other studies lacking clinical information were excluded. Clinical and genetic data were collected in a separate dataset (Table S1 in Supplemental Material).

2.5 Standard Protocol Approvals and Patient Consents

This study was performed according to ethical principles for medical research involving human subjects stated in the Declaration of Helsinki. Patients or their legally authorized representatives consented to participate in the study. The study was approved by Marche Region Ethics Committee (CERM).

3. RESULTS

We enrolled 16 newly diagnosed patients (8 females and 8 males), with a median age of 10.2 years (interquartile range [IQR]: 17.5 months-19 years; 8 months–43 years) (table 1).

Eleven patients had de novo *SCN1A* pathogenic variants leading to neonatal/early infantile DEE with seizure onset at a median age of 2 months (IQR: 2 days–2.5 months; 1 day–5 months) in nine (patients 2, 3, 5, 8, 9, 10, 11, 12, 16), and infantile/childhood focal epilepsy (FE) in two, with seizure onset at 9 and 36 months (patients 4, 7). Four patients presented familial pathogenic variants causing childhood-onset FE (median 7.5 years; IQR: 4.5-10.5 years; 4-11 years), and an 18-month child with early infantile-onset FE carried a variant inherited from his affected father (Figure S1 in Supplemental Material).

The median follow-up after seizure onset was 7.7 years (IQR: 17 months–14.5 years; 5.5 months–33 years).

3.1 De novo *SCN1A* pathogenic variants

- Nine patients with neonatal and early infantile DEE presented with focal motor (9/9; 100%), tonic (8/9; 88.8%), myoclonic (8/9; 88.8%), and bilateral tonic-clonic seizures (9/9; 100%). Over time other seizure types were epileptic spasms (4/9; 44.4%), focal dysautonomic (4/9; 44.4%), and migrating seizures (2/9; 22.2%). Four had episodes of status epilepticus (SE) (44.4%). One patient died of sudden unexplained death in epilepsy (SUDEP) at 27 years. Four of them also had hyperkinetic movement disorder. All patients had daily seizures at onset; over time, seizures persisted but with periods of reduced frequency or seizure freedom in 77.7%.

  VEEG recordings revealed poorly organized background activity (BA) in most (6/9; 66.6%), with focal/multifocal discharges in 77.7% (7/9), migrating pattern in 22.2% (2/9), and diffuse epileptiform abnormalities in 22.2% (2/9).

  Brain MRI was unrevealing in 66.5% (6/9), delayed myelination was present in 22.2% (2/9), while one patient with a longer follow-up showed progressive brain atrophy (patient 11).

- Two patients with infantile/childhood FE presented with predominant focal motor seizures with a clonic component, usually of alternating side involvement, both had focal to bilateral seizures. Onset was characterized by daily/weekly seizures, while sporadic events persisted over time with long periods of better control. VEEG recording was characterized by focal/multifocal epileptiform discharges. Neuroimaging was unrevealing in both.

3.2 Familial SCN1A pathogenic variants

- The 18-month child (patient 1) carrying a familial p.V157I variant manifested focal seizures at one month of age and global delay with hypotonia. Seizures were rapidly controlled with phenobarbital (PB) which was withdrawn at 12 months. At 19 months, he was seizure-free off medication, able to walk, presented mild diffuse hypotonia, good non-verbal communication skills, and speech characterized by a limited number of single words. The repeated MRI was normal. He inherited the variant from his 34-year-old father, who had 3 seizures in the first year of life and is now healthy with normal cognition and motor function. *SCN1A* variant was also identified in the paternal uncle, who had few seizures in infancy; he is in his late twenties and leads a normal functioning, working life. Blood sample from additional family members was not available.
- Patient 6, harboring the p.N1338H variant inherited from her healthy father, manifested with refractory focal seizures at 11 years, both spontaneous and reflex to tactile stimuli causing frequent falls; repeated brain MRI revealed a possible blurring over the left frontal-mesial lobe.
- Two siblings and their father, harboring the p.R1892L variant, presented with childhood-onset sporadic focal motor seizures and auditory aura. VEEG recording revealed focal epileptiform abnormalities. Brain MRI was normal in all. NGS epilepsy gene panel excluded the presence of an LGI gene mutation.

None of the 16 patients presented classical or prolonged febrile seizures.

3.3 Treatment options and response

In this cohort, all patients but one received SCBs, which were effective or mildly effective in 73% of them. Carbamazepine (CBZ) was administered to twelve patients, and it was mildly effective in four with DEE and effective in five with childhood FE who gained seizure freedom for long periods, with sporadic relapses related to low plasma levels. Lacosamide (LCM) was administered to four patients, and three DEE were responders. Other effective treatments were VPA (5/11) and ACTH (2/4). One familial patient (patient 1) was seizure-free without medication. At the age of 25 years, patient 6 underwent stereo-EEG recording of focal seizures followed by left frontal-mesial lobectomy. Histology revealed only gliosis. Following the surgical procedure, the patient continued to have seizures at a reduced frequency and without falls; when CBZ was added, she had long periods of seizure freedom.

3.4 Development and additional features

Developmental delay (DD) was evident in the nine patients with neonatal/early infantile DEE who had periods of regression resulting in a moderate to severe disability at the last follow-up. The two patients with infantile/childhood FE and de novo *SCN1A* variants showed mild motor DD and borderline cognitive functioning. The two siblings and their father (patients 13-15) with childhood FE had normal cognitive functioning. The adult patient carrying a familial *SCN1A* variant (patient 6), despite a long history of focal refractory epilepsy and frontal lobectomy, had normal cognitive functioning. The familial patient (patient 1) with early-infantile FE at 18 months had a mild DD.

