Progress in understanding and treating *SCN2A*-mediated disorders

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Overview

Our understanding of the genetic causes of neurodevelopmental disorders has increased dramatically in the past decade. In that time, the gene *SCN2A* has risen in prominence as one of the leading causes of neurodevelopmental disorders. Remarkably, *SCN2A* is associated with a range of disorders, including Autism Spectrum Disorder (ASD), developmental delay, and early-onset seizures (before the first year of life) of varying severity. Moreover, the functional consequences of mutations appear to predict phenotype and, to some extent, severity.

SCN2A encodes the neuronal voltage-gated sodium channel $Na_V1.2$, a sodium channel isoform involved in the initiation and propagation of action potentials in a range of neuron classes. The well-defined biology of this channel, the strong genotype-phenotype correlations, and the potential for developing channel-specific drugs, make it a leading candidate for understanding the etiology of these disorders and for developing treatments for neuropsychiatric disorders. Following an *SCN2A*-dedicated conference organized by Simons VIP (https://sfari.org/resources/autism-cohorts/simons-vip), we summarize the current state of research, future directions, and consider potential challenges.

The *SCN2A* gene

The Sodium Channel, Voltage-Gated, Type II, Alpha (*SCN2A*) gene is located on the positive strand of chromosome 2 (2q24.3) in humans, between the closely related gene *SCN3A* and the nuclear protein gene *CSRNP3*. The closely related genes *SCN1A* and *SCN9A* are within 1,000kbp downstream. The *SCN2A* mRNA transcript contains 27 exons with 8,430 nucleotides, which is processed to 26 coding exons (exon 1/27 includes 5`UTR only) with 6,018 nucleotides. The processed mRNA encodes a 2,005 amino acid protein called $Na_V1.2$. Due to the large last coding exon, only stop codons in the first 1,591 amino acids would be expected to induce nonsense-mediated decay (Figure 1A) (Nagy and Maquat, 1998).

There are two known isoforms that use different copies of coding exon 5/26 that differ by one amino acid at position 209: Asparagine (Asn/N) vs. Aspartic acid (Asp/D)

(Kasai et al., 2001) (Figure 1A). These isoforms are called 5N and 5A respectively. Of note, *SCN2A* contains two non-canonical splice sites (AT-AC instead of GT-AG) between coding exons 2:3/26 and 23:24/26, suggesting involvement of the minor spliceosome (U12) (Parada et al., 2014; Wu and Krainer, 1999). These non-canonical splice sites are conserved in the mouse genome (mm10) and observed in multiple voltage-gated ion channels (Wu and Krainer, 1999).

