Received: 17 June 2025 | Revised: 10 December 2025 | Accepted: 31 December 2025

DOI: 10.1002/epi.70100

Epilepsia®

RESEARCH ARTICLE

Mechanisms of *SCN2A* loss of function do not predict presence or phenotype of epilepsy

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Funding information
RogCon; Murdoch Children's Research Institute (MCRI); Victorian State Government Operational Infrastructure Support Program; Medical Research Future Fund (MRFF), Grant/Award Number: GHFMCDI000002; Praxis Precision Medicines, Inc.

Abstract

Objective: *SCN2A* loss-of-function (LoF) variants are associated with epilepsy (onset age ≥ 3 months), intellectual disability (ID), and autism spectrum disorder (ASD). Despite numerous identified variants and the description of phenotypic subgroups, relationships between $Na_v1.2$ channel dysfunction and clinical phenotypes remain unclear. This study examined how distinct LoF mechanisms relate to phenotypic outcomes.

Methods: Whole-cell patch-clamp electrophysiology was used to characterize 15 presumed LoF *SCN2A* variants. Mechanism–phenotype correlations were assessed in 33 patients with these variants (six recurrent) and 41 patients with 15 previously characterized LoF variants (four recurrent). Phenotypic subgroups were categorized as later onset epilepsy–midinfancy (onset between 3 and 18 months), later onset epilepsy–childhood (onset after 18 months), ID/ASD without epilepsy, and "other" for unclassified cases.

Results: Of the 15 electrophysiologically characterized *SCN2A* variants, 11 caused total $Na_v1.2$ LoF, three caused partial LoF, and one showed mixed LoF and gain-of-function (GoF) effects. Among previously published variants, seven showed total LoF, five partial LoF, and two mixed LoF/GoF, and one was undetermined. Across both cohorts, seven of 10 recurrent variants (70%) were associated with multiple phenotypic subgroups. Partial or total $Na_v1.2$ LoF variants were identified in all subgroups. Notably, a midinfancy epilepsy phenotype was observed in 22 of 24 individuals (92%) carrying a mixed LoF variant,

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with phenotype data unavailable for seven additional individuals. A novel LoF-associated phenotype—episodic ataxia with or without developmental delay or ID—was identified in five of six individuals with the L1650P variant. Although episodic ataxia has been previously associated with GoF variants in *SCN2A*, this is the first reported instance in individuals with a confirmed LoF variant.
Significance: Distinct *SCN2A* LoF phenotypes cannot be reliably linked to specific biophysical mechanisms, as both total and partial $Na_v1.2$ LoF occurs across diverse phenotypes. For efficient personalized treatment, it is crucial not to rely solely on clinical phenotype to predict the underlying LoF mechanism.

KEYWORDS
clinical phenotype, de novo variant, epilepsy, loss of function, $Na_v1.2$ channel, patch-clamp, *SCN2A*

1 | INTRODUCTION

SCN2A encodes the voltage-gated sodium channel $Na_v1.2$, a key protein in brain development and the initiation and propagation of neuronal action potentials.[1] Variants in *SCN2A* are associated with various neurodevelopmental disorders, driven by either gain or loss of function (GoF or LoF) of the $Na_v1.2$ channel.[2–5] GoF variants typically cause seizure onset before 3 months of age, leading to early onset epilepsies of varying severity.[6] These forms often respond well to sodium channel-blocking (SCB) antiseizure medications (ASMs).[3]

In contrast, LoF variants are associated with later onset (LO) epilepsy phenotypes, as well as intellectual disability (ID) and/or autism spectrum disorder (ASD) without epilepsy (ID/ASDwoE). Epilepsies due to LoF variants typically have onset at or after 3 months of age. For these LO cases, SCB treatment is rarely effective and may even exacerbate the condition.[3]

Overall, three LO/LoF phenotypic subgroups have been described: LO epilepsy–midinfancy (LO-MI), LO epilepsy–childhood (LO-C), and ID/ASDwoE. The epilepsy in people with LO-MI is a developmental and epileptic encephalopathy (DEE) with onset in mid–late infancy (typically as infantile epileptic spasms syndrome [IESS]). In people with LO-C, epilepsies are more heterogeneous and can be DEEs or milder epilepsies in individuals with ID and/or ASD.[3,5–8] Although the presence and type of epilepsy varies between phenotypic subgroups, ID and/or ASD are common to all. Communication abilities and adaptive function are similarly severely impacted across subgroups, but motor abilities vary, with significantly lower levels of function in the LO-MI subgroup.[6] Other clinical features may also be seen, some in all subgroups (e.g., hypotonia, gastrointestinal symptoms) and some predominantly in one subgroup (e.g., choreoathetosis in the LO-MI subgroup).[9]

Key points

- *SCN2A* LoF phenotypes stem from total, partial, or mixed (LoF/GoF) $Na_v1.2$ channel dysfunction.
- The relationship between clinical phenotype and $Na_v1.2$ channel dysfunction is inconsistent.
- A novel *SCN2A* LoF phenotype—episodic ataxia with or without cognitive impairment—was linked to the L1650P variant.
- For personalized treatment, predicting the underlying LoF mechanism should not rely solely on phenotypic grouping.

$Na_v1.2$ mutations can impact the channel's structure, biophysical properties, and expression by affecting protein folding, intracellular interactions, and cellular compensatory mechanisms.[10] Most *SCN2A* variants associated with ASD are protein-truncating due to premature stop codons,[8] whereas epilepsy-associated variants are typically missense mutations, often clustering in transmembrane segments and connecting loops, or protein-truncating.[6,8] However, epilepsy cases with nonsense, frameshift, and splice-site variants have also been reported,[3] suggesting the relationship between genetic mechanism and phenotype may be less consistent.

To date, relatively few variants associated with LO *SCN2A* have undergone electrophysiological evaluation. A better understanding of LoF mechanisms and correlation with phenotypic subgroups is needed to develop and implement mechanism-based therapy.

In this study, we conducted clinical and biophysical analyses of 15 *SCN2A* variants presumed to be associated with LoF based on their associated phenotypes. Using these data, along with previously characterized LoF variants, we sought to (1) confirm the LoF disease mechanism for the novel variants, (2) identify distinct LoF mechanisms, and (3) establish correlations between specific *SCN2A* variants and/or LoF mechanism with phenotypic subgroups.

2 | MATERIALS AND METHODS

2.1 | Standard protocol approvals, registrations, and patient consents

The Human Research Ethics Committees of the Royal Children's Hospital and Austin Health Melbourne, Australia, and the Ethics Committee of the University Hospital of Bonn, Germany, approved this study. Written informed consent was obtained from all individuals whose previously unpublished clinical data are presented here.

2.2 | Patients and variant selection

The variants studied were identified in patients enrolled in an *SCN2A* Natural History Study (NHS) and were presumed to be LoF based on their associated phenotypes. Nine of 15 variants had not been previously characterized biophysically, whereas six variants have been previously characterized using a different cell line. We also reviewed the Simons Foundation Autism Research Initiative (SFARI) database and the medical literature to identify additional patients with these variants. Furthermore, we searched the literature for *SCN2A* variants previously shown to cause LoF and identified additional patients with those variants within our NHS cohort and in SFARI. The novel and previously characterized variants presented here include a range of variant types—both missense and protein-truncating—and span various phenotypic subgroups.

2.3 | Phenotypic group definition/ clinical data

We used phenotypic data collected from parent and/or clinician questionnaires, medical records, and medical interviews with a pediatric neurologist (K.B.H., M.W.) for NHS, SFARI data from individuals identified through this source, and data from the medical literature (where available) for previously reported individuals. Data extracted included detail of the epilepsy (including onset age, seizure types, epilepsy syndrome, seizure outcome) and its treatment, developmental data (including developmental trajectory, presence and severity of developmental delay [DD] or ID, and where available, Vineland Adaptive Behavior Scale-3 [VABS-3] parent/caregiver form adaptive behavior composite (ABC) scores and gross motor and communication functional classification system levels), and ASD diagnosis. Using these data, patients were assigned into phenotypic subgroups as follows: LO-MI, defined by seizure onset between 3 and 18 months of age (excluding cases of self-limited neonatal–infantile epilepsy typically associated with GoF variants); LO-C, with seizure onset after 18 months; ID/ASDwoE; "other" (for phenotypes that did not align with these categories); or "unknown" (where phenotypic data were minimal or unavailable).

2.4 | Mammalian cell culture and transfection

Chinese hamster ovary (CHO) cells were cultured in Dulbecco Modified Eagle Medium/Nutrient Mixture F-12, supplemented with 10% (vol/vol) fetal bovine serum, 50 IU/mL penicillin, and 50 µg/mL streptomycin, in T25 cm^2 flasks kept in a 37°C humidified incubator with 5% CO_2 as previously described.[4] At ~80% confluency, cells were transiently cotransfected with wild-type or mutant $Na_v1.2$ channel constructs encoding the adult isoform (5 µg) and enhanced green fluorescent protein (eGFP; 1 µg; Clontech) using Lipofectamine 3000 (Thermo Fisher Scientific). After 24 h at 37°C and 5% CO_2, the culture medium was refreshed, and the cells were maintained at 30°C with 5% CO_2. Additionally, three $Na_v1.2$ variants were studied in the presence of cotransfected auxiliary β_1- and β_2-subunits (4 µg each). At 48–72 h posttransfection, cells were gently detached using TrypLE Express Reagent (Thermo Fisher Scientific) and plated onto 13-mm glass coverslips (Menzel-Glaser, Thermo Fisher Scientific). Cells were then incubated at 30°C in 5% CO_2 until use for electrophysiological recordings.

2.5 | Fluorescence-based immunolabeling for confocal microscopy

CHO cells were transfected with wild-type, M951R, or L1650P constructs (without eGFP) and incubated as described above. At 48–72 h posttransfection, cells were plated onto glass coverslips and fixed in 4% paraformaldehyde/sucrose solution for 15 min on ice. Cells were then blocked for 1 h at 4°C in Tris-buffered saline containing 4% nonfat milk and .1% Triton, followed by incubation with monoclonal mouse anti-$Na_v1.2$ supernatant (1:50, clone K69/3, Neuromab). After three

washes, cells were incubated with goat antimouse Alexa 488 (1:500, catalog A21131, Thermo Fisher Scientific) for 2 h at room temperature. Nuclei were counterstained with 4′,6-diamidine-2′-phenylindole dihydrochloride (1:5000, catalog D9542, Sigma-Aldrich). Coverslips were mounted onto slides using ProLong Glass Antifade (Invitrogen) and stored at 4°C. Images were acquired using a Zeiss LSM 900 confocal laser scanning microscope. High-resolution three-dimensional images were obtained with the Airyscan module and C Plan-Aprochromatic 63×/1.4 NA oil immersion lens.

