Commonalities and distinctions between two neurodevelopmental disorder subtypes associated with SCN2A and SCN8A variants and literature review

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Abstract
This study was aimed to analyze the commonalities and distinctions of voltage-gated sodium channels, Nav1.2, Nav1.6, in neurodevelopmental disorders. An observational study was performed including two patients with neurodevelopmental disorders. The demographic, electroclinical, genetic, and neuropsychological characteristics were analyzed and compared with each other and then with the subjects carrying the same genetic variants reported in the literature. The clinical features of one of them argued for autism spectrum disorder and developmental delay, the other for intellectual disability, diagnoses confirmed by the neuropsychological assessment. The first patient was a carrier of SCN2A (p.R379H) variant while the second was carrier of SCN8A (p.E936K) variant, both involving the pore loop of the two channels. The results of this study suggest that the neurodevelopmental disorders without overt epilepsy of both patients can be the consequences of loss of function of Nav1.2/Nav1.6 channels. Notably, the SCN2A variant, with an earlier expression timing in brain development, resulted in a more severe phenotype as autism spectrum disorder and developmental delay, while the SCN8A variant, with a later expression timing, resulted in a less severe phenotype as intellectual disability.

Keywords
autism spectrum disorders, EEG, epilepsy, intellectual disability, SCN2A, SCN8A, whole-exome sequencing

1 | INTRODUCTION

Voltage-gated sodium channels (VGSC) exert a key function in neuronal excitability and their specific clustering at axon initial segments (AISs) and nodes of Ranvier is essential for effective action potential initiation and propagation (Catterall, 2012; O’Brien & Meisler, 2013). The most genes linked with epilepsy frequently investigated are...
SCN1A, SCN2A, SCN3A (located on 2q24.3), and recently SCN8A (located on 12q13.13) which encode Na\textsubscript{1.1}, Na\textsubscript{1.2}, Na\textsubscript{1.3}, and Na\textsubscript{1.6} respectively. Overall, they are responsible for a significant number of early-onset genetic epilepsies characterized by a wide phenotypic spectrum, ranging from mild treatable seizure disorders to severe epileptic encephalopathies (EEs). Notably, epileptic phenotype SCN1A-related ranges from benign febrile seizures (FS) to severe Dravet syndrome, epileptic phenotype SCN2A-related ranges from self-limiting benign familial neonatal-infantile epilepsy (BFNIE) to severe Ohtahara syndrome, the SCN8A-related epileptic phenotype is linked to a spectrum ranging from severe early infantile epileptic encephalopathies (EIEE) to benign familial infantile epilepsy (BFIE) (Brunklaus et al., 2020; Musto et al., 2020; Wolff et al., 2017).VGSCs share a similar amino acid sequence and structure, but their genetic variants lead to different dysfunctions as SCN1A is mostly expressed in inhibitory neurons, whereas SCN2A/8A is mainly expressed in excitatory neurons. In addition, SCN1A-related epileptic phenotype is mainly linked to loss of function (LoF) of Na\textsubscript{1.1}, whereas the SCN2A/8A-related epileptic phenotypes are mostly linked to gain of function (GoF) of Na\textsubscript{1.2}, and Na\textsubscript{1.6}. Further, Na\textsubscript{1.2} and Na\textsubscript{1.6}, while being structurally and functionally similar, differ from each other in the age of expression, being Na\textsubscript{1.2} present early from the prenatal late second trimester to 1–2 years of age, when it is largely replaced by Nav1.6 (Sanders et al., 2018).

During the last decade, the increasing use of next-generation sequencing (NGS) technologies, including whole-exome sequencing (WES) for the diagnostic evaluation of patients with epileptic syndromes, led to more than 300 patients diagnosed with SCN8A mutations and 276 subjects with SCN2A mutations with gain-of-function hyperactivity as the predominant pathogenic mechanism (Meisler, 2019; Sanders et al., 2018; Solé et al., 2020). In addition, a broadening of the phenotypic spectrum SCN2/8A-related, that includes heterogeneous neurodevelopmental disorders (NDD), is arising in a way quite evident (Larsen et al., 2015; Wagnon et al., 2017). Interestingly, a case series of 21 patients with SCN2A-associated Episodic Ataxia (EA) and epilepsy have been recently described suggesting a feasible upcoming identification of a specific phenotype/genotype also supported by an increasing targeted therapeutic approach such as acetazolamide in some patients (Dilena et al., 2017; Schwarz et al., 2019).