Four patients with neonatal/early infantile DEE (4/9; 44.4%) developed an early hyperkinetic movement disorder (MD), comprising hyperkinesia with perioral and upper limbs involvement, dystonic posturing, myoclonic jerks, and chorea. Additional features in DEE patients were hypotonia (3/9; 33.3%), pyramidal signs and spastic quadriplegia (4/9; 44.4%).

The three patients with familial childhood FE also suffered from migraine without aura.

3.5 Genetic analysis

The study includes thirteen *SCN1A* missense variants, ten are de novo, and three are inherited; eleven occur singularly, whereas two are recurrent, including the inherited variant identified in the affected siblings and father.

The p.R1636Q variant, detected in two, was previously reported in several other patients with variable phenotypes, including DS,[29,30] Lennox-Gastaut Syndrome,[31] and DEE, myoclonic seizures, dystonia, and spasticity.[10,32] At the same position but with a different amino-acid change, the p.R1636P variant detected in one of our patients was reported in a proband with EIMFS.[33] The p.T226M variant, detected in one, was associated with DEE in several other patients.[9,10] The p.A420D variant identified in one of our patients was reported by Butler et al.,[32] without phenotypic description. The p.T162I variant detected in one of our patients was associated with DS.[34] The p.N1338H variant was not previously reported, but a frameshift variant at position p.N1338 is associated with DS.[35] As expected, the frameshift mutation causing LoF is associated with a more severe, DS-like phenotype. Eight variants are novel and never reported.

3.6 Functional study

Whole-cell patch-clamp experiments were performed for variants p.T162I (neonatal DEE), p.T1501A (infantile FE), and p.R1892L (childhood FE). The mean values of the functional properties are shown in table 2. Figure 1 (A-L) displays representative current traces of WT and mutant channels elicited with depolarizing voltage steps. WT and mutants had similar maximal current density consistent with a similar plasma-membrane targeting (figure 1E). IV curves showed similar features for WT and mutants, but for p.T162I, whose current started to activate at more negative potentials (Figure S2 in Supplemental Material). Consistently, the activation curve of p.T162I was negatively shifted by 6.6mV, indicating a GoF (figure 1F-G). The slope of the activation curves of the mutants was similar to that of the WT (figure 1H). The inactivation curve of p.T1501A and p.R1892L was positively shifted by 9.5mV and 7.7mV respectively (figure 1I-J), consistent with a GoF, which is larger for p.T1501A than p.R1892L. The inactivation curve of p.T162I was not shifted, but it was steeper than

that of the WT, although this modification does not have clear functional relevance (figure 1I-J-K). The variant p.T1501A induced a faster recovery from fast inactivation, with 70% reduction in the time constant of recovery at -80 mV (figure 1L), which is consistent with a GoF. We evaluated the kinetics of activation and of decay of the current elicited with a depolarizing step to -10 mV (figure 2A-E). Activation kinetics was not modified. The exponential fit of the decay showed that the time constant of p.T1501A is 1.53-fold slower (figure 2D; table 2), consistent with a GoF. The variant p.T1501A was the only one modifying the amplitude of the persistent component of the current (INa_P), inducing a 3.94-fold increase (figure 2B, E), which is consistent with a GoF. To reproduce neuronal dynamic conditions during a series of action potentials and to evaluate the overall effect of the variants, we performed action potential-clamp experiments recording ~~Na^+~~ action currents in tsA-201 cells, using as voltage command the action potential discharge of a fast spiking GABAergic neuron (figure 3A-E). These experiments evaluated the overall effect of the variants, with the potential to identify dysfunctions that were not evident or not included (e.g., slow inactivation) in the classical parameters, which we previously characterized applying voltage steps. Our action potential clamp data correlated with the results obtained with the classical voltage step protocols. The amplitude of the first action current was not statistically significant, but p.T1501A showed a strong trend toward an increase, whereas p.T162I and p.R1892L showed a looser trend (figure 2F; table 2). The effect was more prominent at the end of the discharge because p.T1501A and p.T162I showed a 7.05 and 2.35-fold increase, respectively, and p.R1892L showed a strong trend towards an increase (figure 3G; table 2). Action potential-clamp experiments performed with cells co-expressing ~~with the alpha subunits~~ β1 and β2 accessory subunits showed similar features (Figure S3 in Supplemental Material), consistent with previous studies[18,25,26,36]. Our functional study was performed in tsA-201 cells. Further studies are warranted to better elucidate the effect of GoF $Na_V1.1$ epilepsy variants on neuronal excitability including exploiting gene targeted animal models. A neuronal cell background could modify functional effects, increasing them, possibly in a variant-specific manner. However, in our previous studies ~~comparing~~ functional properties in tsA-201 cells and cortical neurons in culture,

we observed that a neuronal cell background was able to rescue some folding/trafficking defective $Na_v1.1$ mutants increasing current density/membrane targeting, but did not significantly modify the effect that $Na_v1.1$ variants had on gating properties[18,19,37]. Thus, since the GoF $Na_V1.1$ epilepsy variants that we studied do not reduce current density/membrane targeting, it is likely that their overall functional effect is similar in a neuronal background.

3.5 Literature review

We identified 44 unrelated subjects (18 females; 26 males) from ten previous studies (Table S1). Clinical details were available for re-analysis for 42 probands with phenotypes other than DS and GEFS+ (Table 3).[7-10,38-42] Forty patients carried *SCN1A* de novo missense variants, and the remaining four had de novo deletions of chromosome 2q24.3.