2.6 | Electrophysiology

Whole-cell patch-clamp experiments were performed as previously described.[4] Briefly, the cells were superfused at a constant rate of .3 mL/min with extracellular solution containing 145 mmol·L^{-1} NaCl, 5 mmol·L^{-1} CsCl, 2 mmol·L^{-1} $CaCl_2$, 1 mmol·L^{-1} $MgCl_2$, 5 mmol·L^{-1} glucose, 5 mmol·L^{-1} sucrose, and 10 mmol·L^{-1} hydroxyethylpiperazine ethane sulfonic acid (HEPES; pH adjusted to 7.4 with NaOH and osmolarity adjusted to ~305 mOsm/kg). The intracellular solution contained 1 mmol·L^{-1} $MgCl_2$, 120 mmol·L^{-1} CsF, 5 mmol·L^{-1} CsCl, 5 mmol·L^{-1} NaCl, 11 mmol·L^{-1} ethyleneglycoltetraacetic acid, 10 mmol·L^{-1} HEPES, 1 mmol·L^{-1} $CaCl_2$, and 2 mmol·L^{-1} Na_2ATP (pH adjusted to7.3 with CsOH and osmolarity adjusted to ~290 mOsm/kg). Sodium currents were recorded using an Axopatch 200B amplifier (Molecular Devices), controlled by a pCLAMP 10/DigiData 1440 acquisition system (Molecular Devices). Experiments were performed at 23 ± .5°C and adjusted with a TC344B Dual Automatic Temperature Controller (Warner Instruments). Patch electrodes were pulled from borosilicate glass capillaries (GC150TF-7.5, Harvard Apparatus) and exhibited resistance values ranging from 1.2 to 1.5 MΩ. Series resistance values, typically of .8–3.8 MΩ, were 80%–85% compensated. Leak and capacitive currents were corrected using a −P/4 subtraction protocol, except when determining steady-state inactivation and recovery from fast inactivation. Currents and potentials were low-pass filtered at 10 kHz and digitized at 50 kHz. CHO cells exhibited a mean cell capacitance of 11.75 ± 3.21 pF (n = 77). Cells expressing inward peak sodium current (I_{Na}) amplitudes smaller than 10 nA were included in the data analysis, whereas cells exhibiting larger peak I_{Na} amplitudes (approximately 20% of all patched cells exhibiting partial LoF or mixed LoF/GoF biophysical characteristics) were excluded.[11]

To maintain rigor in the biophysical analyses of activation, inactivation, and recovery from fast inactivation, peak sodium current amplitudes smaller than 2 nA or larger than 10 nA were excluded for the wild-type, partial LoF (V198D, Y428C, R1635Q), or mixed LoF/GoF (A1773T) variants, consistent with our previously established protocols. However, for current density analyses of wild-type, V198D, Y428C, R1635Q, and A1773T variants, we included all recordings with current densities greater than 50 pA/pF (corresponding to ~.6 nA peak sodium current) as well as currents exceeding 10 nA, because lower values were considered representative of endogenous CHO cell currents. For the remaining variants shown in Figure 1B, we included all current amplitudes, most of which are considered endogenous.

Current density–voltage relationships were determined by plotting peak inward I_{Na} amplitudes normalized to cell capacitance against membrane potential values in the range between −80 mV and +70 mV. Activation and inactivation curves were obtained as previously described.[4] Briefly, normalized conductance values (G/G_{max}) were plotted against membrane potential to obtain activation curves. The voltage dependence of steady-state fast inactivation was determined using 100-ms voltage prepulses in 5-mV increments, followed by a 10-ms voltage step to −10 mV to assess I_{Na} availability. Steady-state activation and inactivation curves were fitted using the Boltzmann equation:

$$\frac{G}{G_{max}} = \frac{1}{\left[1 + e^{(V - V_{.5})/k}\right]} \quad (1)$$

where V is the membrane potential, $V_{.5}$ is the half-maximal activation or inactivation values ($V_{.5,act}$ or $V_{.5,inact}$, respectively), and k is the slope factor. The time course of recovery from fast inactivation was determined from a holding potential of −120 mV using a paired-pulse protocol. A 10-ms prepulse to −10 mV (P1) was applied to fast inactivate I_{Na}, followed by a test pulse to −10 mV (P2) to measure I_{Na} availability after variable recovery intervals. Data were fitted to a single exponential function to obtain the time constant, τ, as follows:

$$\frac{I}{I_{max}} = 1 - e^{-t/\tau} \quad (2)$$

where t is time (the time between P1 and P2).

2.7 | Distinguishing LoF mechanisms leading to $Na_v1.2$ channel dysfunction

The diversity of molecular and biophysical mechanisms contributing to LoF was defined as follows: total LoF, characterized by a completely nonfunctional or absent $Na_v1.2$ protein; partial LoF, due to reduced $Na_v1.2$ channel expression and current density, or from altered voltage dependence that diminishes channel function;

and mixed LoF and GoF, where distinct biophysical alterations exert opposing effects on channel function. Notably, even in the presence of mixed biophysical properties, the net effect may still be LoF, leading to reduced neuronal excitability.

2.8 | Data and statistical analysis

Electrophysiological data were analyzed using Clampfit 9.2 (Molecular Devices) and Prism 9.2 (GraphPad Software). Data are shown as mean ± SD. *N* values represent the number of individuals, whereas *n* values represent the number of independent experiments. Statistical analysis was performed using Student *t*-tests or one-way analysis of variance followed by Dunnett post hoc test. Differences between groups were considered statistically significant if $p < .05$.

3 | RESULTS

3.1 | Variants

3.1.1 | Characterization of 15 variants, including six overlapping with prior studies

We biophysically evaluated 15 *SCN2A* variants presumed to cause LoF based on associated phenotypes in 33 patients. Among these, seven are protein-truncating (either frameshift or nonsense), whereas the remaining eight are missense. Of the 15 variants, six (R119I, Y428C, R937C, M951R, L1650P, and A1773T)—all missense—had been previously studied biophysically (Table 1). The variants are distributed throughout the coding DNA sequence of the *SCN2A* gene, encompassing the transmembrane domains associated with voltage sensing (V198D, R1235*, R1635*, and R1635Q), ion permeation (Y428C, W1348* and A1773T), pore loops (R937C and M951R), cytoplasmic linkers (S486fs, F537fs, Y1498del, and L1650P), and the N-terminus (R102* and R119I; Figure 1A). Six of the variants were recurrent (R102*, Y428C, R937C, R1235*, L1650P, and A1773T). All variants were identified as having arisen de novo or had unknown inheritance (with the parent not tested), except for the L1650P variant, which was inherited from an affected parent in two unrelated families, and R119I, which was present in mosaic form in an unaffected parent of one proband.

3.1.2 | Previously characterized variants

A review of the literature identified additional 15 *SCN2A* variants in 41 patients that have been previously confirmed to cause LoF (Figure 1A and Table 1).[8,11,13] Among these, four variants were recurrent (D195G, R379H, R853Q, and E1211K), whereas 11 variants were each identified in only one individual (D12N, D82G, S686fs, G899S, R937H, C959*, G1013*, C1386R, T1420M, P1622S, and S1656P).

3.2 | Biophysical characterization of $Na_V1.2$ channel variants

In voltage clamp experiments, we determined the magnitude of the expressed current and assessed the voltage dependence and the time course of recovery from fast inactivation of each variant relative to wild type. Collectively, all variants demonstrated LoF (Table 1). Consistent with previous literature,[8] the frameshift variants (S486fs, F537fs) and protein-truncating variants (R102*, R1235*, W1348*, Y1498del, and R1635*) exhibited minimal residual currents, likely reflecting the activity of endogenously expressed sodium channels in CHO cells (Figure 1B).[17] Similarly, the missense variants R119I, R937C, M951R, and L1650P also displayed relatively small currents (Figure 1B). In summary, 11 of 15 variants demonstrated reduced sodium current densities, indicating total LoF. Conversely, four missense variants—V198D, Y428C, R1635Q, and A1773T—resulted in current densities comparable to wild type (Figure 2A and Table 2).

The electrophysiological properties of these four conducting variants were further investigated. Compared to wild type, the A1773T variant exhibited a 11-mV hyperpolarizing shift in the half-maximal activation voltage ($V_{.5,act}$), which corresponds to GoF (Figure 2B and Table 2). However, the half-maximal inactivation voltage ($V_{.5,inact}$) of the A1773T variant exhibited a significant 23-mV hyperpolarizing shift relative to wild type, which is expected to stabilize inactivation at physiologically relevant membrane potential values, leading to an LoF (Figure 2B and Table 2). To determine whether the shift of the activation or the inactivation of the A1773T variant contributes most to the channel dysfunction, we performed voltage clamp (ramps and pulse trains) and dynamic action potential clamp experiments. Ramp protocols of different speeds revealed that A1773T channels undergo enhanced closed-state inactivation, leading to markedly reduced sodium current availability compared with wild type (Figure S1). Furthermore, dynamic action potential clamp experiments[11] showed that the A1773T variant significantly decreases firing frequency and overall excitability, despite its GoF activation shift (Figure S2). These results confirm that the LoF properties of A1773T outweigh its GoF activation shift.

TABLE 1 Clinical phenotypes and biophysical impact of 30 later onset $Na_v1.2$ variants present in 74 individuals.

Variant	Variant type	Variant inheritance	Phenotype					$Na_v1.2$ channel LoF mechanism	References
			Later onset epilepsy–midinfancy	Later onset epilepsy–childhood	ID/ASD without epilepsy	Unknown	Other		
D12N	Missense	1 de novo		1				Partial	8
D82G	Missense	1 de novo				1		Partial	8
R102*	Frameshift	3 de novo		2	1			Total	Current article
R119I	Missense	Inherited (mosaic unaffected parent)			1			Total	12, current article
D195G	Missense	2 de novo	2					Total	4
V198D	Missense	1 de novo			1			Partial	Current article
R379H	Missense	2 de novo				2		Total	8
Y428C	Missense	1 de novo; 1 UK	1	1				Partial	12, current article
S486fs	Frameshift	1 UK	1					Total	Current article
F537fs	Frameshift	1 de novo			1			Total	Current article
S686fs	Frameshift	1 de novo				1		Total	8
R853Q	Missense	14 de novo; 6 UK	14	1	1	4		Mixed	4,11,13,14
G899S	Missense	1 de novo	1					Partial	3
R937C	Missense	4 de novo			4			Total	12,14, current article
R937H	Missense	1 de novo				1		Total	8
M951R	Missense	1 UK	1					Total	12, current article
C959*	Frameshift	1 de novo			1			Total	8
G1013*	Frameshift	1 de novo			1			Total	8
E1211K	Missense	4 de novo; 2 UK	5			1		Mixed	4,8,15
R1235*	Frameshift	2 de novo; 2 UK		2	2			Total	Current article
W1348*	Frameshift	1 UK	1					Total	Current article
C1386R	Missense	1 de novo				1		Total	8
T1420M	Missense	1 de novo			1			Partial	8
Y1498del	Frameshift	1 de novo			1			Total	Current article
P1622S	Missense	1 de novo		1				Partial	3
R1635*	Frameshift	1 de novo	1					Total	Current article
R1635Q	Missense	1 de novo			1			Partial	Current article

TABLE 1 (Continued)

Variant	Variant type	Variant inheritance	Phenotype					$Na_v1.2$ channel LoF mechanism	References
			Later onset epilepsy–midinfancy	Later onset epilepsy–childhood	ID/ASD without epilepsy	Unknown	Other		
L1650P	Missense	2 de novo; 1 UK; 3 inherited	1				5	Total	12, current article
S1656P[a]	Missense	1 de novo	1					No biophysical data available	Clinical information available 16
A1773T	Missense	2 de novo; 3 UK	3			2		Mixed	12,14, current article

Abbreviations: ID/ASD, intellectual disability/autism spectrum disorder; LoF, loss-of-function; UK, unknown.