These findings made it difficult to outline a genotype/phenoype correlation due to increasing phenotypic variability since today carriers of SCN8A variants include individuals with distinct clinical features ranging from EE until isolated cognitive impairment, the latter linked to loss of channel function as the predominant pathogenic mechanism. In the same way, no precise correlation between phenotypic severity and genetic variant has been highlighted despite more than 150 missense mutations of SCN8A has been identified (Meisler, 2019; Solé et al., 2020). So, the electrophysiological analysis is a useful procedure to predict the clinical consequences and the pathogenic mechanism of a variant since, today, it represents a crucial step for a more specific and effective treatment.

However, although the functional characterization gives the obvious benefits, it entails challenging laboratory investigations that currently interfere with widespread use in clinical care and as a result, only 14 functional characterizations of missense Na\textsubscript{1.6} variants are reported today, 12 of which recognized as GoF (Solé et al., 2020). Similarly, only about 20 SCN2A variants have been electrophysiologically assessed (Sanders et al., 2018). To overcome these limitations, studies aimed at identifying the relationship between phenotype and location of known and functionally characterized variants have recently been carried out. The results revealed that LoF missense variants with channel loss of function often localize to the pore region (especially the S5–6 region); while variants with a gain-of-function are identified in the voltage sensor (S4), in DIII–IV linking, and in C-terminal regions. The former was identified in individuals with neurodevelopmental disorders without epilepsy, the latter in patients with the epileptic phenotype (O’Brien & Meisler, 2013; Wagnon et al., 2017).

The growing number of identified SCN2A/SCN8A variants, against the few available functional studies, have opened new investigative hypotheses aimed to build a powerful and accurate map of pathogenic variants as a surrogate of functional analysis and with positive effects on the therapeutic choice (Gardella & Møller, 2019; Johannesen et al., 2019).

We think that strengthening recent research lines, focused on the identification of distinct hotspots with recurrent mutations and on their concurrent increase in size, could be a productive way to identify and better understand the role of the environment, of the genetic background, and/or of the modifier genes on phenotypic expression (Schwarz et al., 2019). As previously suggested, an overall assessment that takes into account both genetic data and detailed clinical features such as developmental delay/regression, electroclinical pattern with the age of onset, sensitivity to AEDs, neuropsychological profile, other comorbidities, and the clinical outcome would also facilitate systematic identification and a better understanding of the SCN2A/SCN8A-related phenotypic spectrum.

Here we report two patients, carrying SCN8A E936K and SCN2A R379H variants, with NDD and distinct EEG patterns without epilepsy, notably the former suffers from an intellectual disability (ID) and the latter from development delay (DD) and autism spectrum disorder (ASD).
2  METHODS

An observational study was performed enrolling patients with neurodevelopmental disorders without overt epileptic seizures due to missense SCN2A/8A variants referred to the Child Neuropsychiatry Unit of University Hospital of Palermo, Italy. Clinical information was retrospectively and prospectively collected by interviews with patients and their families and from clinical documents. The patients underwent neurological and psychiatric evaluation, electroencephalographic recording, and detailed neuropsychological assessment with standardized formal tools.

Written informed consent, including an agreement for publication of data in scientific journals, was obtained from the patient's parents. This study was approved by the ethics committee Palermo 1 of “Paolo Giaccone” University Hospital of Palermo, Italy.

3  RESULTS

We identified 4 patients carrying SCN2A/8A variant referred to the Department of Child Neuropsychiatry. Two of them, mother and daughter, carrying a de novo SCN8A (NM_014191.3) c.5630A>G; p.N1877S variant in C-terminal, had moderate ID and rare epileptic focal seizures responsive to antiepileptic drugs. These features were suggested to be more consistent with the diagnosis of intermediate SCN8A-related epilepsy subtype, so both patients were excluded from the study. Then, we included in the study a subject with SCN8A variant and ID and a child with SCN2A variant and DD and ASD.

3.1  Patient 1

The proband, a 10-year and 5 months-old girl, was the third offspring born to healthy Italian unrelated parents whose cognitive, social, and academic functioning was adequate. Her family history was remarkable for learning disability and psychiatric disorder in her mother's sister and brother respectively. She was born at the 38th week of gestation by elective cesarean section following an uneventful pregnancy. Her birth weight was 3500 g (75th percentile), height cm 49 (10th percentile), head circumference 33 cm (25th percentile) and her clinical postnatal course was uneventful. Head control, sitting without support, and walking were achieved at 3, 10, and 22 months, respectively; she spoke the first words at 1 year followed by slow lexical development. Her academic skills remained impaired, and she received permanent educational support at school.