Seventeen patients had neonatal DEE, associated with hyperkinetic movement disorder in seven. Eleven patients also had arthrogryposis, multiple contractures, hip dysplasia and talipes, two additional subjects who died in utero showed prenatal detection of arthrogryposis. Twenty-five patients presented with early infantile DEE, with and without MD. Multiple seizure types persisted over time and were refractory to all prescribed ASMs. Treatment details were reported for thirty-eight patients. Commonly prescribed ASMs included BZP (73.6%), PB (55.2%), LEV (65.7%), TPM (52.6%), VPA (63.1%), STP (38.3%), VGB (20.5%), cannabidiol (23.6%), fenfluramine (13.1%), acetazolamide (11.7%), zonisamide (8.8%), ACTH (8.8%), perampanel (5.8%), pyridoxine (5.8%), gabapentin (7.9%), rufinamide (5.8%), ESM (2.9%), felbamate (2.9%), cannabis oil (2.9%), and bromide (2.9%). Thirty-two patients received more than one SCB (15 CBZ, 16 PHT, 9 LTG, 11 oxcarbazepine, 3 LCM) without seizure aggravation. Carbamazepine was effective in 46.6% (7/15; two neonatal DEE, and five early infantile DEE), PHT in 50% (8/16; three neonatal DEE, five early infantile DEE), LTG in 33.3% (3/9; early infantile DEE), oxcarbazepine in 63.6% (7/11; 5 neonatal DEE, 2 early infantile DEE), LCM in 100% (3/3; neonatal DEE)

4. DISCUSSION

This study, including clinical and functional analysis of 16 novel patients and re-analysis of 44 previously reported subjects carrying *SCN1A* disease-causing variants with GoF effect, refines the clinical spectrum beyond the classical DS and GEFS+, increases the number of patients with *SCN1A*-related phenotypes, and describes their response to ASMs.

4.1 *SCN1A* and DEE phenotypes

Nine subjects, all carrying *de novo* variants, manifested a DEE. They exhibited early infantile focal clonic, tonic, myoclonic, and bilateral tonic-clonic seizures. Four patients also had spasms or focal dysautonomic seizures, and two presented migrating seizures. Unlike DS, these patients had multiple seizures per day at onset and concomitant cognitive impairment. Indeed, motor development and cognitive functions were impaired, ranging from mild to severe. Impaired cognition was present from the first weeks of life, confirming that *SCN1A* disease-causing variants underlie seizures and abnormal development. However, seizures, especially when very frequent and associated with an abnormal EEG, worsen motor and cognitive development. Our patient carrying the p.R1636Q variant presented a mild delay at the onset, when seizures were controlled with CBZ. At 14 months, she went through an 'encephalopathic phase', seizures reappeared, and EEG evolved to a continuous spike-wave pattern during sleep and wakefulness, accompanied by a decline of cognitive skills. Treatment with steroids ended the 'encephalopathic phase', leading to the re-acquisition of motor and cognitive abilities.

Approximately 40% of DEE patients developed an early MD, including hyperkinesia with perioral and upper limbs involvement, dystonic posturing, myoclonic jerks, and chorea.

4.2. *SCN1A* variants and focal epilepsy

Seven patients manifested focal epilepsies. Two carried de novo *SCN1A* variants, whereas five were inherited. Focal epilepsies presented from early infancy to childhood with focal motor, focal to bilateral, focal with auditory aura, and reflex seizures. Only two patients have ongoing sporadic seizures. Cognitive functioning was normal in five, and two showed mild DD. Two siblings and

their father also presented with sporadic episodes of migraine without aura that might be related to the *SCN1A* variant in this family.

4.3 Literature review and comparison of cohorts

The literature review identified 44 patients, 42 with available clinical information and carrying *SCN1A* pathogenic variants not associated with DS or GEFS+.[7-10,38-42] Common findings with our cohort include: a) early-onset seizure; b) frequent focal and focal to bilateral seizures; c) epileptic spasms occurring only in a quarter of patients. Unlike our cohort, migrating seizures are more prominent and observed in about 40%. Eleven patients with neonatal DEE had arthrogryposis, multiple contractures, and hip dysplasia and talipes, and two additional patients who died in utero showed prenatal detection of arthrogryposis. None of the patients included in our cohort manifested arthrogryposis. Status epilepticus presented in most, and premature death occurred in 21% of reported patients. In our series, only one patient died of SUDEP at 27 years. All patients but one had severe or profound DD with hyperkinetic MD and hypotonia in the majority of them.

One of the main objectives of our study was to investigate the response to ASMs, especially to SCBs. Data analysis showed a striking, 'unexpected', response to SCBs, considering the genetic etiology and previous reports.[14-16] More than 70% of patients included in our cohort responded to SCBs, comprising four with childhood FE who were seizure-free for long periods. The remaining patients treated with SCBs were not aggravated. In the previously reported patients, 76% received more than one SCBs without seizure aggravation, and about 50% of them were responders.

We performed functional studies on three *SCN1A* variants representative of the phenotypic spectrum of this cohort, observing a clear GoF for all of them. The variant p.T1501A showed the largest effect, inducing GoF modifications in five biophysical parameters, whereas p.T162I and p.R1892L modified a single parameter. The analysis of the overall effect of the variants on the function of $Na_V1.1$ by means of action-potential-clamp experiments confirmed that p.T1501A induces the largest GoF, followed by p.T162I and p.R1892L. Functional effects do not correlate with phenotype severity since the most severe among the three tested variants is associated with

p.T162I, which causes neonatal DEE. The GoF induced by these variants is moderate compared to those causing hemiplegic migraine,[11,18,19,25,43] confirming that the amount of GoF is not directly correlated to phenotype severity. All three patients carrying the GoF tested variants, associated with different phenotypes with variable severity, shared a beneficial response to therapeutic doses of SCBs, supporting our hypothesis that they could be effective medications counteracting channel excess of function and possible neuronal hyperexcitability.