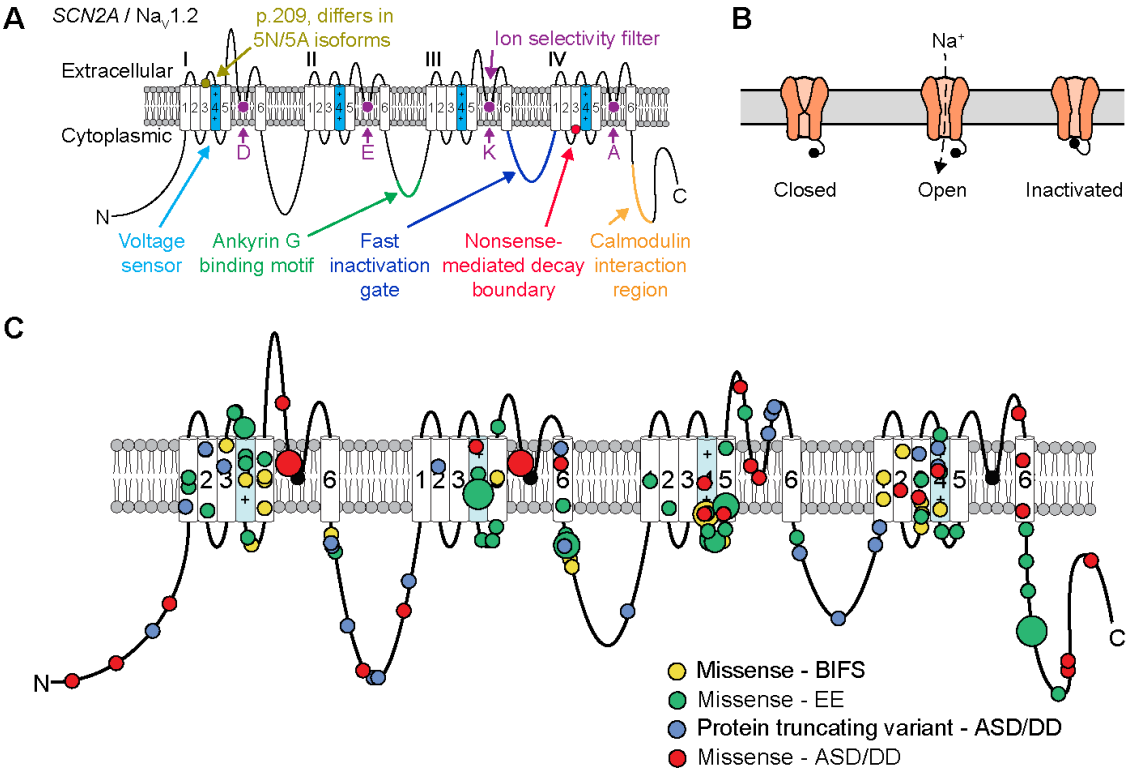

Figure 1. Overview of *SCN2A* / Na$_V$1.2. A) Na$_V$1.2 sits in the cell membrane and is composed of four repeating subunits (I-IV) each of which contains a voltage sensor (light blue) and a pore loop with an amino acid making up the DEKA ion selectivity filter (purple). The cytoplasmic loop between repeats III and IV is the fast inactivation gate (dark blue, see **B**). Two isoforms are known, differing by one amino acid at p.209 (dark yellow) (Kasai et al., 2001). The channel binds to Ankyrin G (*ANK3*), which anchors it to the membrane (green) (Bouzidi et al., 2002), and interacts with calmodulin, which may have a regulatory role (orange) (Mori et al., 2000). Stop codons before the nonsense mediated decay boundary (red) are predicted to prevent protein translation, while those after this boundary may not. **B)** Na$_V$1.2 is closed at rest. Sufficiently positive voltage opens the channel, allowing sodium flux, after which the channel is blocked by the fast inactivation gate (**A**). As the membrane voltage returns to rest the channel resets. **C)** Location of variants in Benign Infantile Familial Seizures (BIFS, yellow), Epileptic Encephalopathy (EE, green), and Autism Spectrum Disorder/Developmental Delay (ASD/DD, blue and red). Larger circles indicate recurrent mutations.

The structure and function of *SCN2A* / Na$_V$1.2

SCN2A encodes the neuronal sodium channel Na$_V$1.2, which is one of several sodium channels involved in action potential initiation and propagation in neurons. *SCN2A* is one of four sodium channel isoforms expressed throughout the central nervous system, alongside *SCN1A* (Na$_V$1.1), *SCN3A* (Na$_V$1.3), and *SCN8A* (Na$_V$1.6). Structurally, sodium channels are comprised of 4 repeating subunits, each with 6 transmembrane domains (Figure 1) (De Lera Ruiz and Kraus, 2015). The 4th transmembrane domain is sensitive to voltage differences between the inside and outside of the cell, and will initiate conformational changes in the channel that lead to channel “activation” when the voltage

becomes more positive. Activation opens the central ion selectivity pore, allowing for electrical conduction by way of sodium flux into the cell (Figure 1B). Shortly thereafter, a gate physically blocks the pore, and the channel enters a conformation termed “fast inactivation”. From here, the membrane voltage hyperpolarizes, which helps remove the inactivation gate to “recover from inactivation”. While exceptions have been noted, sodium channels typically must go through all three steps to then be primed for subsequent activation (Armstrong, 2006).

These steps through activation, conduction, inactivation, and recovery from inactivation vary across sodium channel isoforms, both in timing and voltage sensitivity. This is due to small differences in the amino acid structure of the channels. As one could imagine, variation induced by *de novo* missense mutation can also alter the biophysical properties of the channel, especially if such mutations are in parts of the protein that are important for sensing voltage or permitting ion flux (Figure 1C). Furthermore, mutations at key sites for interactions with beta (e.g. *SCN1B*) and other auxiliary subunits (e.g., FHF-14), modulatory systems, and scaffolding proteins can all impact $Na_V1.2$ activity (Yan et al., 2014). How these mutations can affect different aspects of channel function is described in detail below.