[a]Biophysical data for the S1656P variant is unavailable.

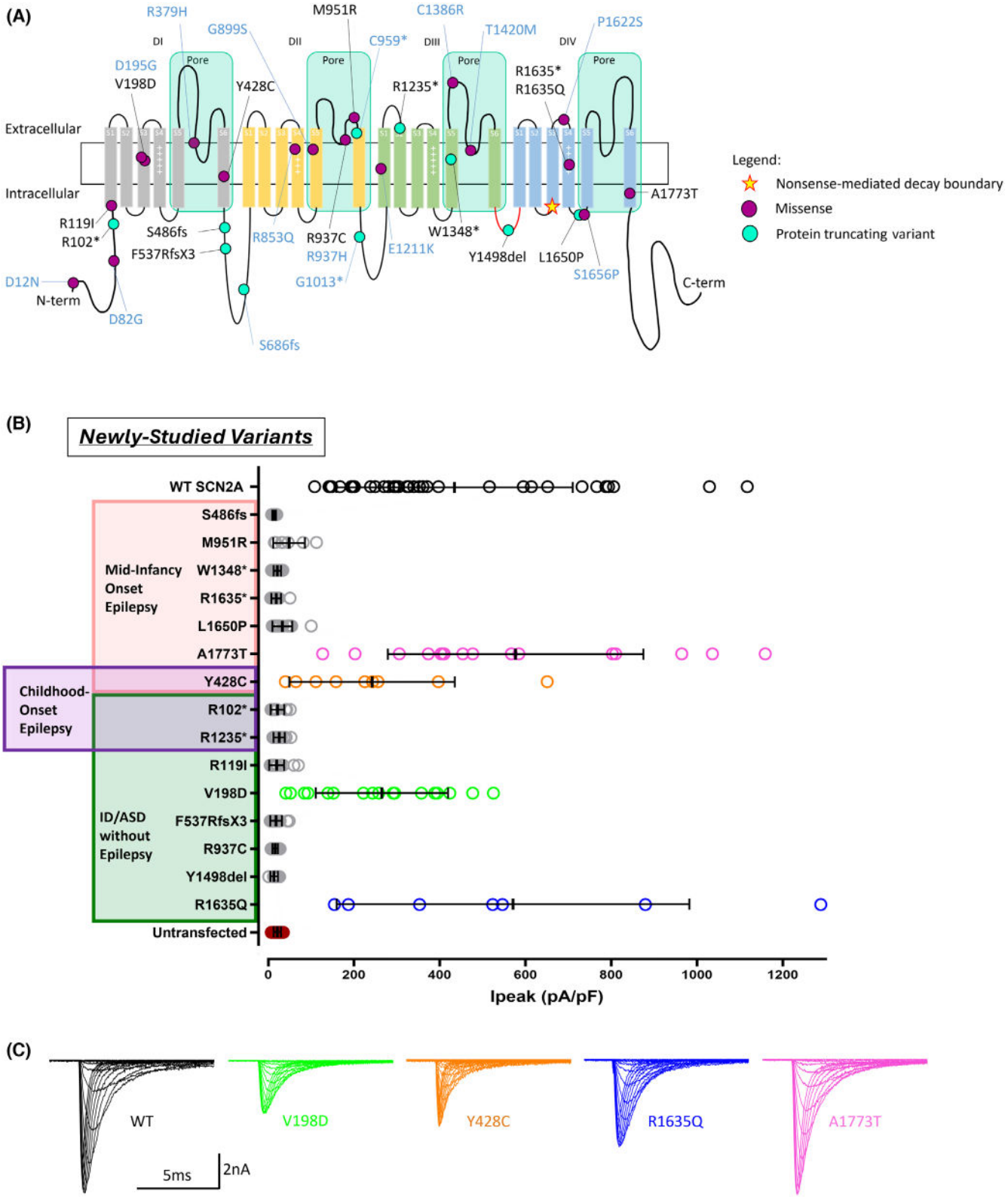
Relative to wild type, the V198D, Y428C, and R1635Q variants had limited effects on the $V_{.5,act}$ values, resulting in small shifts of the corresponding activation curves. However, V198D and Y428C exhibited shallower activation slope factors (k_{act}) compared to wild type, suggesting a reduced voltage sensitivity in the transition from the closed to open state of the channel (Figure 2B and Table 2).[18] Additionally, these variants caused significant hyperpolarizing shifts in the $V_{.5,inact}$ values, indicating a clear LoF (Figure 2B and Table 2).

Overall, the disruption of fast inactivation in the conducting variants was more pronounced than any effect on activation, further shifting channel availability toward nonconducting states and contributing to the LoF phenotype. Recovery from fast inactivation, assessed using the paired-pulse protocol described in the Materials and Methods section, was slower for the R1635Q variant compared to wild type, indicating an LoF. In contrast, the A1773T, V198D, and Y428C variants exhibited recovery times similar to wild type (Figure 2C and Table 2).

In summary, these four conducting variants demonstrated unaltered functional expression but altered voltage dependence relative to wild type. Their LoF mechanisms can be classified as partial (V198D, Y428C, and R1635Q) and mixed (A1773T), distinguishing them from channel variants that lack functional current expression (Table 1).

The absence of detectable currents in several missense variants could reflect either a trafficking defect or a properly trafficked but nonconducting channel. To explore this, we transfected CHO cells with *SCN2A* constructs encoding wild-type, M951R, or L1650P Nav1.2 channels and performed confocal imaging of immunolabeled cells (Figure S3). Wild-type $Na_v1.2$ channels displayed a dotted distribution across the plasma membrane. The M951R variant was detectable but appeared more clustered and also perinuclear, whereas the L1650P variant showed no detectable expression, consistent with a trafficking defect and/or reduced protein stability.

We investigated whether peak current expression in nonconducting variants could be rescued by cotransfection with β-subunits. These auxiliary subunits are known to modulate the gating and kinetics of sodium channels and enhance α-subunit expression at the plasma membrane.[19] Three nonconducting variants, M951R, R1635*, and Y428C, were selected for cotransfection with β_1- and β_2-subunits in CHO cells to assess whether functional channel expression could be restored. In addition, the Y428C variant was also studied in human embryonic kidney 293 T cells. The R1635* frameshift variant is located downstream of amino acid 1591 in the α-subunit protein, and thus, should not undergo nonsense-mediated



decay.[5] As shown in Figure S4, coexpression of β_1- and β_2-subunits with these α-subunits did not increase current density compared to expression of the α-subunits alone, suggesting that β-subunit modulation does not enhance the functional expression of these $Na_v1.2$ LoF variants.

3.3 | Clinical data, phenotypic subgroups, and association of $Na_v1.2$ channel variants with genotype

The 74 individuals with a newly or previously characterized variant (Table 1) were aged .8–38 years at last review

FIGURE 1 Localization of *SCN2A* variants and their impact on membrane current expression in transiently transfected mammalian cells. (A) Predicted locations of the 15 variants biophysically assessed in this study (black labels) alongside 15 previously evaluated variants (blue labels). Missense variants are indicated by purple circles, whereas protein-truncating variants are represented by cyan circles. The channel's four domains (DI–DIV), each containing six transmembrane segments (S1–S6), are color-coded. The nonsense-mediated decay boundary (star) and the fast inactivation gate (red) are highlighted. (B) Peak current density values for all variants evaluated in this study. Colors indicate conducting variants relative to wild-type (WT; black): A1773T (pink), R1635Q (blue), V198D (green), and Y428C (orange). Nonconducting variants are shown in gray, and nontransfected control cells are indicated in red. The phenotypic groups for the variants are represented by shaded boxes. Notably, Y428C is associated with individuals classified phenotypically as later onset epilepsy–midinfancy and later onset epilepsy–childhood (LO-C), whereas R102* and R1235* are linked with individuals with LO-C and intellectual disability/autism spectrum disorder (ID/ASD) without epilepsy. Data for individual experiments and the mean ± SD (black error bars) are shown for each variant. For clarity, additional phenotypic groups for the L1650P and A1773T variants (see Table 1) are not included in the figure. (C) Representative sodium current traces of the conducting variants. Only the first 10 ms of the current traces, elicited in the voltage range between −80 mV and +20 mV, are shown. Horizontal scale bar = 5 ms.

(median = 7 years, with age unknown in 10 individuals). Individual patient data are provided in Tables 3 and 4.

Of these, 47 individuals (63%) individuals had epilepsy, with age at onset ranging from .25 to 5 years (median = .83 years, with onset age unknown for 12 individuals). Onset epilepsy syndromes were IESS in 26 of 47 (55%), epilepsy with myoclonic–atonic seizures in one (2%), unclassified epilepsies in 11 (23%), and unknown epilepsy syndrome in nine (19%). In the 11 individuals with unclassified epilepsies, nine had more than one seizure type reported; seizure types were generalized in five, focal in two, mixed focal and generalized in two, and unknown in two. Patients were taking a median of 3 ASMs at last review (range = 1–7, inclusive of dietary and hormonal antiseizure treatments) and had tried a median of 7 ASMs in their lifetimes. SCB ASM had been used by 28 individuals (53 uses: lamotrigine, 14; carbamazepine, 8; lacosamide, 8; zonisamide, 8; oxcarbazepine, 7; phenytoin, 6; rufinamide, 2). Seizure worsening was reported with 16 of 53 (30%) SCB ASMs used, and deterioration of development was reported in two instances where seizures were improved or unchanged. Other than rufinamide, where the impact on seizures was unknown, seizure worsening was reported with each SCB ASM in at least one individual. Conversely, improvement of seizures was reported with 12 SCB ASM uses, predominantly lamotrigine (five uses) and zonisamide (three uses). Seizures were ongoing within the past 3 months in 27 of 37 (73%) individuals (seizure outcome unknown for 10 individuals with epilepsy). Of the 10 of 37 individuals with no seizures for more than 3 months, seven had been seizure-free for more than 1 year, with seizure freedom achieved at age 2.5–10 years (age at seizure control unknown in one); three younger individuals had been seizure-free for 4–6 months (from age 1.7–2.1 years).

Thirty-three (45%) patients had a clinical ASD diagnosis. For the 65 patients who had available developmental data, all but three had DD or ID. Thirty individuals had detailed developmental data collected through the *SCN2A* NHS. Of these, 27 (90%) had at least one period of developmental plateau/regression. At most recent review, 24 of 24 (100%, six were too young) had a communication functional classification system level of III–V (indicating limited communication abilities even with familiar partners such as family members). Motor abilities were more variable, with gross motor functional classification system levels of I–II (ambulant) in 14 of 24 (58%) and III–V (minimally to nonambulant) in 10 of 24 (42%, six were too young). The VABS-3 parent/caregiver form was completed for 22 individuals, with ABC scores of 20–89 (median = 47). A VABS-3 ABC score of 47 indicates adaptive function >3 SD below the normative mean (VABS-3 mean = 100, SD = 15).