Two additional *SCN1A* pathogenic variants identified in our cohort (T226M and R1636Q) were recently found to cause moderate GoF[9,10,42], similarly to the variants that we have characterized here. Overall, five of the thirteen *SCN1A* variants detected in our cohort lead to a moderate GoF. There is a striking genotype-phenotype correlation in patients carrying the recurrent p.R1636Q variant, because they all showed early-onset DEE with variable seizure semiology and manifested a favorable response to SCBs treatment.

4.4 Loss-of-function vs gain-of-function *SCN1A* variants: is there a functional effect-phenotype correlation?

Numerous functional studies of *SCN1A* missense variants have demonstrated a predominant LoF, which is expected to result in hyperexcitability of neuronal networks because $Na_V1.1$ channels are particularly important for the function of inhibitory interneurons.[1,44,45] Available data suggest that complete LoF variants leading to haploinsufficiency are more likely to cause severe DS phenotype, whereas phenotypes might be milder and fall within the GEFS+ spectrum when the LoF is partial, [27,45-47] but seizures may worsen the phenotype.[48] Although strong LoF is considered the main effect, there are studies that have observed mixed GoF and LoF effects for a few DS missense variants.[44,49] The pharmacological implication of the discovery of pathogenic *SCN1A* haploinsufficiency in DS has contributed to the clinical management of DS and GEFS+ patients. In general, SCBs like CBZ, PHT, and LTG are not effective or can possibly worsen seizures; thus, all guidelines advise their discontinuation in patients with DS and *SCN1A* variants.[14,50,51]

Instead, most *SCN1A* GoF variants cause a spectrum of disorders beyond the DS/GEFS+ dichotomy, which extends from mild focal epilepsies to hemiplegic migraine and to severe DEE.[10,16,18,19,25,28] Few studies have described GEFS+ *SCN1A* variants causing $Na_V1.1$ GoF,[52,53] although this finding is controversial.[2] The pharmacological implication of this observation is that GoF-associated phenotypes might benefit from the treatment with SCBs counteracting the increased function of $Na_V1.1$ channels ([10], present study).

More studies are needed to understand how the moderate GoF observed in $Na_V1.1$ variants can cause such a wide phenotypic range, including very severe phenotypes. We might, however, speculate that the larger GoF of hemiplegic migraine variants could block seizure onset because it induces strong hyperexcitability of GABAergic neurons leading to extracellular K^+ accumulation and spreading depolarization of the entire neuronal network, as we have previously shown [28,54]. Notably, spreading depolarization has been proposed as a general antiseizure mechanism[55,] and the milder hyperexcitability induced by GoF $Na_V1.1$ epilepsy variants would not be sufficient to induce it. Phenotypic variability in patients might also be ~~conditioned~~ by interactions with other genetic variants, as recently observed in a family with large phenotypic variability[56]. Additional source of functional and phenotypic variability might be the recently observed dimer interaction between Na_V alpha subunits[57] which might modify the functional properties of homomeric or heteromeric dimers, possibly in a variant-specific manner.

5. CONCLUSION

Clinical analysis of the 16 plus 40 patients included in the study highlights that the boundaries of GoF *SCN1A* disorders are wider than previously thought, although it is not clear yet how this functional effect can cause these phenotypes. Seizures can arise from the first day of life, be very severe from the onset and occur without triggers. On the other extreme, pathogenic variants are associated with childhood-onset, mild epilepsies, and normal cognition. Overall, focal epilepsies

were diagnosed in 43.7% of probands, suggesting that focal seizures are a major clinical feature of our cohort. This result supports the recommendation to include *SCN1A* testing in the diagnostic workup of pediatric, familial and non-familial, focal epilepsies. This phenotypic evidence is in line with a recent paper reporting an *SCN1A* gene overexpression in brain tissue of temporal lobe epilepsy patients with a specific genotype (rs7587026)[58].

A relevant observation of our study is that it 'unmasks the myth' that SCBs should not be used in patients with *SCN1A* disease-causing variants. There are patients in whom, due to the type of variant causing a GoF, SCBs might be beneficial because the variant they carry induces GoF, and functional analysis to evaluate the consequences of variants can be relevant in some cases to tailor the appropriate ASM.

Ethical Publication Statement

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.

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

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Disclosure of Conflicts of Interest.

None of the authors has any conflict of interest to disclose.

Authors' contribution:

Sara Matricardi, Sandrine Cestèle, Massimo Mantegazza, and Carla Marini conceptualized and designed the study. Sara Matricardi, Marina Trivisano, Benedetta Kassabian, Roberta Vittorini, Margherita Nosadini, Elisabetta Cesaroni, Sabrina Siliquini, Cristina Marinaccio, Francesca Longaretti, Barbara Podestà, Francesca Felicia Operto, Concetta Luisi, Stefano Sartori, Clementina Boniver, Nicola Specchio, Federico Vigevano, and Carla Marini selected and enrolled patients, critically reviewing all medical charts and records. Sandrine Cestèle, Nathalie Leroudier, and Massimo Mantegazza performed the functional analysis. Sara Matricardi and Carla Marini performed the literature review and were involved with the dataset and analysis. Sara Matricardi, Sandrine Cestèle, Massimo Mantegazza, and Carla Marini drafted the manuscript. All the Authors are responsible for each case data collection and diagnostic process. All Authors edited the manuscript.