Box 1, terms used to describe neuronal voltage:
A certain set of terms is used to describe neuronal voltage dynamics. A neuron not actively generating action potentials is said to sit at “resting membrane potential,” or V_{rest}. For most neurons, there is a small voltage differential between the inside and outside of the cell at V_{rest}, with the inside being more negative than the outside (typically -60 to -90 millivolts, abbreviated as “mV”). Channels that allow positive ions into the cell, such as sodium channels, are said to “depolarize” the membrane potential, as they bring the membrane potential closer to 0 mV. Conversely, ion channels that allow positive ions to leave the cell (potassium channels) “hyperpolarize” the membrane potential away from 0 mV. This is sometimes called “repolarization”, especially in the context of action potential generation.

Developmental changes in $Na_V1.2$ expression across brain regions

$Na_V1.2$ channels are expressed across the central nervous system. In cortical structures, $Na_V1.2$ are co-expressed with $Na_V1.6$ predominately in excitatory, glutamatergic neurons. Inhibitory interneurons largely express $Na_V1.1$ instead of $Na_V1.2$ (Catterall et al., 2010; Ogiwara et al., 2007), though both $Na_V1.1$ and $Na_V1.2$ co-express in the somatostatin-expressing interneuron subclass in human tissue (Tian et al., 2014). Similar distributions across excitatory and inhibitory cells are found in hippocampus (Lorincz and Nusser, 2010; Ogiwara et al., 2007).

The 5N isoform of *SCN2A*/$Na_V1.2$ is expressed in the fetal brain, while the 5A isoform is expressed in adult brain (Kasai et al., 2001). The timing and nature of this transition has not been characterized in detail. The adult 5A isoform makes the channel more sensitive, resulting in increased neuron excitability (Gazina et al., 2015; Xu et al., 2007).

In addition to changes in isoforms, the distribution of $Na_V1.2$ changes markedly over development in excitatory pyramidal cells. Early on, $Na_V1.2$ is the only sodium channel isoform expressed in the axon initial segment (AIS) (Boiko et al., 2003; Gazina et al., 2015; Osorio et al., 2005), an axonal subcompartment located proximal to the soma that is the site of action potential initiation (Bender and Trussell, 2012). In humans, this early developmental period spans from the late 2nd trimester, when cortex is beginning to

form, to 1-2 years of age. At this time, $Na_V1.2$ in the distal AIS as well as the rest of the axon is largely replaced by $Na_V1.6$ (*SCN8A*), which has a lower voltage threshold for activation. Consequently, the distal AIS becomes the site of action potential initiation in mature neurons (Kole et al., 2008; Kole and Stuart, 2012). $Na_V1.2$, now restricted to the region of AIS most proximal to the soma, takes on a new role. Rather than initiating action potentials, $Na_V1.2$ channels now function as a reserve pool of channels, right next to the cell body, waiting for $Na_V1.6$ channels to initiate action potentials. Once initiated, action potentials propagate bidirectionally, down the axon and back towards the soma. On its way, action potentials are boosted by the reserve pool of $Na_V1.2$ channels, which is thought to help action potentials backpropagate into the somatodendritic compartment (Hu et al., 2009). There, backpropagating action potentials may influence activity dependent gene transcription, synaptic integration, and synaptic plasticity.

Another prominent site of $Na_V1.2$ expression is the cerebellar cortex (Martínez-Hernández et al., 2013). Here, $Na_V1.2$ channels are co-expressed with $Na_V1.6$ in unmyelinated axons of excitatory granule cells that form parallel fiber synapses on Purkinje neurons. In contrast to the developmental loss of $Na_V1.2$ in cortical myelinated axons, $Na_V1.2$ expression persists throughout development, with an apparent peak in density within the second and third postnatal weeks in mice (Y. Liao et al., 2010; Martínez-Hernández et al., 2013). This suggests that $Na_V1.2$ serves different roles in mature neurons with myelinated and unmyelinated axons.