Phenotypic subgroups were as follows: LO-MI in 31 individuals (42%), LO-C in nine individuals (12%), ID/ASDwoE in 16 individuals (22%), and "other" in five individuals (7%). Clinical data were insufficient to determine phenotypic group for two patients with variants characterized in this study (3%) and 11 patients with previously characterized variants (15%).[8,20–23]

Phenotypic features were consistent with those previously reported for the LO-MI, LO-C, and ID/ASDwoE subgroups. The LO-MI group had seizure onset at median = .8 years (range = .3–1.5 years); IESS was the onset epilepsy syndrome in 26 of 30 (87%, one unknown). At last review, 22 of 29 (76%, two unknown) had ongoing seizures within the past 3 months (at >weekly frequency in 10/12 [83%] in whom frequency was known) and were taking 1–7 ASMs (median = 3, inclusive of dietary therapies). Those in the LO-C subgroup had seizure onset at median = 2.5 years (range = 1.6–5 years). The epilepsy syndromes were unclassified in most, and seizures were ongoing in five of eight (63%, one unknown; weekly or lower frequency in 2/2 individuals [three had unknown frequency]), on 1–4 ASMs (median = 2, unknown in four). We did not observe differences in ASM response between the LO-MI and LO-C subgroups, although small numbers preclude detailed analysis. Importantly, seizure exacerbations with SCB ASM were identified in both

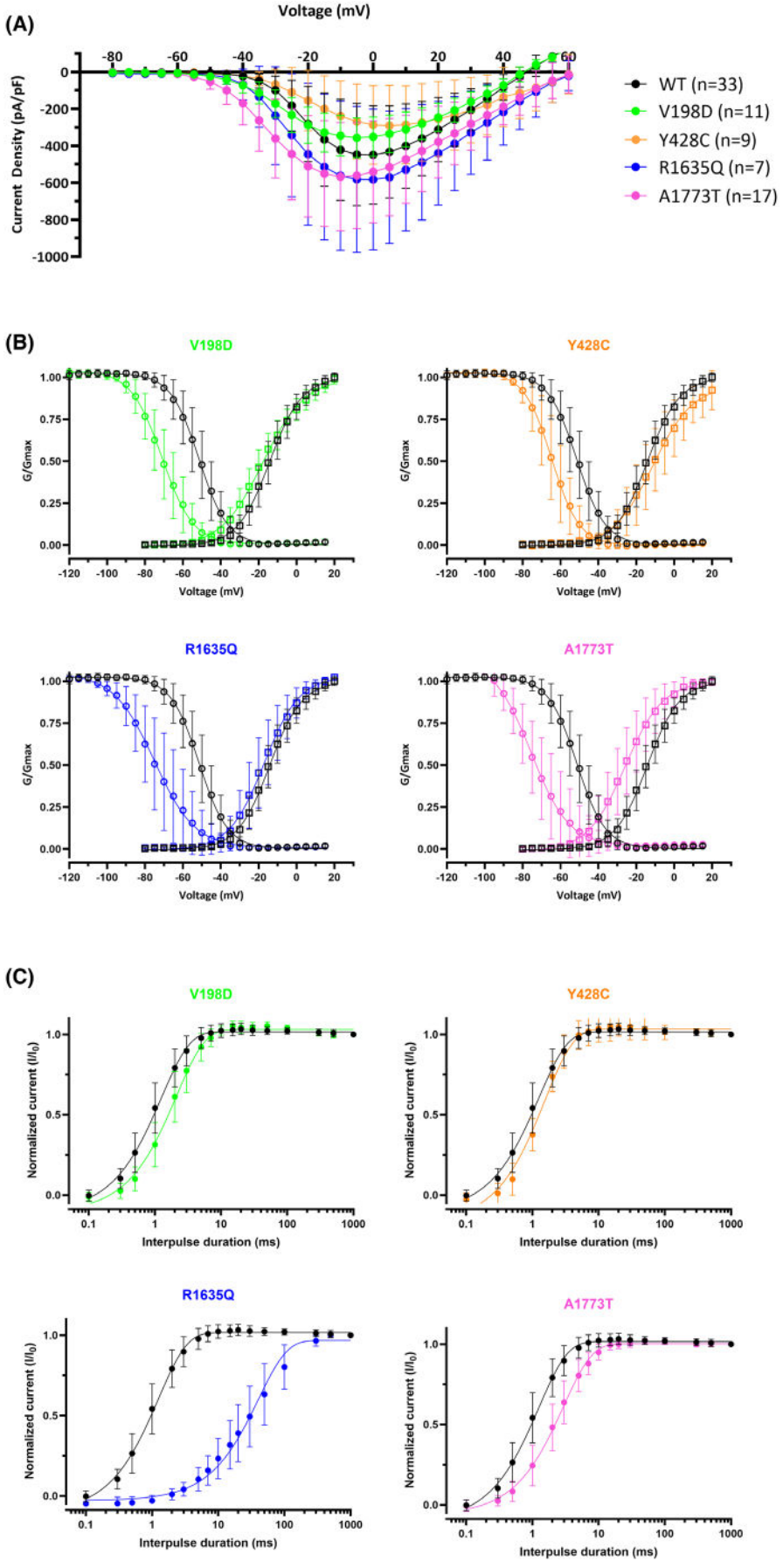


FIGURE 2 Biophysical characteristics of conducting later onset variants linked to midinfancy or childhood onset epilepsy and to intellectual disability/autism spectrum disorder without epilepsy, relative to wild-type (WT). (A) Current density-voltage relationships of V198D (green), Y428C (orange), R1635Q (blue), and A1773T (pink) variants compared to WT (black). Individual mean current density values are shown in Table 2. (B) Voltage dependence of activation (squares) and inactivation (circles) was determined from a holding potential of −120 mV. Currents were activated using 40-ms depolarizing voltage steps in 5-mV increments at 1 Hz. Steady-state inactivation was determined using 100-ms conditioning steps ranging from −120 to +20 mV followed by 20-ms test pulses to −10 mV at .1 Hz. Normalized conductance (G/G_{max}) data were fitted to Equation 1 (Materials and Methods); fitting parameters are listed in Table 2. $Na_v1.2$ variants are color-coded and WT shown in black. (C) Recovery from fast inactivation. Recovery time constants (τ) were obtained by fitting the recovery time course to Equation 2. Data are mean ± SD, with *n* representing the number of experiments. Statistical evaluations are provided in Table 2.

TABLE 2 Biophysical characteristics of V198D, Y428C, R1635Q, and A1773T $Na_v1.2$ variants, categorized according to clinical phenotype.

		Midinfancy onset epilepsy	Midinfancy onset epilepsy and childhood onset epilepsy	ID/ASD without epilepsy	
Characteristic	**WT**	**A1773T**	**Y428C**	**V198D**	**R1635Q**
Current density, pA/pF	−421.60 ± 273.78	−567.65 ± 292.96	−238.36 ± 189.70	−345.85 ± 110.08	−561.71 ± 404.35
p	–	.24	.25	.87	.59
n	33	17	9	11	7
Activation					
$V_{.5,act}$, mV	−14.03 ± 4.51	−25.56 ± 8.14****	−10.77 ± 6.95	−18.06 ± 4.75	−17.16 ± 7.61
p	–	<.001	.48	.22	.61
k_{act}, mV	8.20 ± .72	8.79 ± .89	9.54 ± 2.06**	10.75 ± .73****	8.00 ± .69
p	–	.21	.003	<.001	.98
n	31	16	9	11	7
Inactivation					
$V_{.5,inact}$, mV	−51.67 ± 5.71	−73.66 ± 10.84****	−64.35 ± 6.52**	−70.74 ± 6.71****	−73.65 ± 10.81****
p	–	<.001	.006	<.001	<.001
k_{inact}, mV	6.72 ± 1.24	7.67 ± .71*	6.28 ± .92	7.12 ± .90	8.15 ± 1.38 **
p	–	.02	.87	.75	.010
n	31	16	5	10	7
Recovery					
τ at −120 mV, ms	1.31 ± .47	3.05 ± 1.08	1.56 ± .20	2.17 ± .77	49.99 ± 31.13****
p	–	.96	>.99	>.99	<.001
n	31	16	4	10	7

Note: Data are represented as mean ± SD. Current density values are reported at −10 mV. Asterisks indicate statistically significant differences relative to WT.

Abbreviations: ID/ASD, intellectual disability/autism spectrum disorder; *n*, number of cells measured; $V_{.5act}$ and $V_{.5inact}$, membrane potential for half-maximal activation and inactivation, respectively; k_{act} and k_{inact}, slope of activation and inactivation curve, respectively; WT, wild-type.

*$p < .05$, **$p < .01$, ***$p < .005$, ****$p < .001$, one-way analysis of variance with Dunnett post hoc test.

phenotypic subgroups (and in people with a range of epilepsy syndromes).

Of the 74 patients, 20 carried nonrecurrent variants, which were distributed across the following phenotypic groups: LO-MI (S486fs, G899S, M951R, R1635*, S1656P), LO-C (D12N, W1348*, P1622S), ID/ASDwoE (R119I, V198D, F537fs, C959*, G1013*, T1420M, Y1498del, R1635Q), and unknown (D82G, S686fs, R937H, C1386R; Tables 1, 3, and 4). For the 10 recurrent variants, three were associated with only one phenotypic group (D195G, R379H, R937C) and three were primarily associated with a single phenotypic group but were also observed in individuals with either an unknown (E1211K, A1773T) or "other" (L1650P) phenotype, whereas four were associated with more than one phenotypic group (R102*, Y428C, R1235*, R853Q; Tables 1, 3, and 4).

The L1650P variant was identified in an individual classified as LO-MI, as well as five patients categorized as "other" who did not fit into any previously described "LoF" phenotypic group. These five individuals were from two families (each proband had an affected parent and one an affected sibling). Each individual shared similar clinical features, including episodic ataxia ($n = 4$) or episodic hemiplegia ($n = 1$) without epilepsy. Episodes had onset at ages 12 months–3 years (median = 15 months) and lasted 2 days–4 weeks. One individual was felt to have benefitted from acetazolamide with flunarizine after nonresponse to corticosteroids. Other individuals did not have benefit from acetazolamide ($n = 2$) or corticosteroids ($n = 1$). Only one individual had developmental concerns (mild motor delay) prior to episode onset. At last review, one of the five patients had normal cognitive function, three had borderline ID (intelligence quotients of 71, 78, and 78), and the youngest individual had mild developmental delay at age 2 years.[24,25]

In addition to phenotypic variability among different individuals carrying the same recurrent variant, we also identified phenotypic variability between individuals with distinct biophysical outcomes (i.e., total, partial, or mixed LoF; Tables 1, 3, and 4).