At the age of 5 years and 10 months, she was referred to our Child Neuropsychiatric Department, because of developmental delay, learning, and language difficulties. At last admission (age 10 years and 5 months), the neurological examination showed head circumference 48,5 cm (<3rd percentile), height cm.129 (<3rd percentile), weight kg 25 (3rd percentile), normal deep tendon reflexes, moderate motor, and coordination impairment, clumsiness with specific difficulties such as get off the sidewalks or stairs, but she was able in the basic tasks of daily activities; in addition, she exhibited shyness, low frustration tolerance, irritability.

The level of intellectual abilities, assessed with the Wechsler Intelligence Scale for Children IV Edition (WISC IV), showed that all Index scores were in the extremely low range except the Verbal Comprehension Index that was in the extremely low borderline range (VCI 63–77) emerging as a significant individual strong point.

The NEPSY II, a neurodevelopmental neuropsychological battery, administration showed affected attention, memory, learning, visuospatial, and visuomotor abilities (scaled score 1–3). The child's performances fell in the “well below expected level.” However, the child maintained some control of execution and ability to monitor her own behavior showing herself capable of formulating basic thoughts.

Adaptive functioning was assessed using the Vineland Adaptive Behavior Scale-Second Edition (VABS-II). Adaptive Behavior Composite, Communication, and Daily Living Skills scores were 35, 30, and 43 respectively showing a low adaptive level. On Socialization domain her scores were in the moderately low adaptive level (60) (Table 1 and Supplementary material).

Brain MRI performed at 6 years of age showed no abnormalities.

Wakefulness and sleep EEG showed bilateral discharges of high amplitude sharp waves and slow waves in the parieto-temporo-occipital regions increasing during sleep associated with generalized sequences of 3 Hz spike and wave complexes well identified in the anterior regions without related clinical events. These abnormalities were low sensitive to intermittent photic stimulation and hyperventilation (Figure 1a,b).

Panel NGS sequencing studies for 171 genes associated with developmental and brain malformations, was performed in trios (proband and his parents) and showed a heterozygous de novo SCN8A (NM_014191: c. 2806G>A p.E936K2) variant. The SCN8A p.E936K emerged as the explanation for the disease pathophysiology, being fully consistent with the phenotype of SCN8A encephalopathy and confirmed de novo in the trio by Sanger sequencing. The variant c.2806G>A results in the substitution of negatively charged Glutamic
| WISC IV | Standard score | 95% CI | Classification | NEPSY II | Scaled score | Classification | Scaled score |
|---------|----------------|--------|----------------|----------|--------------|----------------|--------------|
| IQ      | 40             | 37–49  | Extremely low  | Attention/executive |              | List memory immediate | 1            | Impaired     |
| VCI     | 68             | 63–77  | Extremely low-borderline | Auditory attention | <2 percentile | Impaired | List memory delayed | 1            | Impaired     |
| PRI     | 50             | 46–62  | Extremely low  | Visual attention | 4            | Impaired | Memory for names immediate | 1            | Impaired     |
| WMI     | 49             | 46–64  | Extremely low  | Design fluency   | 6            | Borderline | Memory for names delayed | 1            | Impaired     |
| PSI     | 47             | 45–65  | Extremely low  | Inhibition A composite | 1            | Impaired | Narrative memory | 2            | Impaired     |
| GAI     | 54             | 50–62  | Extremely low  | Inhibition B composite | 1            | Impaired | Sentence repetition | 2            | Impaired     |
| CPI     | 36             | —      | Extremely low  | Inhibition C composite | 1            | Impaired | Social perception |              |             |
| Verbal comprehension tasks | Scaled score |       | Animal sorting | 5          | Borderline | Theory of mind | 1            | Impaired     |
| Similarities | 5             |       | Borderline | Language and communication |              | Affect recognition | 1            | Impaired     |
| Vocabulary | 4             |       | Impaired | Comprehension of instructions | 1            | Impaired | Visuospatial functions |              |             |
| Comprehension | 5             |       | Borderline | Speeded naming composite | 4            | Impaired | Design copying | 1            | Impaired     |
| Information | 1             |       | Impaired | Phonological processing | 1            | Impaired | Block construction | 1            | Impaired     |
| Perceptual reasoning tasks | |       | Impaired | Semantic fluency | 2            | Impaired | Picture puzzles | 1            | Impaired     |
| Block design | 1             |       | Impaired | Phonological fluency | 4            | Impaired | Geometric puzzles | 1            | Impaired     |
| Picture concepts | 1             |       | Impaired | Repetition of nonsense words | 1            | Impaired | Visual motor precision composite | 4            | Impaired     |
| Matrix reasoning | 5             |       | Borderline | Oromotor sequences | 1            | Impaired |              |              |             |
| Working Memory tasks | |       | Impaired | Learning and memory |              | Vineland-II | Scaled score | Classification |
| Digit Span | 1             |       | Impaired | Memory for faces immediate | 1            | Impaired | Communication | 30           | Low adaptive level |
| Letter/number sequencing | 1             |       | Impaired | Memory for faces delayed | 2            | Impaired | Daily living skills | 43           | Low adaptive level |
acid residue 936 with a positively charged Lysine. This variant is predicted to cause loss of normal protein function being in the DII S5-S6 pore loop. Additionally, the c.2806G>A variant is not observed in large population cohorts (GnomAD database). The variant was identified in only 1 individual on ClinVar (accession number: VCV000520968.2) and considered “uncertain significance.” In addition, the c.2806G>A variant in the SCN8A was previously reported only once as a pathogenic variant in a patient with “intermediate” SCN8A-related epilepsy [10]. The variant is predicted “disease causing” by Mutation Taster, “Likely Pathogenic” by Varsome, and “probably damaging” by Poliphen 2.