SCN2A-mediated disorders

Variants in *SCN2A* are associated with three distinct disorders (Ben-Shalom et al., 2017; Wolff et al., 2017): 1) Epileptic Encephalopathy (EE), defined by early onset seizures, before 12 months of age, followed by neurodevelopmental delay. 2) Benign infantile familial Seizures (BIFS), characterized by early onset seizures before 12 months of age that resolve by 2 years of age without overt long-term neuropsychiatric sequelae. 3) Autism Spectrum Disorder/Developmental Delay (ASD/DD), characterized by global developmental delay, with social and language milestones particularly delayed; up to a third of children in this category identified to date also develop late-onset seizures with worsening developmental delay.

Table 1: Frequency of *SCN2A*-related disorders in the general population and literature

	EE	**BIFS**	**ASD/DD**
Cases per 100,000 births	120 (Gaily et al., 2016; Ronen et al., 1999)	20 (Gaily et al., 2016; Ronen et al., 1999)	2,500 (Christensen et al., 2016)
Population frequency	0.12%	0.02%	2.5%
Percent of cases with *SCN2A* variant	1.2% (EuroEPINOMICS-RES Consortium et al., 2014)	11% (Zara et al., 2013)	0.3% (Sanders et al., 2015)
Estimate of *SCN2A*-related cases per 100,000 births	1.4	2.2	7.5
Expected *SCN2A*-related births per year in the USA	56	88	298
Year *SCN2A* association documented	2009 (Ogiwara et al., 2009)	2002 (Heron et al., 2002)	2012 (Sanders et al., 2012)

Published mutations	107 cases	24 families	92 cases

While most children with *SCN2A* variants fit into one of these categories, there are some exceptions, including EE with choreoathertoid movements in R853Q mutations (Kobayashi et al., 2016; Samanta and Ramakrishnaiah, 2015), late-onset episodic ataxia (Leach et al., 2016; Y Liao et al., 2010; Schwarz et al., 2016), intermediate EE (Howell et al., 2015), and schizophrenia (Carroll et al., 2016; Fromer et al., 2014). Further work is required to understand how these disorders relate to the other three distinct categories and the role of cerebellar $Na_V1.2$ in ataxia, hypotonia, and other symptoms.

In interpreting the clinical features of the disorders associated with *SCN2A* it is important to consider how the cases were discovered and the consequent ascertainment bias. Early-onset seizures are distinctive, require specialist treatment at an early age, and have a well-recognized genetic origin that helps guide treatment (Wirrell et al., 2015). In contrast, developmental delay and ASD are often diagnosed in the community and, despite strong evidence of a genetic basis (Sandin et al., 2014; Tick et al., 2015), genetic testing that would discover *SCN2A* mutations is not routine. Considering the frequency of these disorders and the fraction mediated by *SCN2A* mutations, we would expect ASD/DD-related mutations to be 5-fold more common than EE-related mutations (Table 1); however, approximately equal numbers of cases have been described for EE and ASD/DD. Furthermore, within each disorder, there is likely to be a bias towards genetic testing in the more severe cases. As a result, we are probably under-sampling mutations with milder effects on $Na_V1.2$ function and overestimating the frequency of late-onset seizures in the ASD/DD group.

Towards a model of *SCN2A* pathology

An integrated analysis of genetic and electrophysiological data led to a model to account for the three disorders associated with *SCN2A* (Figure 2) (Ben-Shalom et al., 2017). Variants that increase sodium flux ($Na_V1.2$ gain of function) lead to EE and BIFS, while those that reduce sodium flux ($Na_V1.2$ loss of function) lead to ASD/DD (Figure 2). The degree to which the gain of function variants increase sodium flux distinguishes EE from BIFS, with severe mutations leading to EE and milder mutations leading to BIFS. This model is supported by several strands of evidence, including: 1) Electrophysiological analysis of 15 variants; 2) The restriction of protein truncating variants, resulting in loss of function, to ASD/DD cases; 3) The presence of multiple recurrent missense mutations within the three groups, but none between the three groups; and 4) The clustering of EE/BIFS mutations around the voltage sensor and ASD/DD missense mutations around the pore loop (Figure 1C). The subsequent publication of 71 novel *SCN2A* variants from European clinics (Wolff et al., 2017) further supported the model proposed in Ben-Shalom *et al.* across all four of these strands of evidence.

This model has therapeutic implications. Since both increased and decreased $Na_V1.2$ activity result in serious phenotypes, it is important that therapies aimed at modifying this activity can be prevented from over-correcting, resulting in the opposite phenotype.