Individuals with the variants exhibiting mixed LoF, R853Q, E1211K, and A1773T, were classified as LO-MI

TABLE 3 Genotype, phenotypic group, selected clinical features, and predicted functional impact of later onset $Na_v1.2$ variants in this study.[a]

Variant (*n*), c., p.	Patient source[b]	Patient age at review, years	Age at epilepsy onset, years	Seizure type(s)	Epilepsy syndrome (at onset)	Seizure outcome	Antiseizure treatments used (current treatments underlined)
R102* (3) c.304C>T Arg102*	NHS ID 31	8.1	5	FBTCS	Unclassified	Ongoing seizures (weekly)	LEV, CBD, KD, CLB, LTG
	Kamiya 2004	29	1.6	F, FBTCS	Unclassified	Seizure-free (since age 10 years)	PB, VPA, CBZ
	Zhang 2020 (pt DDD4K.03614), and DDD 2015 and Richardson 2021 (both using Decipher ID 263714)	13	No epilepsy	–	–	–	–
R119I (1) c.356G>T Arg119Ile	NHS ID 41	14.2	No epilepsy	–	–	–	–
S486fs (1) c.1456_1457del Ser486fs	NHS ID 61	6.3	1.2	Sp, T	IESS	Seizure-free (since age 4 years)	STP, VPA, CLB, CZP, LEV, PNL, VGB
F537fs (1) c.1608_1624del17 Phe537fs	NHS ID 102	2.3	No epilepsy	–	–	–	–
R937C (4) c.2809C>T Arg937Cys	NHS ID 10	5.6	No epilepsy	–	–	–	–
	Rauch 2012 (pt ZH60991) and Ben-Shalom 2017	18	No epilepsy	–	–	–	–
	Li 2016	UK	No epilepsy	–	–	–	–
	Iossifov 2014 (pt 14280) and Ben-Shalom 2017	9	No epilepsy	–	–	–	–
M951R (1) c.2852T>G Met951Arg	NHS ID 12	7.7	.8	Sp, T, TCS	IESS	Ongoing seizures (multiple weekly)	BRV, CBD, CLB, LCS, LEV, OXC, TPM, VGB, ZNS, PNL, ACTH
R1235* (4) C.3703C>T Arg1235*	NHS ID 46	13.2	3	Unknown (multiple types)	Unclassified	Ongoing seizures (>monthly)	CLB, LTG, TPM, VPA, KD, RUF
	NHS ID 89	3.3	No epilepsy	–	–	–	–
	NHS ID 43	9.6	No epilepsy	–	–	–	–
	Wolff 2017 (pt 59)	14	3.3	FS, TCS, At	Unclassified	Ongoing seizures (frequency UK)	CLB, LEV, LTG, VPA, ZNS (current treatments UK)
W1348* (1) c.4043G>A Trp1348*	NHS ID 87	2.5	1.8	TCS, Ab	Unclassified	No seizures for 4 months	TPM, CLB, LEV
Y1498del (1) c.4492_4494delTAC Tyr1498del	NHS ID 14	11.1	No epilepsy	–	–	–	–
R1635* (1) c.4903C>T Arg1635*	NHS ID 88	1.3	.8	Sp	IESS	Ongoing seizures (daily)	VPA, PNL, VGB, CZP
L1650P (6) c.4949C>T Leu1650Pro	NHS ID 21, also Trump 2016	19	.8	F, TCS, Sp, T, M	Unclassified	Ongoing seizures (<monthly)	VPA, LTG, CBZ, CLB, LCS, LEV, PHT, TPM
	Fazeli 2018 proband (pt 1), Schwarz 2019 (pt 14), German NHS SCN2A_P82	10.6	No epilepsy	–	–	–	–

Seizure response to sodium channel-blocking ASMs	Worsening or seizure freedom with other antiseizure treatments?	DD/ID at last review	ASD diagnosis	History of plateau/ Regression	VABS-3 ABC score	Gross motor functional classification system level	Communication function classification system level	Phenotypic group
LTG: seizures improved, development worse	–	Yes	Yes	Yes	–	II	IV	Later onset epilepsy–childhood
CBZ: no benefit	–	Yes (severe)	Yes					Later onset epilepsy–childhood
–	–	Yes (UK severity)	UK					ID/ASD without epilepsy
–	–	Yes	Yes	Yes	49	I	III	ID/ASD without epilepsy
Not used	STP most effective ASM	Yes	No	Yes	39	II	V	Later onset epilepsy–midinfancy
–	–	Yes	Yes	Yes	68	I	IV	ID/ASD without epilepsy
–	–	Yes	Yes	Yes	–	I	IV	ID/ASD without epilepsy
–	–	Yes (moderate)	UK					ID/ASD without epilepsy
–	–	Yes (UK severity)	UK					ID/ASD without epilepsy
–	–	UK	Yes					ID/ASD without epilepsy
LCS and OXC: seizures worse; ZNS: seizures reduced	–	Yes	No	Yes	32	IV	IV	Later onset epilepsy–midinfancy
LTG: seizures reduced; RUF: unknown effect	–	Yes	Yes	Yes	–	I	III	Later onset epilepsy–childhood
–	–	Yes	Yes	Yes	–	I	IV	ID/ASD without epilepsy
–	–	Yes	Yes	Yes	51	I	IV	ID/ASD without epilepsy
ZNS: seizures reduced; LTG: no change	–	Yes (moderate)	Yes					Later onset epilepsy–childhood
Not used	TPM most effective ASM	Yes	No	Yes	57	I	IV	Later onset epilepsy–childhood
–	–	Yes	Yes	Yes	33	I	IV	ID/ASD without epilepsy
Not used	–	Yes	Too young	Yes	57	Too young (unable to sit unaided)	Too young (babble)	Later onset epilepsy–midinfancy
LTG: seizures improved; PHT: seizures worse; LCS and ZNS: seizures improved but very agitated; CBZ: effect unknown	–	Yes	No	Yes	20	V	IV	Later-onset epilepsy – midinfancy
–	–	IQ 78	No					Other (episodic ataxia/ episodic hemiplegia phenotype)

(Continues)

TABLE 3 (Continued)

Variant (*n*), c., p.	Patient source[b]	Patient age at review, years	Age at epilepsy onset, years	Seizure type(s)	Epilepsy syndrome (at onset)	Seizure outcome	Antiseizure treatments used (current treatments underlined)
	Fazeli 2018 proband's mother (pt 2), Schwarz 2019 (pt 16), German NHS SCN2A_P83	28	No epilepsy	–	–	–	–
	Fazeli 2018 proband's brother (pt 3), Schwarz 2019 (pt 15), German NHS SCN2A_P81	8.3	No epilepsy	–	–	–	–
	Passi 2020, proband	2	No epilepsy	–	–	–	–
	Passi 2020, proband's father	UK (adult)	No epilepsy	–	–	–	–
V198D (1) c.593T>A Val198Asp	NHS ID 33	4.9	No epilepsy	–	–	–	–
Y428C (2) c.1283A>G Tyr428Cys	NHS ID 97	14.8	.5	M, T, Ab	Unclassified	Ongoing seizures (<monthly)	VPA, CBZ, CLB, LEV, TPM, VGB
	Sahli 2019 (pt 3)	7	1.8	T	Unclassified	Ongoing seizures (UK frequency)	CLB, VPA
R1635Q (1) c.4904G>A Arg1635Gln	NHS ID 35	10	No epilepsy	–	–	–	–
A1773T (5) c.5317G>A Ala1773Thr	NHS ID 48	2.3	.8	Sp, T	IESS	No seizures for 6 months	TPM, VPA, VGB, KD, CLB, CZP, LCS, LEV, PNL
	NHS ID 53	11.2	.8	Sp, T, TCS, At, unclassified	IESS	Ongoing seizures (multiple weekly)	CLB, LTG, VPA, KD, CBD, LCS, LEV, PHT, TPM, VGB, ACTH, PNL
	NHS ID 85	1.5	.6	Sp, T	IESS	Ongoing seizures (multiple daily)	CLB, TPM, VPA, FLB, LTG, PB, VGB, ACTH
	Ko Ep Res 2018 (pt 72)	UK	UK age	UK	UK	UK	UK
	Zhang 2020 (pt DDD4K.00 631)	UK	UK if epilepsy present	UK	UK	UK	UK

Note: Six variants (R102*, Y428C, R937C, R1235*, L1650P, and A1773T) were recurrent, whereas the remaining nine variants (R119I, V198D, S486fs, F537fs, M951R, W1348*, Y1498del, R1635*, and R1635Q) were nonrecurrent.

Abbreviations: Ab, absence seizures; At, atonic seizures; –, not applicable; ABC, adaptive behavior composite; ASD, autism spectrum disorder; ASM, antiseizure medication; BRV, Brivaracetam; CBD, Cannabidiol; CBZ, Carbamazepine; CLB, Clobazam; CZP, Clonazepam; DD, developmental delay; F, focal seizures; FBTCS, focal to bilateral tonic-clonic seizures; FLB, Felbamate; FS, febrile seizures; ID, intellectual disability; IESS, infantile epileptic spasms syndrome; KD, Ketogenic diet; LCS, Lacosamide; LEV, Levetiracetam; LTG, Lamotrigine; M, myoclonic seizures; NHS, Natural History Study; OXC, Oxcarbazepine; PB, Phenobarbital; PHT, Phenytoin; PNL, Perampanel; pt, patient; RUF, Rufinamide; Sp, spasms (epileptic spasms); STP, Stiripentol; T, tonic seizures; TCS, tonic-clonic seizures; TPM, Topiramate; UK, unknown; VABS-3, Vineland Adaptive Behavior Scale-3; VGB, Vigabatrin; VPA, Valproic acid (also valproate); ZNS, Zonisamide.

[a]Note that six variants—R119I, Y428C, R937C, M951R, L1650P, and A1773T—were also studied by Berg et al. in a transient human embryonic kidney cell expression system, using an automated patch-clamp approach to assess channel properties (Brain 2024;147:2761–2774).

[b]Patient source: R102*: Kamiya et al., J Neurosci 2004;24:2690–2698; Shu et al., American Journal of Medical Genetics Part A 2021;185:2119–2125/Large-scale discovery of novel genetic causes of developmental disorders (DDD) Nature 2015;519:223–228/Richardson et al. Am J Med Genet A 2022;188:867–877. R937C: Rauch et al., Lancet 2012;380:1674–1682/Ben-Shalom et al., Biol Psychiatry 2017;82:224–232; Li et al., Mol Psychiatry 2016;21:290–297; Iossifov et al., Nature 2014;515:216–221/Ben-Shalom et al., Biol Psychiatry 2017;82:224–232. R1235*: Wolff et al., Brain 2017;140:1316–1336. L1650P: Trump et al., J Med Genet 2016;53:310–317; Fazeli et al., Neuropediatrics 2018;49:379–384/Schwarz et al., Eur J Paediatr Neurol 2019;23:438–447; Passi and Mohammad, Brain and Development 2021;43:166–169.