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Figure 1. Representative whole-cell sodium current families for $hNa_v1.1$-WT (**A**), $hNa_v1.1$-T162I (**B**), $hNa_v1.1$-T1501A (**C**), $hNa_v1.1$-R1892L (**D**) recorded with 100-ms-long depolarizing voltage steps from -80 to +60 mV in 5 mV increments from a holding potential of -100 mV. (**E**) Current density calculated for cells transfected with the $hNa_v1.1$-WT or with the mutant channels. (**F**) Mean voltage dependence of activation, the lines are mean Boltzmann fits. Comparison of the voltage of half activation $V_{1/2}$ (**G**) and of the activation slope factor, k_a (**H**) obtained from the Boltzmann fits. (**I**) Mean voltage dependence of inactivation, the lines are Boltzman fits. Comparison of the voltage of half inactivation (**J**) and of the inactivation slope factor, k_h, (K) obtained from the Boltzmann fits. (**L**) Time constant (in ms) of the recovery from fast inactivation at -80 mV, obtained from the exponential fits of recovery curves.

Figure 2. Comparison of mean normalized currents elicited with a 150-ms-long depolarizing step to -10 mV from a holding potential of -100 mV. (**A**) $hNa_v1.1$-WT (black), $hNa_v1.1$-T162I (red), (**B**) $hNa_v1.1$-WT (black), $hNa_v1.1$-T1501A (blue), (**C**), $hNa_v1.1$-R1892L (green). The left insets show a 9-ms window of the current traces in order to compare the activation kinetics and the current decay; the right insets show the traces between 70 and 80 ms to compare the INa_P. (**D**) Comparison of the time constants (tau in ms) of the current decay at -10 mV (single exponential fits at the indicated potential). (**E**) Comparison of the persistent current quantified as mean current between 70 and 80 ms of the 150-ms-long depolarizing step (expressed as % of I_T max).

Figure 3. Action sodium currents recorded using as voltage stimulus an action potential discharge of a GABAergic fast spiking neuron, shown in panel (**A**). Action ~~Na^+~~ currents for $hNa_v1.1$-WT (**B**), $hNa_v1.1$-T162I (**C**), $hNa_v1.1$-T1501A (**D**), $hNa_v1.1$-R1892L (**E**); traces are presented as mean current densities (CD), for clarity error bars are not shown. (**F**) Comparison of the peak current density of the first action current. (**G**) Comparison of the peak current density at the end of the discharge (mean of the 3 last action currents).

Figure S1 in Supplemental Material. The pedigrees of inherited variants.

Figure S2 in Supplemental Material. Current density(CD)-voltage curves of peak sodium current for hNa$_v$1.1-WT, hNa$_v$1.1-T162I, hNa$_v$1.1-T1501A and hNa$_v$1.1-R1892L. Data points are displayed as mean±SEM.

Figure S3 in Supplemental Material. Action sodium currents recorded using as voltage stimulus an action potential discharge of a GABAergic fast spiking neuron, shown in panel (**A**)~~, in tsA-201 cells co-expressing hβ1 and hβ2 subunits~~. Action ~~Na$^+$~~ currents for hNa$_v$1.1-WT+ hβ1+hβ2 (**B**), hNa$_v$1.1-T162I+ hβ1+hβ2 (**C**), hNa$_v$1.1-T1501A+ hβ1+hβ2 (**D**), hNa$_v$1.1-R1892L+ hβ1+hβ2 (**E**); traces are displayed as mean current densities (CD), for clarity error bars are not shown. (**F**) Comparison of the peak current density of the first action current. (**G**) Comparison of the peak current density at the end of the discharge (mean of the 3 last action currents).