3.2 | Patient 2

The patient is the only child born to apparently healthy unrelated parents at the 39th week of gestation, following an uneventful pregnancy, and delivery. The APGAR scores were 10–10 at 5 and 10 minutes respectively; his birth weight was 3640 g (75th percentile), height 52.4 cm (90th percentile), head circumference 35.2 cm (75th percentile) and his clinical postnatal course was uneventful. His family history was burdened with autism in one third-degree paternal female cousin. Developmental milestones of our patient were delayed: head control, sitting without support, and walking were achieved at 3, 8, and 18 months, respectively. He vocalized undifferentiated nasal sounds at 7 months and at present his language is jargon. He is currently under speech and neuropsychomotor therapy.

At the age of 3 years and 2 months the child was referred to our Child Neuropsychiatric Department because of developmental delay, behavioral, and communicative difficulties.

On admission, the neurological examination showed head circumference 50 cm (25th percentile), height cm.105 (<3rd percentile), weight kg 16 (3rd percentile), normal deep tendon reflexes, moderate motor, and coordination impairment, clumsiness.

He showed inconsistent eye contact and erratic response to name. He failed to integrate gaze with other communication behaviors. He showed limited range of facial expression directing toward others, infrequent initiation of joint attention, or of communicative bids for social or regulatory purposes. The child displayed decreased object exploration, restricted interests in a few toys and in the variety of play acts. He showed stereotyped fingertip movements (shaking and rubbing), and repetitive objects manipulation (stack of blocks). Rarely, he vocalized nasal/guttural and vowel sounds, almost always associated with an intentional communication of discomfort, and
occasionally exhibited a very low variety of conventional gestures.

To assess the developmental functioning was administered the Bayley Scales of Infant and Toddler Development Third Edition (Bayley III; Ferri et al., 2015) where all composite scores fell into the extremely low range of functioning: Cognitive scale 55, Language scale 49, Motor scale 42, Socioemotional scale 60, and General Adaptive Composite 55. Motor and language skills were delayed to a greater degree than cognitive and social skills (p < 0.05). The Adaptive Behavior Subscale Communication was significantly worse compared to other adaptive skills (p < 0.05). Short Sensory Profile (McIntosh, 1999), which measures the sensory processing patterns, showed atypical sensory reactivity, predominantly in sensory modulation (Table 2 and Supplementary material). Communication, social interaction, play, and restricted and repetitive behaviors were investigated by a semistructured, standardized assessment instrument (ADOS 2 module 1) which showed impairment in social–emotional reciprocity, poor empathy, and comments on others’ emotions, reduced use of eye contact, poor initiation of social interaction, along with repetitive games and use of objects. Scores in the

**FIGURE 1** EEG: SCN8A patient (a,b); SCN2A patient (c,d). Both EEG showed bilateral discharges of high amplitude sharp waves and slow waves in the parieto-temporo-occipital regions increasing during sleep associated with right frontal-central short sequences of 3.5–4 Hz spike and wave complexes.
domains of social affect (15), restricted and repetitive behaviors (2), and combined (17) supported a diagnosis of autism (cut off >16). The comparison score (6) indicated a moderate level of autism-related symptoms (Table 2 and Supplementary material).