Seizure response to sodium channel-blocking ASMs	Worsening or seizure freedom with other antiseizure treatments?	DD/ID at last review	ASD diagnosis	History of plateau/ Regression	VABS-3 ABC score	Gross motor functional classification system level	Communication function classification system level	Phenotypic group
–	–	IQ 71	No					Other (episodic ataxia/ episodic hemiplegia phenotype)
–	–	IQ 78	No					Other (episodic ataxia/ episodic hemiplegia phenotype)
–	–	Yes (mild)	Too young					Other (episodic ataxia/ episodic hemiplegia phenotype)
–	–	No	Not reported					Other (episodic ataxia/ episodic hemiplegia phenotype)
–	–	Yes	No	No	49	II	III	ID/ASD without epilepsy
CBZ: seizures worse	TPM: seizure-free, seizures recurred after weaning it; VGB, LEV, and CLB: seizures worse	Yes	No	Yes	25	I	III	Later onset epilepsy–midinfancy
Not reported	–	Yes (UK severity)	UK					Later onset epilepsy–childhood
–	–	Yes	Yes	No	49	I	IV	ID/ASD without epilepsy
LCS: seizures worse	TPM and KD reported most effective	Yes	No	Yes	44	III	V	Later onset epilepsy–midinfancy
PHT: seizures improved; LTG: seizures improved; LCS: no change	CBD: seizures worse	Yes	Yes	Yes	–	IV	IV	Later onset epilepsy–midinfancy
LTG: no change	–	Yes	Too young	Yes	62	Too young (sits)	Too young (cooing)	Later onset epilepsy–midinfancy
UK	UK	UK	UK					UK (epilepsy and DD/ID, no further details)
UK	UK	Yes	UK					UK (ID/ASD with or without epilepsy)

TABLE 4 Genotype, phenotypic group, selected clinical features, and predicted functional impact of later onset $Na_v1.2$ variants previously studied.

Variant (*n*), c., p.	Patient source[a]	Patient age at review, years	Age at epilepsy onset, years	Seizure type(s)	Epilepsy syndrome (at onset)	Seizure outcome	Antiseizure treatments used (current treatments underlined)
D195G (2) c.584A>G Asp195Gly	NHS ID 34	2.1	1	Sp, unclassified	IESS	Ongoing seizures (multiple daily)	CBD, FLB, VPA, CLB, LCS, VGB, ZNS, KD, ACTH
	NHS ID 69	5.7	.8	Sp, FIAS	IESS	Ongoing seizures (daily)	CLB, CZP, PB, KD, ACTH, VGB, CBD, LEV, OXC
R379H (2) c.1136G>A Arg379His	Ben-Shalom 2017 (pt AU026A)	>2 years	UK	UK	UK	UK	UK
	Ben-Shalom 2017 (pt DEASD_0143_001)	>2 years	UK	UK	UK	UK	UK
S686fs (1) c.2058 + A Ser686fs	Ben-Shalom 2017 (pt 10C109819)	>2 years	UK	UK	UK	UK	UK
R937H (1) c.2810G>A Arg937His	Ben-Shalom 2017 (pt NDAR_INVTZ957VTW_ wes1)	>2 years	UK	UK	UK	UK	UK
C959* (1) c.2877C>A Cys959X	Ben-Shalom 2017 (pt 11892. p1)	7	No epilepsy	–	–	–	–
G1013* (1) c.3037G>T Gly1013X	Ben-Shalom 2017 (pt 11114. p1)	8	No epilepsy	–	–	–	–
C1386R (1) c.4156T>C Cys1386Arg	Ben-Shalom 2017 (pt 13544. p1)	7	UK	UK	UK	UK	UK
D12N (1) c.34G>A Asp12Asn	Ben-Shalom 2017 (pt 14545. p1)	11	2.5	TCS	UK	UK	UK
D82G (1) c.245A>G Asp82Gly	Ben-Shalom 2017 (pt ASDFI_372)	>2 years	UK	UK	UK	UK	UK
R853Q (20) c.2558G>A Arg853Gln	NHS ID2, Berecki 2018 (pt 2)	10.7	.7	T, Sp, M, At, unclassified	IESS	Ongoing seizures (daily)	CBD, CLB, CZP, FLB, LEV, OXC, PHT, TPM, VPA, VGB, ZNS, KD, ACTH, PNL
	NHS ID 5	4.5	.7	Sp, T, F	IESS	Seizure-free (since age 3.5 years)	CBD, STP, CLB, LEV, NZP, PB, VGB, VPA, KD, PNL
	NHS ID 7, Berecki 2018 (pt 3)	12	.5	Sp, T, F, M	IESS	Ongoing seizures	Used >10 antiseizure treatments, current treatments unknown
	NHS ID 24	5.6	No epilepsy	–	–	–	–
	NHS ID 120	2.2	.6	Sp, TCS	IESS	No seizures for 4 months	VGB, TPM, IV methylprednisolone, CZP, LEV, VPA, ACTH
	NHS ID 121	1.3	.8	Sp, T, unclassified	IESS	Ongoing seizures (multiple daily)	TPM, KD, CBD, CLB, PHT, TPM, VGB, PNL

Seizure response to sodium channel-blocking antiseizure medications	Worsening or seizure freedom with other antiseizure treatments	DD/ID at last review	ASD diagnosis	History of plateau/ regression	VABS-3 ABC score	Gross motor functional classification system level	Communication function classification system level	Phenotypic group
LCS: seizures worse; ZNS: no change	–	Yes	No	Yes	41	V	V	Later onset epilepsy–midinfancy
OXC: seizures markedly worse	–	Yes	No	Yes	44	V	IV	Later onset epilepsy–midinfancy
UK	UK	UK	Yes					UK (ID/ASD with or without epilepsy)
UK	UK	UK	Yes					UK (ID/ASD with or without epilepsy)
UK	UK	UK	Yes					UK (ID/ASD with or without epilepsy)
UK	UK	UK	Yes					UK (ID/ASD with or without epilepsy)
–	–	UK	Yes					ID/ASD without epilepsy
–	–	UK	Yes					ID/ASD without epilepsy
UK	UK	Yes (moderate)	Yes					UK (epilepsy and DD/ID, no further details)
UK	UK	Yes (severe)	Yes					Later onset epilepsy–childhood
UK	UK	UK	Yes					UK (ID/ASD with or without epilepsy)
PHT: seizures worse; OXC: no change (but devt worse); ZNS: no change	VGB: seizures worse	Yes	No	Yes	23	IV	IV	Later onset epilepsy–midinfancy
Not used	Seizures ceased without contemporaneous ASM changes	Yes	No	Yes	38	IV	IV	Later onset epilepsy–midinfancy
PHT: no change	VGB: seizures worse	Yes	No	Yes	–	V	III	Later onset epilepsy–midinfancy
–	–	Yes	No	No	42	IV	IV	ID/ASD without epilepsy
Not used	ACTH ceased spasms; after recurrence, spasms ceased with monthly pulses of IV methylprednisolone	Yes	No	Yes	53	Too young (unable to sit unaided)	Too young (babbles)	Later onset epilepsy–midinfancy
PHT: UK effect	VGB: seizures worse	Yes	Too young	Yes	66	Too young (unable to sit unaided)	Too young (cooing)	Later onset epilepsy–midinfancy

TABLE 4 (Continued)

Variant (*n*), c., p.	Patient source[a]	Patient age at review, years	Age at epilepsy onset, years	Seizure type(s)	Epilepsy syndrome (at onset)	Seizure outcome	Antiseizure treatments used (current treatments underlined)
	NHS ID 126	1.8	.7	Sp, T, unclassified	IESS	Ongoing seizures (multiple daily)	CLB, LEV, KD, CBD, LTG, ZNS, ACTH
	Allen 2013 (family kf)	.8	.5	Sp, T, F, AtAb	IESS	UK	UK
	Berecki 2018 (pt 1)	6	.5	Sp, T	IESS	Ongoing seizures (infrequent)	CBZ, VGB, others unknown
	Kong 2019 (pt 41)	UK	.6	EM, Sp, T	Unclassified	Ongoing seizures (UK frequency)	CBZ, MXT, VPA (current treatments unknown)
	Samanta 2015	2	.7	Sp, F, M	IESS	Ongoing seizures (UK frequency)	CLP, CZP, LEV, LTG, TPM, KD (current treatments unknown)
	Wolff 2017 (pt 46)	6.5	.7	Sp, AtAb, T, F	IESS	Ongoing seizures (UK frequency)	CBZ, FLB, LTG, LEV, TPM, VPA, ACTH (current treatments unknown)
	Nakamura 2013 (pt 99)/ Kobayashi 2016	UK (>6 yrs)	.8	Sp	IESS	Seizure-free (since age 6 years)	LTG, CLB, NZP, VPA, ACTH (current treatments unknown)
	Allen 2013 (family bg)	2	.8	Sp	IESS	UK	UK
	Wolff 2017 (pt 50)	8	1.1	Sp, T, "autonomic," M	IESS	Ongoing seizures (UK frequency)	ACZ, CLB, ESM, LEV, PB, STM, TPM, VGB, KD, steroids (current treatments unknown)
	Wolff 2017 (pt 56)	25	3	T, Sp	Unclassified	Ongoing seizures (UK frequency)	VPA, ACTH (current and other treatments unknown)
	Butler 2017 (pt 45)	UK	UK	UK	UK	UK	UK
	Li 2016 (pt 1)	UK	UK	UK	UK	UK	UK
	Li 2016 (pt 2)	UK	UK	UK	UK	UK	UK
	Long 2019 (pt P045)	3.7 (deceased)	UK	UK	UK	UK	UK
G899S (1) c.2695G>A Gly899Ser	Wolff 2017 (pt 38)	7	.3	HC, TCS, F, AtAb	Unclassified	Yes (UK frequency)	CLB, CZP, LCS, LEV, OXC, PB, RUF, TPM, VPA, ZNS (current treatments unknown)
T1420M (1) c.4259C>T Thr1420Met	Ben-Shalom 2017 (pt 13642. p1)	9	No epilepsy	–	–	–	–
P1622S (1) c.4864C>T Pro1622Ser	Wolff 2017 (pt 52)	3	1.8	TCS, M-A, T, AtAb, M	EMAtS	Ongoing seizures (UK frequency)	CLB, ESM, LEV, LTG, OXC, PB, TPM, VGB, KD (current treatments unknown)
E1211K (6) c.3631G>A Glu1211Lys	NHS ID 64	7.8	1.5	Sp, unclassified		Seizure-free (since age 2.5 years)	KD, CBD, CBZ, CLB, CZP, LTG, LEV, PHT, TPM, VPA
	NHS ID 123	1.2	.5	Sp	IESS	Ongoing seizures (multiple daily)	TPM, VPA, VGB, PNL, CLB, LEV, PB, ZNS