Table 1: Clinical and genetic features of the 16 novel, unpublished, patients

Patient ID	*SCN1A* variant	Current age & Gender	Age at sz onset	Follow-up	Sz type	Epilepsy Syndrome	ASMs prescribed & other treatments	Effective treatments	Sz off-set	Development, other features
1 *	c.469G>A; p.V157I; familial	19m M	1 m	14m	F motor	Early Infantile FE	PB	PB	2m; sz freedom no ASM	Mild DD
2	c.485C>T; p.T162I	13y 8m F	1 d	13y 8m	F motor; F to bilateral; Tonic; Myoclonic; Spasms	Neonatal DEE	PB, CBZ, CLB, VGB, VPA, LEV, ESM, PER	CBZ	Sz reduction	Severe ID, Spastic quadriplegia
3	c.677C>T; p.T226M	8m M	2.5 m	5.5m	F motor; F to bilateral; Tonic; Myoclonic; F dysautonomic; spasms; SE	Early Infantile DEE	PB, Pyridoxine, CBZ, NZP, PHT, LEV, VPA, CLB, STP	VPA+CLB+ STP	Sz reduction	Severe DD, Hypotonia, Hyperkinetic MD
4	c.1172C>T; p.T391I	10y 1m F	36 m	7y	F motor; F to bilateral;	Childhood FE	VPA, LEV, CBZ, ACZ, CLB, ESM, TPM	CBZ, TPM	Sz freedom	Mild ID
5	c.1259C>A; p.A420D	12m F	2 m	10m	F motor; F to bilateral; F dysautonomic; Migrating; Tonic; Myoclonic, SE	Early Infantile DEE	Pyridoxine, CBZ, LEV, LCM, MDZ, PHT, VGB, VPA, ACTH, KD, PB, STR, CLB	CLB, PB, VPA	Sz reduction (last sz 3/12/20 due to VPA discontinuation)	Severe DD, Hyperkinetic MD
6 *	c.4012A>C; p.N1338H; familial	39y F	11 y	15y	F motor; F to bilateral; reflex sz	Childhood FE	CLB, CNZ, CBZ, OXC, VGB, ZNS, PER, LCS, PB, PHT, LEV, ESM, LTG, VPA, TPM; FLB, VGB, KD, VNS, Epilepsy Surgery	CBZ, VPA, KD	Sz reduction	Normal Cognitive functioning
7	c.4501 A>G; p.T1501A	24y M	9 m	23y 3m	F motor; F to bilateral	Infantile onset FE	VPA, LEV, LCM, CBZ	LCM, VPA	Transitory sz freedom	Borderline cognitive functioning
8	c.4907 G>A; pR1636Q	1y 8m F	1 m	1y 7m	F motor; Tonic; Myoclonic, F dysautonomic	Early Infantile DEE	Pyridoxine; PB, VPA, CBZ, CLB, ACTH	CBZ, ACTH	Prolonged Sz freedom	Moderate DD, Hyperkinetic MD, Hypotonia
9	c.4907 G>C; p.R1636P + c.3386C>T; p.T129M	1y 3m F	2 d	1y 3m	F motor; Tonic; Myoclonic; F dysautonomic; Migrating; SE	Neonatal DEE	Pyridoxine; PLP, PB, LEV, PHT, MDZ, Thiopental, Ketamine, LCM, VPA, CLB	LCM, VPA	Sz reduction	Severe DD, Hypotonia
10	c.4907 G>A; p.R1636Q	14y 3m M	2 d	14y 1m	F motor; F to bilateral; Tonic; Myoclonic	Neonatal DEE	PB, CBZ, CLB, VPA, CNZ, HC, LEV, ESM, LTG	CBZ, VPA	Sz reduction	Severe ID, Pyramidal signs
11	c.5006C>A; p.A1669E	10y 3m M	5 m	9y 10m	F motor; F to bilateral; Tonic; Myoclonic, Spasms	Early infantile DEE	Pyridoxine, LEV, TPM, VGB, VPA, CBZ, ACTH, HC, PB, ESM, CLB	None	Ongoing sz	Severe ID, Spastic quadriplegia, Hyperkinetic MD
12	c.5020G>C; p.G1674C	Deceased at 27y; M	2 m	26y10m	F motor; F to bilateral; Tonic; Myoclonic, SE	Early infantile DEE	Pyridoxine, LEV, PB, LTG, VPA, CNZ	None	Ongoing sz	Severe ID, Spastic quadriplegia
13*	c.5675G>T p.R1892L; familial	10y 11m M	5 y	5y 11m	F motor; F to bilateral;	Childhood FE	CBZ	CBZ	Sz freedom	Normal Cognitive functioning; Migraine
14*		8y 6m F	4 y	4y 6m	F motor	Childhood FE	CBZ	CBZ	Sz freedom	Normal Cognitive functioning; Migraine
15*		43y 1m M	10 y	33y 1m	F motor; F to bilateral;	Childhood FE	PB, CBZ	CBZ	Sz freedom	Normal Cognitive functioning; Migraine
16	c.5732 A>G; p.K1911R	8y 9m F	3 m	8y 6m	F motor; F to bilateral; spasms	Early Infantile DEE	VGB, ACTH, VPA, NTZ, HC, TPM, CLB, PDN, PER, CNZ, LZP, LTG, KD, LCM	ACTH, CBZ, LCM	Sz reduction	Moderate ID, ADHD

Abbreviations: ACTH: adrenocorticotropic hormone; ACZ: acetazolamide; ADHD: attention deficit hyperactivity disorder; ASM: antiseizure medication; CBZ: carbamazepine; CLB: clobazam; CNZ: clonazepam; d: days; DD: developmental delay; DEE: developmental epileptic encephalopathy; ESM: ethosuximide; F: focal; FE: focal epilepsy; FLB:

felabamate; HC: hydrocortisone; ID: intellectual disability; KD: ketogenic diet; m: months; MD: movement disorder; MDZ: midazolam; LCM: lacosamide; LEV: levetiracetam; LTG: lamotrigine; LZP: lorazepam; NTZ: nitrazepam; PB: phenobarbital; PDN: prednisone; PER: perampanel; PLP: pyridoxal phosphate; Sz: seizures; TPM: topiramate; VPA: valproate; VGB: vigabatrin; VNS: vagus nerve stimulation; y: years. *= familial pathogenic SCN1A variants

	WT	T162I	T1501A	R1892L
Current Density (pA/pF)	153.6±20.3 (n=16)	147.0±11.7 (n=11)	115.0±10.5 (n=13)	111.1±15.6 (n=9)
Va (mV)	-27.6±1.2 (n=16)	**-34.2±0.9** (n=11) $p = 3*10^{-4}$	-30.3±0.8 (n=13)	-24.9±1.2 (n=9)
Ka (mV)	6.9±0.4 (n=16)	6.7±0.3 (n=11)	7.2±0.4 (n=13)	7.6±0.5 (n=9)
Vh (mV)	-63.3±0.9 (n=13)	-61.7±1.1 (n=11)	**-53.8±1.1** (n=13) $p = 3*10^{-7}$	**-55.6±1.3** (n=9) $p = 9*10^{-5}$
Kh (mV)	6.3±0.3 (n=13)	**4.9±0.2** (n=11) p = 0.008	5.5±0.3 (n=13)	6.8±0.4 (n=9)
τ Recovery from Fast Inactivation at -80mV (ms)	3.1±0.3 (n=6)	2.1±0.2 (n=6)	**0.93±0.04** (n=9) $p = 3*10^{-6}$	2.7±0.4 (n=6)
τ Decay at -10mV (ms)	0.53±0.02 (n=16)	0.48±0.02 (n=11)	**0.81±0.04** (n=13) $p < 3*10^{-10}$	0.53±0.02 (n=9)
INa_P (%INa_Tmax)	1.6±0.3 (n=16)	2.2±0.4 (n=11)	**6.3±0.3** (n=13) $p < 10^{-10}$	1.5±0.2 (n=9)
CD First peak AP discharge (pA/pF)	-30.9±6.7 (n=9)	-50.5±10.3 (n=8)	-53.4±5.2 (n=9)	-41.2±5.3 (n=9)