Wakefulness and sleep EEG showed bilateral discharges of high amplitude sharp waves and slow waves in the parietal–temporal-occipital regions increasing during sleep associated with right frontal-central short sequences of 3.5–4 Hz spike and wave complexes (Figure 1c,d).

Panel NGS sequencing studies for 171 genes associated with developmental and brain malformations, was performed in trios (proband and his parents) and showed a heterozygous de novo SCN2A (NM_021007.2: c.1136G>A; p.R379H variant. The SCN2A p.R379H emerged as the explanation for the disease pathophysiology, being fully

### Table 2 Neuropsychological profile patient 2

| Scale                   | Composite score | Classification       |
|-------------------------|-----------------|----------------------|
| Cognitive               | 55              | Extremely low        |
| Language                | 49              | Extremely low        |
| Motor                   | 42              | Extremely low        |
| Socioemotional          | 60              | Extremely low        |
| General adaptive composite | 55             | Extremely low        |

| Ados 2 toddler module   |                  |                      |
|-------------------------|-------------------|----------------------|
| Social affect           | 15                |                      |
| Restricted and repetitive behaviors | 2            |                      |
| Combined domain total   | 17                | >Cut off autism      |

### Sensory profile

| Factors                          | Score | Classification       |
|----------------------------------|-------|----------------------|
| Sensory seeking                  | 67    | Typical performance  |
| Emotionally reactive             | 58    | Typical performance  |
| Low endurance/tone               | 31    | Definite difference  |
| Oral sensory sensitivity         | 27    | Probable difference  |
| Inattention/distraictibility     | 17    | Definite difference  |
| Poor registration                | 24    | Definite difference  |
| Sensory sensitivity              | 9     | Definite difference  |
| Sedentary                        | 13    | Typical performance  |

### Sensory processing

|                  | Score | Classification       |
|------------------|-------|----------------------|
| Auditory         | 29    | Probable difference  |
| Visual           | 32    | Typical performance  |
| Vestibular       | 46    | Typical performance  |
| Tactile          | 73    | Typical performance  |
| Multisensory     | 23    | Probable difference  |
| Oral sensory     | 40    | Probable difference  |

### Sensory modulation

| Factors                                         | Score | Classification       |
|-------------------------------------------------|-------|----------------------|
| Sensory processing related to endurance/tone    | 31    | Definite difference  |
| Modulation related to body position and movement| 32    | Definite difference  |
| Modulation of movement affecting activity level | 22    | Typical performance  |
| Modulation of sensory input affecting emotional responses | 11 | Definite difference |
| Modulation of visual input affecting emotional responses and activity level | 16 | Typical performance |
| Emotional–social responses                      | 63    | Probable difference  |
| Behavioral outcomes of sensory processing       | 18    | Probable difference  |
| Items indicating thresholds for response        | 7     | Definite difference  |
consistent with the phenotype of SCN2A-related syndrome and confirmed de novo in the trio by Sanger sequencing. The variant c.1136G>A causes the substitution of positively charged Arginine residue 379 with a positively charged Histidine. This variant is predicted to cause loss of normal protein function being in the DI S5-S6 linker, pore loop. Additionally, the c.1136G>A variant is not observed in large population cohorts (GnomAD database, ClinVar). The c.1136G>A variant in the SCN2A was previously reported only twice as a pathogenic variant in patients with SCN2A-related ASD (De Rubeis et al., 2014). The variant is predicted “disease causing” by Mutation Taster, “probably damaging” by Poliphen 2, “Pathogenic” by Varsome.

4 | DISCUSSION

The VGSCs are complex proteins consisting of primary pore-forming α subunits of 250 kDa and one or two auxiliary β subunits of 30–40 kDa. The channel proteins of α-subunit include nearly 2000 amino acid residues organized in four homologous domains (DI-DIV), each of which contains six transmembrane segments (S1-S6). They are characterized by similar structural and functional properties that confer a high degree of protein sequence conservation (Anderson & Greenberg, 2001; Catterall, 2012; Sanders et al., 2018).