Seizure response to sodium channel-blocking antiseizure medications	Worsening or seizure freedom with other antiseizure treatments	DD/ID at last review	ASD diagnosis	History of plateau/ regression	VABS-3 ABC score	Gross motor functional classification system level	Communication function classification system level	Phenotypic group
LTG: seizures worse; LCS: no change; RUF: UK effect; ZNS: no change	–	Yes	Too young	Yes	–	Too young (unable to sit unaided)	Too young (no cooing)	Later onset epilepsy–midinfancy
UK	UK	Yes (severe)	UK					Later onset epilepsy–midinfancy
CBZ: seizures reduced	VGB: seizures worse	Yes (severe)	UK					Later onset epilepsy–midinfancy
CBZ: no change	–	Yes (severe)	UK					Later onset epilepsy–midinfancy
LTG: no change	–	Yes (severe)	UK					Later onset epilepsy–midinfancy
CBZ: seizures worse; LTG: no change	–	Yes (severe)	UK					Later onset epilepsy–midinfancy
LTG: seizure-free	NZP: worsened	Yes (severe)	UK					Later onset epilepsy–midinfancy
UK	UK	Yes (severe)	UK					Later onset epilepsy–midinfancy
LTG: no change; OXC: no change	–	Yes (severe)	UK					Later onset epilepsy–midinfancy
Not used	–	Yes (severe)	UK					Later onset epilepsy–childhood
UK	UK	Yes	UK					UK (epilepsy and DD/ID, no further details)
UK	UK	Yes	UK					UK (epilepsy and DD/ID, no further details)
UK	UK	Yes	UK					UK (epilepsy and DD/ID, no further details)
UK	UK	Yes	UK					UK (epilepsy and DD/ID, no further details)
OXC: seizures worse: LCS: no change; ZNS: no change	–	Yes (severe)	Yes					Later onset epilepsy–midinfancy
–	–	No	Yes					ID/ASD without epilepsy
OXC: seizures worse; LTG: seizures worse	–	Yes (moderate)	Yes					Later onset epilepsy–childhood
PHT: seizures worse; CBZ: no change; LTG: no change	Seizures ceased without contemporaneous ASM additions	Yes	Yes	Yes	–	II	IV	Later onset epilepsy–midinfancy
OXC: seizures worse; ZNS: seizures worse; LTG: UK effect	–	Yes	Too young	Yes	89	Too young (unable to sit unaided)	too young (cooing)	Later onset epilepsy–midinfancy

TABLE 4 (Continued)

Variant (*n*), c., p.	Patient source[a]	Patient age at review, years	Age at epilepsy onset, years	Seizure type(s)	Epilepsy syndrome (at onset)	Seizure outcome	Antiseizure treatments used (current treatments underlined)
	Ogiwara 2009 (pt 1)	22	.9	Sp, T, TCS	IESS	Ongoing seizures (UK frequency)	CLB, PB, VPA, ACTH (current treatments unknown)
	Wolff 2017 (pt 49)	4	.9	Sp, M, FS	IESS	Ongoing seizures (UK frequency)	LEV, LTG, STH, VGB, KD, steroids (current treatments unknown)
	Wong 2015	6	1.3	Sp	IESS	Seizure-free (since age 4 years)	MAD, CLB, LEV, VGB, VPA, ACTH
	van der Werf 2020 (no pt. ID)	UK	UK	UK	UK	UK	UK
S1656P (1) c.4966T>C Ser1656Pro	Suddaby 2019	38	.8	UK	UK	Controlled	CBZ, LEV

Note: Four variants (D195G, R379H, R853Q, and E1211K) were recurrent, whereas the remaining 11 variants (D12N, D82G, S686fs, G899S, R937H, C959*, G1013*, C1386R, T1420M, P1622S, and S1656P) were nonrecurrent.

Abbreviations: –, not applicable; ABC, adaptive behavior composite; ACZ, Acetazolamide; ACTH, Adrenocorticotropic hormone; ASM, antiseizure medication; ASD, autism spectrum disorder; At, Atonic seizures; AtAb, Atonic-Absence seizures; CBZ, Carbamazepine; CBD, Cannabidiol; CLB, Clobazam; CLP, Clorazepate; CZP, Clonazepam; DD, developmental delay; Devt, development; EM, Epileptic myoclonic seizures; EMAtS, Epilepsy with Myoclonic Atonic Seizures; ESM, Ethosuximide; F, Focal seizures; FIAS, Focal Impaired Awareness Seizures; FS, Febrile seizures; FLB, Felbamate; HC, Hemiclonic seizures; ID, intellectual disability; IESS, Infantile Epileptic Spasms Syndrome; IV, intravenous; KD, Ketogenic diet; LCS, Lacosamide; LEV, Levetiracetam; LTG, Lamotrigine; M, Myoclonic seizures; M-A, Myoclonic-Atonic seizures; MAD, Modified Atkins Diet; MXT, Methsuximide; NHS, Natural History Study; NZP, Nitrazepam; OXC, Oxcarbazepine; PB, Phenobarbital; PHT, Phenytoin; PNL, Perampanel; pt, patient; RUF, Rufinamide; STH, Sulthiame; STM, Stimulant therapy; STP, Stiripentol; Sp, Spasms (epileptic spasms); T, Tonic seizures; TCS, Tonic-Clonic Seizures; TPM, Topiramate; UK, unknown; VABS-3, Vineland Adaptive Behavior Scale-3; VGB, Vigabatrin; VPA, Valproic acid; ZNS, Zonisamide.

[a]Patient source: R379H, S686fs, R937H, C959*, G1013*, C1386R, D12N, D82G, and T1420M: Ben-Shalom et al., Biol Psychiatry 2017;82:224–232. R853Q: Berecki et al., Proceedings of the National Academy of Sciences 2018;115:E5516; Allen et al., Nature 2013;501:217–221; Kong et al., Clinica Chimica Acta 2018;483:14–19; Samanta & Ramakrishnaiah, Acta Neurologica Belgica 2015;115:773–776; Wolff et al., Brain 2017;140:1316–1336; Nakamura et al., Neurology 2013;81:992–998/Kobayashi et al., Brain Dev 2016;38:285–292; Butler et al., Pediatr Neurol 2017;77:61–66; Li et al., Mol Psychiatry 2016;21:290–297; Long et al., Frontiers in Neurology 2019;10;G899S. P1622S: Wolff et al., Brain 2017;140:1316–1336. E1211K: Ogiwara et al., Neurology 2009;73:1046–1053; Wolff et al., Brain 2017;140:1316–1336; Wong et al., Brain and Development 2015;37:729–732; van der Werf et al., Eur J Hum Genet 2020:1726–1733. S1656P: Suddaby et al., Psychiatr Genet 2019;29:91–94. Please see SCN2A International Natural History Study Database for all NHS patient sources.

in 22 of 24 (92%) cases with available phenotypic data (phenotype was unknown in seven individuals). In contrast, both partial and total LoF was observed across all three phenotypic groups. There was a tendency for total LoF in the ID/ASDwoE group (12/16 individuals with this phenotype, 75%), but six individuals with the LO-MI phenotype also carried total LoF variants, including two with protein-truncating mutations (S486fs and R1635*; Table 1). Additionally, the recurrent R853Q missense variant, which causes mixed LoF, was also identified in a 5-year-old individual with an ID/ASDwoE phenotype, further underscoring the limited correlation between biophysical impact and clinical presentation (Tables 3 and 4).

Notably, the recurrent L1650P variant, associated with a novel phenotype of episodic ataxia with or without DD/ID and resulting in total LoF, was also found in an individual with an LO-MI phenotype and severe ID. This highlights that, beyond distinct phenotypic groupings, individual variants can give rise to disorders of markedly different severity.

We mainly considered relationships between phenotype and LoF mechanism by phenotypic subgroups, which reflect presence and age at onset of epilepsy, and provide useful clinical information about epilepsy syndrome (IESS vs. other) and developmental outcome severity (lower or higher motor function). However, we also considered relationships between LoF mechanisms and specific epilepsy features; we saw no difference in the proportion with ongoing seizures at last review by LoF mechanism (total LoF, 8/11 [73%]; partial LoF, 4/4 [100%]; mixed LoF, 15/21 [71%]), we did not identify consistently beneficial ASMs, and seizure exacerbations with SCB ASMs were reported across all three LoF mechanisms.

4 | DISCUSSION

Disorders caused by LoF variants in *SCN2A* have a range of clinical features, generally clustering into one of three phenotypic categories: midinfancy onset epilepsy (a DEE),

Seizure response to sodium channel-blocking antiseizure medications	Worsening or seizure freedom with other antiseizure treatments	DD/ID at last review	ASD diagnosis	History of plateau/ regression	VABS-3 ABC score	Gross motor functional classification system level	Communication function classification system level	Phenotypic group
Not used	–	Yes (severe)	Yes					Later onset epilepsy–midinfancy
LTG: seizures reduced	–	Yes (severe)	UK					Later onset epilepsy–midinfancy
Not used	Seizure-free on MAD							Later onset epilepsy–midinfancy
UK	UK	Yes (UK severity)	UK					UK (epilepsy and DD/ID, no further details)
CBZ: seizure-free (in combination)	LEV: seizure-free (in combination)	Yes (UK severity)	Yes					Later onset epilepsy–midinfancy

childhood onset epilepsy (variably a DEE), and ID/ASD without epilepsy.[3,6] To date, these LO phenotypes resulting from *SCN2A* LoF have been less extensively studied than the early onset phenotypes typically caused by *SCN2A* GoF variants. The relationship between the underlying LoF mechanisms in Nav1.2 channels and the resulting clinical phenotypes has been unclear.

In this study of 15 LoF *SCN2A* variants, supported by 15 previously characterized variants, we confirm that phenotypes with onset at age 3 months or later reliably predict $Na_v1.2$ channel LoF, with biophysical analysis validating the presumption of LoF in all assessed variants. However, the identified LoF mechanisms—total, partial, or mixed—did not strongly correlate with specific phenotypic groups (LO-MI, LO-C, ID/ASDwoE, or other). As different LoF mechanisms may require distinct precision therapies, our findings underscore the importance of not relying solely on clinical phenotype to guide personalized treatment strategies.

4.1 | From nonsense to dysfunction: Molecular mechanisms underlying $Na_v1.2$ LoF

The mechanisms underlying $Na_v1.2$ LoF, as revealed by our biophysical analyses, were diverse and included total LoF (e.g., R102*, R119I, S486fs, F537fs, R937C, M951R, W1348*, Y1498del, R1235*, R1635*, L1650P), partial LoF (e.g., Y428C, V198D, R1635Q), and mixed LoF (e.g., A1773T). Truncating variants, particularly those introducing premature stop codons or frameshifts before residue 1591, are likely subject to nonsense-mediated decay (NMD).[5,8,26] Consistent with this, heterologous expression of R102*, S486fs, F537fs, S686fs, C959*, G1013*, R1235*, W1348*, and Y1498del did not produce measurable sodium currents, suggesting a complete loss of functional channel expression. Other variants such as R1635* and L1650P may escape NMD, yet still exhibit total LoF, likely due to protein instability or disrupted trafficking.[26]

Despite these distinct molecular mechanisms, which are expected to result in *SCN2A* haploinsufficiency, no consistent clinical phenotype was observed across total LoF variants (Table 1).

Current densities for Y428C, V198D, R1635Q, and A1773T were comparable to those of wild-type channels, but their voltage dependence was altered (Table 2). Among these, V198D and R1635Q are located in transmembrane segments S3 and S4, respectively, which contribute to voltage sensing and gating, whereas Y428C and A1773T reside in S6 segments, which are critical for pore constriction and fast inactivation.[27] Given the established role of domain IV in fast inactivation, it is unsurprising that R1635Q also exhibited slowed recovery from inactivation.[28,29] A1773T caused hyperpolarizing shifts in both activation and inactivation, consistent with a mixed LoF, whereas the other variants displayed hyperpolarized inactivation alone, consistent with partial LoF. Although Y428C was previously reported to exhibit wild-type-like biophysical properties,[12] discrepancies likely reflect differences in expression systems, highlighting the impact of experimental context on functional interpretation.