			(p=0.054)	
Mean CD last 3 peaks AP discharge (pA/pF)	-4.9±0.6 (n=9)	**-11.5±1.7** (n=9) p = 0.027	**-12.7±1.8** (n=9) p = 0.004	-10.2±1.7 (n=9) *(p=0.09)*
CD First peak AP discharge with β1 & β2 (pA/pF)	-32.6±7.3 (n=7)	-50.9±5.9 (n=6)	-56.1±5.6 (n=7) *(p=0.062)*	-43.4±6.2 (n=7)
Mean CD last 3 peaks AP discharge with β1 & β2 (pA/pF)	-5.2±0.9 (n=7)	**-12.1±1.7** (n=6) p = 0.029	**-15.2±2.1** (n=7) p = 0.0007	**-11.2±1.4** (n=7) p=0.048

Table 2. Functional properties of sodium currents recorded from tsA-201 cells. In bold the statistically significant differences in comparison with the WT (see Figures for all the statistical comparisons).

Table 3 Clinical finding of 42 probands reported in literature

Males		24
Females		18
Age at seizure onset		Median: 5 weeks (range: 1 day–16 weeks)
Age at the time of the study		15 months (range: 14 days–19 years)
Length of the follow-up after seizure onset		13 months (range 21 days–18.8 years)
Epilepsy type	*Neonatal DEE*	17: 40.4%
	Early infantile DEE	25: 59.5%
Seizure Types	*Focal motor*	15: 35.7%
	Focal to bilateral	15: 35.7%
	Generalized Tonic-Clonic	14: 33.3%
	Tonic	32: 76.1%
	Myoclonic	20: 47.6%
	Focal with dysautonomic features	8: 19%
	Spasms	5: 11.9%
	Migrating	11: 26.2%
Seizures triggered by fever		13: 30.9%
Status Epilepticus		28: 66.6%
SUDEP		3: 7.1%
EEGs	Focal epileptiform activity	17: 40.4%
	Multifocal epileptiform activity	31: 73.8%
	Migrating pattern	7: 16.6%
	Diffuse epileptiform activity	12: 28.5%
Brain MRI	Normal	27: 64.2%
	Nonspecific abnormalities: dysmorphic corpus callosum, small hippocampi, arachnoid cyst, pineal cyst	10: 23.8%
	Progressive brain atrophy	11: 26.2%
Cognitive functioning	Severe/Profound global impairment	41: 97.6%
Additional features	Hyperkinetic MD	22: 52.3%
	Hypotonia	20: 47.6%
	Microcephaly	11: 26.2%
	Arthrogryposis	11: 26.2%
	Stiffness, clubfeet, hip dysplasia, multiple contractures, and talipes	1: 2.3%

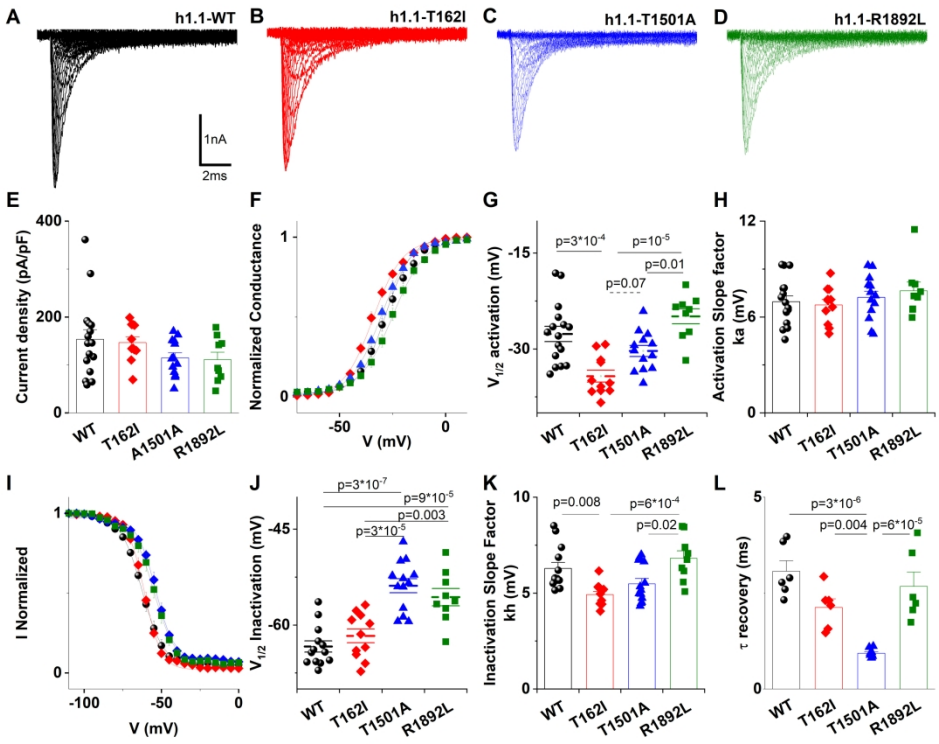

Figure 1. Representative whole-cell sodium current families for hNav1.1-WT (A), hNav1.1-T162I (B), hNav1.1-T1501A (C), hNav1.1-R1892L (D) recorded with 100-ms-long depolarizing voltage steps from -80 to +60 mV in 5 mV increments from a holding potential of -100 mV. (E) Current density calculated for cells transfected with the hNav1.1-WT or with the mutant channels. (F) Mean voltage dependence of activation, the lines are mean Boltzmann fits. Comparison of the voltage of half activation V1/2 (G) and of the activation slope factor, ka (H) obtained from the Boltzmann fits. (I) Mean voltage dependence of inactivation, the lines are Boltzman fits. Comparison of the voltage of half inactivation (J) and of the inactivation slope factor, kh, (K) obtained from the Boltzmann fits. (L) Time constant (☐ in ms) of the recovery from fast inactivation at -80 mV, obtained from the exponential fits of recovery curves.