The recent interest aroused by the involvement of LoF Na1,2.1 and Na1,6 in the two most important and frequent neurodevelopmental phenotypes, such as ASD and ID, has led to justified expectations for its future therapeutic options. Since we believe that objective assessments of the different cognitive and behavioral domains can identify specific and more homogeneous clinical characteristics, therefore more suitable for comparison in case studies, they can better facilitate the understanding of the pathophysiological mechanisms underlying the aforementioned disorders. So, we sought to compare the clinical findings of two of our subjects with ID and ASD respectively with each other, and then with those reported in the literature. Both SCN8A E936K and SCN2A R379H variants are located in the S5-S6 linker (P loop) of DI and DI respectively. Notably, SCN8A E936K alters the DEKA ring responsible for control ion selectivity. Almost all variants across different VGSCs reported in this region appear to be mainly LoF, and notably, the most SCN2A/8A variants associated with NDD without epilepsy are de novo (Ben-Shalom et al., 2017; Larsen al., 2015). The phenotypes of our patients seem consistent with this last statement as the individual carrying the SCN2A R379H variant shows developmental delay and ASD, while the individual carrying the SCN8A E936K variant has ID.

It is emerging that SCN2A is among the genes most frequently associated with ASD, but the underlying neuropathological mechanisms are yet largely unknown. Multiple lines of evidence relative to analyses of spatiotemporal coexpression of ASD genes converge in the early to mid-fetal brain development (10–24 postconceptional weeks); notably, in layer 5/6 of prefrontal and primary motor-somatosensory cortical glutamatergic projection neurons, among first involved in synaptic connections, in striatal medium spiny neurons, and in thalamus and cerebellum during postnatal development, which overall contribute to the formation of cortico-striatal-thalamic circuits, underlying several motor, emotional, cognitive behaviors especially impaired in ASD (Chang et al., 2015; Sanders, 2015; Wilsey et al., 2013).

Recently, it has been shown that Na1,2.2, in addition to early role in action potential initiation and propagation at AISs and at nodes of Ranvier, respectively, is selectively effective in backpropagation of action potential from proximal AISs into soma and dendrites of both immature and mature neocortical pyramidal neurons. Thus, the LoF of Na1,2.2 results in a persistent deficit in dendritic excitability leading to structural and functional changes in synaptic stability, plasticity, integration, and strength. Therefore, it is likely that chronic reduction of neuronal activity may change the dynamics of synaptic plasticity during maturation resulting in aberrant cerebral circuitry that may be detrimental to brain development in ASD and ID patients (Sanders et al., 2018; Spratt et al., 2019).

Among the consequences of the mutated channel protein, a key role concerns the changes associated with the development of early connectivity which typically appears to be characterized by increasing weakening of short-range connections and a simultaneous strengthening of long-range ones. An early imbalance between these two processes due to the LoF of the channel, which leads to a weakening of long-range connections underlying the information integration (Conti et al., 2017), could be influenced by the dysfunction of the SCN2A R379H variant.

However, ASD genes are expressed in multiple cell types and brain areas resulting in atypical patterns of connectivity that vary across systems and time and are not specific to any single brain region or behavioral domain. These findings may account for the phenotypic variability and its different severity, also found among the patients with SCN2A mutations, notably, stronger functional insufficiencies lead to more severe ASD phenotypes. Therefore, it is likely that the distinct phenotypes of our patients, ID, and DD-ASD, may result from different degrees of dysfunction expressed by the relative gene mutations (Chang et al., 2015).

The early haploinsufficiency of Nav1.2 can result in severe impairment of arising connectivity and is consistent
with severe development delay of patient 2, whereas, the haploinsufficiency of Nav1.6 can result in less detrimental impairment of late development and tuning of the connectivity, consistent with the level of the ID of patient 1. In addition, the Nav1.6 patient, exploiting the backpropagation of action potential Nav1.2 mediated, important for proper synaptic development and function, shows a less severe cognitive profile than Nav1.2 patient, and further, the lack of behavior and social impairment predominantly mediated by the backpropagation of action potential Nav1.2 related (Spratt et al., 2019). Although it is now accepted that LoF of SCN2A variants results in ASD/ID, one third of patients also develop late-onset epileptic seizures whose pathophysiological mechanism remains unclear being they are usually associated with GoF of SCN2A variants. It is likely that these events may be related to impaired connectivity or to factors other than the channel functions of SCN2A.