We note that our classification of the LOF mechanisms leading to $Na_v1.2$ channel dysfunction into partial and mixed groups is based on analyses of current densities, voltage dependence of activation and inactivation, and recovery from fast inactivation. This framework has limitations; some variants currently classified as partial LoF may exhibit mixed LoF/GoF properties if additional biophysical features—beyond those assessed in this study—reveal GoF effects. Moreover, certain $Na_v1.2$ variants may display LoF under specific physiological or developmental conditions while retaining normal function under others.

Together, our findings highlight the diverse molecular disruptions underlying $Na_v1.2$ LoF, although the precise relationship between these molecular mechanisms and clinical phenotypes remains incompletely understood.

4.2 | Genotype–phenotype mechanism correlations and their absence

In prior studies, missense variants are more frequently linked to LO epilepsy,[3] whereas truncating variants tend to result in ID/ASDwoE.[8] Our data reveal that the distinct LoF mechanisms, total, partial, and mixed, are all reported in people with each of the three typical LoF phenotypes. In addition, different clinical phenotypes are seen in people with some of the recurrent LoF variants. For example, the R853Q variant appears across LO-MI, LO-C, and ID/ASDwoE categories, whereas other variants such as R102*, Y428C, E1211K, R1235*, L1650P, and A1773T are observed in two distinct phenotypic groups (Table 1).

Although there was no consistent association between LoF mechanism or specific variant and clinical phenotype, it is potentially notable that most individuals with mixed LoF variants have an LO-MI phenotype. It is important to note, however, that the reverse does not hold; that is, the LO-MI phenotypes can be caused by each of the LoF mechanisms. This, and the variability in phenotype among individuals with the same recurrent variant, demonstrate strongly that phenotype should not be used to infer LoF mechanism. Genetic, environmental, or developmental modifiers likely influence disease expression within the range of LoF phenotypes.

4.3 | Novel phenotype

The L1650P variant, identified in an individual with the LO-MI phenotype, was also identified in five individuals who did not fit within established LoF phenotypic groups. These individuals shared similar clinical features of episodic ataxia or episodic hemiplegia and did not have epilepsy. Notably, one exhibited normal cognitive function and three had borderline ID. Episodic ataxia is well described in people with *SCN2A*-related disorders, although previously only associated with GoF variants (eg A263V).[25] The L1650P variant defines a novel genotype–phenotype association, demonstrating that even total LoF mutations can give rise to unexpectedly mild or atypical presentations, further highlighting the complexity of genotype–phenotype relationships in *SCN2A*-related disorders.

4.4 | Treatment implications and precision therapy

Effective seizure control remains elusive for many individuals with LoF *SCN2A* disorders.[3,11,30] The variability in LoF mechanism would support the need for tailored therapies based on the underlying channel dysfunctions—total, partial, or mixed—as each may respond differently to pharmacological or genetic interventions. Currently, the main ASM recommendation for epilepsy in LoF *SCN2A* disorders is avoidance of sodium channel blockers. Our data confirm that seizure exacerbations with SCB ASM can occur with all three types of channel dysfunction. Unfortunately, our data do not provide evidence to support preferential use (as opposed to avoidance) of particular ASMs, whether across all types of LoF or tailored to particular mechanisms of channel dysfunction. Our relatively small patient numbers may have limited our ability to advance understanding of optimal use of currently available treatments; further work on larger cohorts should seek to rectify this.

Although we do not have evidence for variability in treatment response by type of channel dysfunction for currently available treatments (i.e., ASM), consideration of the type of channel dysfunction will be of considerable importance for the development and use of genetic therapies for LoF *SCN2A* disorders. Whereas downregulation strategies show promise in early onset DEE due to channel GoF,[31] LoF phenotypes may require therapies that enhance *SCN2A* expression. CRISPR (clustered regularly interspaced short palindromic repeats)-based activation has restored function in preclinical LoF models[32]; however, the dosage sensitivity of *SCN2A* necessitates precise control to avoid detrimental under- or overexpression, with therapeutic windows likely differing between partial and complete LoF. Patients with total or partial LoF variants may benefit from strategies that enhance $Na_v1.2$ activity, but mixed LoF/GoF variants pose therapeutic challenges. In such cases, allele-specific modulation or combination therapies that balance LoF enhancers with GoF blockers may be required, although no targeted treatments are currently available.

5 | CONCLUSIONS

Improving clinical management of *SCN2A*-associated LoF disorders requires integrating biophysical characterization with phenotypic features, as phenotype alone does not infer the LoF mechanism Ongoing efforts to stratify patients based on variant function and phenotype will be crucial for guiding individualized therapy. Defining the specific mechanism of each variant will ultimately guide precision treatments, prevent harmful interventions, and inform the development of targeted therapies. Ongoing collaboration between research and clinical disciplines remains essential for translating these insights into meaningful outcomes for patients.

AUTHOR CONTRIBUTIONS

Marsha Tan performed in vitro experiments, analyzed data, and revised the manuscript. Beatrice Southby Goad, Meagan Allen, Jill Rodda, and Katherine B. Howell collected, curated, and analyzed data. Kay L. Richards conducted in vitro experiments, analyzed data, and contributed to writing the Methods section. Simone L. Ardern-Holmes, Daniel Bamborschke, Inna Hughes, Kate Riney, Ana Roche Martinez, Angelo Russo, Adriane Sinclair, Stefano Sartori, Marina Trivisano, Angela De Dominicis, and Nicola Specchio collected data. Rikke S. Møller, Ingrid E. Scheffer, Walid Fazeli, Markus Wolff, and Katherine B. Howell analyzed and interpreted the data. Géza Berecki performed in vitro experiments and analyzed data. Ingrid E. Scheffer, Steven Petrou, Katherine B. Howell, and Géza Berecki acquired financial support for the project leading to this publication. Katherine B. Howell and Géza Berecki designed the study and wrote the manuscript. All authors critically reviewed the manuscript, approved the final version for publication, and agree to be accountable for all aspects of the work.

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ACKNOWLEDGMENTS

We are grateful to all families at the participating Simons Searchlight sites as well as the Simons Searchlight Consortium, formerly the Simons VIP Consortium. We appreciate obtaining access to phenotypic and genetic data on SFARI Base. Approved researchers can obtain the Simons Searchlight population dataset described

in this study by applying at https://base.sfari.org. This study was supported by a Medical Research Future Fund Genomic Health Futures Mission project grant to I.E.S., S.P., K.B.H., and G.B., and project funding to the *SCN2A* Natural History study by RogCon and Praxis Precision Medicines to K.B.H. S.P. is supported by an NHMRC fellowship, and K.B.H. by a clinician–scientist fellowship from the Murdoch Children's Research Institute (MCRI). G.B. was partly funded by RogCon. The Florey Institute of Neuroscience and Mental Health and MCRI are supported by a Victorian State Government Operational Infrastructure Support Program.

CONFLICT OF INTEREST STATEMENT

I.H. has received funding from Neurelis, Neurocrine, Marinus and the University of Rochester Medical Center, for clinical trials in which she was principal investigator. K.R. has received honoraria for educational symposia, advisory boards, and/or consultancy work from Eisai, LivaNova, Medlink Neurology, Novartis, and UCB Australia. Her institution has supported clinical trials for Biogen Idec Research, DSLP, Eisai, Epigenyx Therapeutics, GW Research, Janssen-Cilag, Longboard Pharmaceuticals, Marinus Pharmaceuticals, Medicure International, LivaNova, Neurocrine Biosciences, Noema Pharma, Novartis, SK Lifesciences, Takeda Pharmaceutical Company Limited, UCB Australia, UCB Biopharma SRL, and Zogenix. A.R. has received speaker honoraria from Eisai and LivaNova. M.Tr. has received speaker's fees or funding from or has participated on advisory boards for BioMarin, Orion, and Biocodex. N.S. has served on scientific advisory boards for GW Pharma, BioMarin, Arvelle, Marinus, and Takeda; has received speaker honoraria from Eisai, BioMarin, LivaNova, and Sanofi; and has served as an investigator for Zogenix, Marinus, BioMarin, UCB, and Roche. I.E.S. has served on scientific advisory boards for Biocodex, BioMarin, CAMP4 Therapeutics, Chiesi, Eisai, Encoded Therapeutics, Knopp Biosciences, Longboard Pharmaceuticals, Mosaica Therapeutics, Takeda Pharmaceuticals, and UCB; has received speaker honoraria from Akumentis, Biocodex, BioMarin, Chiesi, Eisai, GlaxoSmithKline, LivaNova, Nutricia, Stoke Therapeutics, and Zuellig Pharma; has received funding for travel from Biocodex, BioMarin, Eisai, Encoded Therapeutics, GlaxoSmithKline, Stoke Therapeutics, and UCB; has served as an investigator for Anavex Life Sciences, Biohaven, Bright Minds Biosciences, Cerebral Therapeutics, Cerecin, Cereval Therapeutics, Encoded Therapeutics, EpiMinder, ES-Therapeutics, GW Pharma, Longboard Pharmaceuticals, Marinus, Neuren Pharmaceuticals, Neurocrine BioSciences, Ovid Therapeutics, Praxis Precision Medicines, Shanghai Zhimeng Biopharma, SK Life Science, Supernus Pharmaceuticals, Takeda Pharmaceuticals, UCB, Ultragenyx, Xenon Pharmaceuticals, Zogenix, and Zynerba; has consulted for Atheneum Partners, Biohaven Pharmaceuticals, Care Beyond Diagnosis, Cerecin, Eisai, Epilepsy Consortium, Longboard Pharmaceuticals, Praxis, Stoke Therapeutics, UCB, and Zynerba Pharmaceuticals; and is a nonexecutive director of Bellberry and a director of the Australian Academy of Health and Medical Sciences. She may accrue future revenue on pending patent WO61/010176 (filed: 2008): Therapeutic Compound; has a patent for SCN1A testing held by Bionomics and licensed to various diagnostic companies; and has a patent Molecular Diagnostic/Theranostic Target for Benign Familial Infantile Epilepsy (BFIE), [PRRT2] 2011904493 & 2012900190 and PCT/AU2012/001321 (TECH ID:2012–009). S.P. is cofounder and equity holder in Praxis Precision Medicines and RogCon, which develops precision medicines for neurogenetic disorders. S.P. is a scientific advisor and equity holder in Pairnomix, which is undertaking precision medicine development in epilepsy and related disorders. The remaining authors declare no competing interests. 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 STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Tan M, Goad BS, Allen M, Rodda J, Richards KL, Ardern-Holmes SL, et al. Mechanisms of *SCN2A* loss of function do not predict presence or phenotype of epilepsy. Epilepsia. 2026;00:1–26. https://doi.org/10.1002/epi.70100