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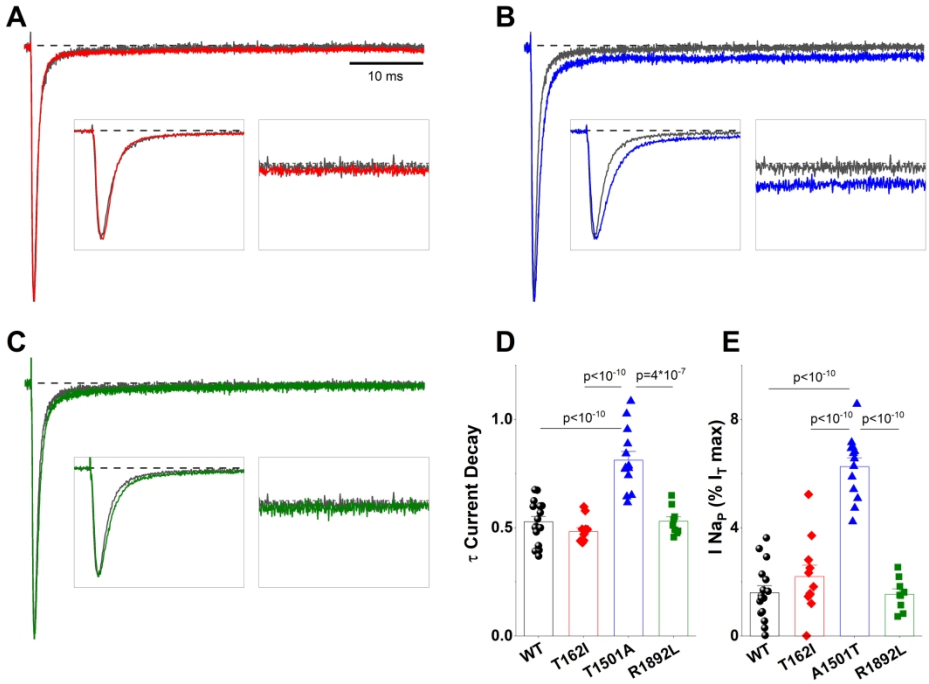

Figure 2. Comparison of mean normalized currents elicited with a 150-ms-long depolarizing step to -10 mV from a holding potential of -100 mV. (A) hNav1.1-WT (black), hNav1.1-T162I (red), (B) hNav1.1-WT (black), hNav1.1-T1501A (blue), (C), hNav1.1-R1892L (green). The left insets show a 9-ms window of the current traces in order to compare the activation kinetics and the current decay; the right insets show the traces between 70 and 80 ms to compare the INaP. (D) Comparison of the time constants (tau in ms) of the current decay at -10 mV (single exponential fits at the indicated potential). (E) Comparison of the persistent current quantified as mean current between 70 and 80 ms of the 150-ms-long depolarizing step (expressed as % of IT max).

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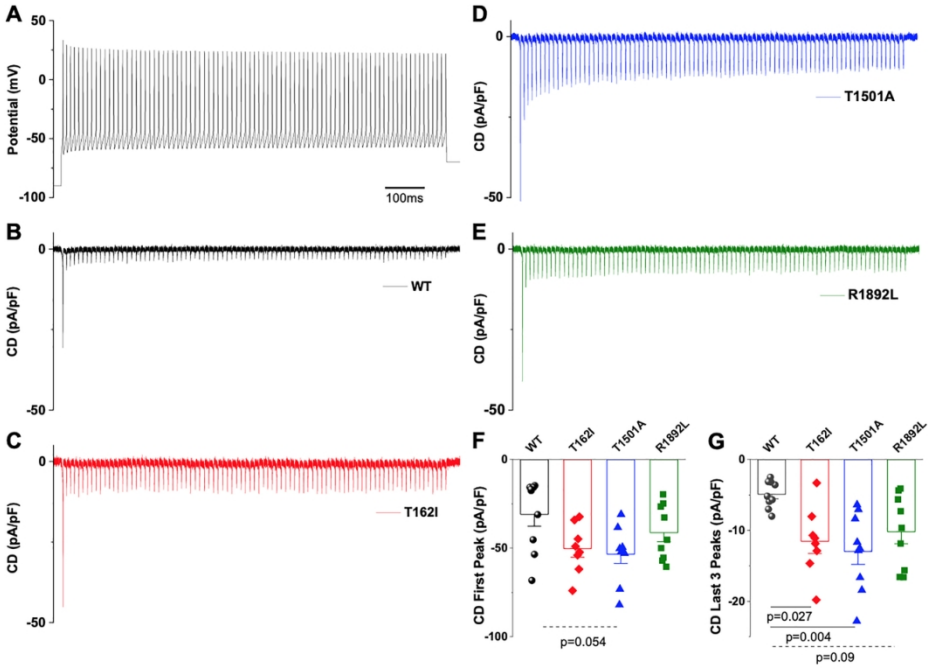

Figure 3. Action sodium currents recorded using as voltage stimulus an action potential discharge of a GABAergic fast spiking neuron, shown in panel (A). Action ~~Na+~~ currents for hNav1.1-WT (B), hNav1.1-T162I (C), hNav1.1-T1501A (D), hNav1.1-R1892L (E); traces are presented as mean current densities (CD), for clarity error bars are not shown. (F) Comparison of the peak current density of the first action current. (G) Comparison of the peak current density at the end of the discharge (mean of the 3 last action currents).

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