In addition, haploinsufficient SCN2A-mice were found to exhibit a mild absence-like epileptic phenotype caused by Na+,1.2 deficiency in cortical excitatory neurons leading to hypoexcitation of downstream inhibitory neurons in the thalamic reticular nucleus which in turn fails to suppress excitatory thalamocortical relay neurons, the hallmark of the pathogenic mechanism of absence epilepsy (Ogiwara et al., 2018). Similarly, Nav1.6-mediate hypoexcitation of cortical circuits results in non-convulsive absence epilepsy in mice and humans (Berghuis et al., 2015; Papale et al., 2009). Notably, loss of SCN8A from inhibitory neurons of the thalamic reticular nucleus in mice leads to hyperexcitation (hypersynchrony) of thalamocortical circuits and non-convulsive absence seizures (Makinson et al., 2017). Both our patients showed an EEG pattern characterized by bilateral discharges of high amplitude sharp waves and slow waves in the parietal–temporal–occipital regions increasing during sleep associated with short sequences of 3–4 Hz spike and wave complexes, generalized in the first patient and focal in the other one, without associated clinical events, supporting the experimental data above mentioned (Makinson et al., 2017).

In addition, the anterior predominant EEG pattern without overt epileptic seizures could mirror a weakness of the synchronous firing spreading, which seems consistent with impaired neuronal connectivity through the cerebral networks (Kaczmarek, 2019; Spratt et al., 2019).

Among the patients with SCN8A variants reported in the literature, we found only an 8-year-old individual with SCN8A E936K, likely inherited by the father suffering from ID and ADHD, whose electroclinical feature was characterized by focal seizures, atypical absence, febrile seizures, beginning at 5 years, sensitive to Levetiracetam, interictal spike–wave in the left frontal, central, and right parietal regions associated with autistic features and behavioral problems (Johannessen et al., 2019). The comparison between the two patients shows that our girl exhibits a less severe phenotype although both could likely share the interictal EEG pattern which in the reported patient has not been described in detail anyway.

Sadly, the comparison between our patient carrying the SCN2A R379H variant with other subjects reported in the literature is not feasible as the other two identical variants so far identified among a series of autistic subjects are lacking in further clinical details of their relative phenotypes (De Rubeis et al., 2014). However, while the delay in the developmental milestone of our SCN2A carrier patient seems consistent with early expression of Na+,1.2, from the prenatal late 2nd trimester, conversely, the early development delay of our SCN8A carrier patient remains to be elucidated. As the expression of Na+,1.6 typically peaks between the first and second year by replacing a large extent to the Na+,1.2 functions, we would have expected a normal psychomotor development in the first 2 years supported by the typical early neuronal excitability by Na+,1.2 and by its compensatory function extended over time.

Despite the knowledge of the pathophysiological mechanisms of Na+ channels are growing, the variability of the genotype/phenotype correlation remains unclear. On the other hand, the understanding of phenotypic variability always suggests carefully considering the epistasis phenomenon resulting from other genetic factors such as in other conditions where the coincidence of different mutated genes could share common targets implicated in the same phenotype (Nardello et al., 2020).

In addition, a recent study documented that biallelic SCNIB variants, leading to alterations in Nav channel gating or kinetics, are predicted to increase neuron excitability and to cause the early infantile epileptic encephalopathy 52 (Scala et al., 2021).

Further, the altered relation of the Nav auxiliary β subunits and Nav channel, the additional effects of reduced early axonal excitability on network functioning, and possibly different environmental factors can play an important role in the phenotype characterization. However, as there is not always a phenotypic overlap, even when the different characteristics of substituting amino acid are taken into account, detailed analyses of the clinical features of patients with recurrent mutations could be useful in characterizing the phenotype for personalized therapeutic options (Brunklaus et al., 2020; Dilena et al., 2017; Schwarz et al., 2019). Therefore, the detailed knowledge of the functioning of the Na channels appears to be an interesting goal that could lead to the discovery of effective molecules in restoring their functions even in the mature brain. Lastly, the current rarity of these disorders would suggest developing an international network, as is usually the case.
with some rare diseases, which allows the exchange of clinical data and helps to identify personalized therapeutic approaches.

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CONFLICT OF INTEREST
None of the authors has any declaration of interest to disclose.

DATA AVAILABILITY STATEMENT
Data can be made available upon request.

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