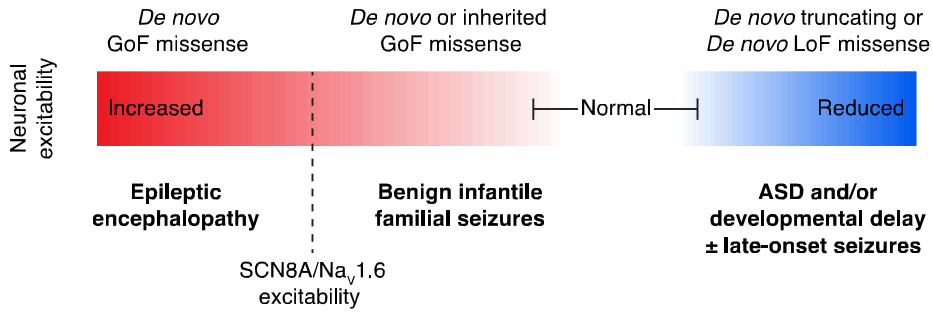

Figure 2. Current model of *SCN2A* pathology. Gain of function (GoF) variants increase glutamatergic neuronal excitability leading to early-onset seizure phenotypes, while loss of function (LoF) variants decrease excitability leading to ASD and/or developmental delay (Ben-Shalom et al., 2017). Seizure severity is correlated with the degree of GoF, with mutations that greatly increase excitability leading to epileptic encephalopathy (EE), while milder variants lead to benign infantile familial seizures (BIFS) that resolve around 1-2 years of age without apparent neurological sequelae. The threshold that distinguishes EE and BIFS variants may be related to neuronal excitability Na$_V$1.6, which replaces Na$_V$1.2 in generating action potentials and has a lower threshold for activation than wild type Na$_V$1.2.

As with any model, there are likely to be exceptions. At least one has come to light: p.K1422E alters the third residue in the DEKA ion selectivity filter (Figure 1A) and is predicted to produce both loss of function and gain of function effects (Heinemann et al., 1992; Schlief et al., 1996). A boy with this mutation developed seizures at 13mths with developmental regression and features of ASD (Sundaram et al., 2013).

Genotype and phenotype appear to be strongly correlated in *SCN2A*. Identification of further mutations, consistent phenotyping, and functional characterization of more variants will hopefully refine this model further and provide additional insights into *SCN2A* pathology and Na$_V$1.2 function (George, 2014).

Children with severe gain of function mutations

Epileptic encephalopathy frequently presents with seizures in the first day after birth, with some families retrospectively reporting rhythmic movements *in utero*, however seizure onset is after three months in 20% of cases (Ben-Shalom et al., 2017). Of note, age of onset is often similar in individuals with the same mutation, suggesting correlation between genotypic and phenotypic severity. Seizure type varies widely between individuals, with most individuals experiencing more than one type of seizure. Some of the children with the earliest onset of seizures are classified as Ohtahara syndrome, based on tonic seizures and EEG burst suppression, while onset after three months often meets criteria for West Syndrome, with infantile spasms and EEG hypsarrhythmia, before being reclassified as Lennox-Gastaut syndrome due to multiple seizure types as the child gets older (Wolff et al., 2017). Development varies from moderate to profound delay. These symptoms are often accompanied by hypotonia and sometimes by dystonia.

Children with loss of function mutations

Early development is generally unremarkable up to about 6mths followed by an increasing delay in meeting motor milestones, for example sitting at 8mths, crawling at 11mths and walking at 18mths, with a more pronounced delay in verbal milestones with first use of words often after 24mths. About a third of documented cases develop seizures, often between the age of 18mths and 4yrs, and these appear to result in more severe delay and sometimes regression, particularly if the seizures are severe. Seizure incidence is likely overestimated in the currently identified population since genetic screening is not common

practice in idiopathic ASD/DD, but can be prompted by additional syndromic features, such as seizures.

While children with LoF mutations do not exhibit a distinctive syndrome, many characteristics are shared. They tend to be contented children that enjoy physical contact with caregivers. They often engage in repetitive actions, including chewing objects, hand gestures, and roaming. Social interaction is rarely initiated and the response to interactions initiated by others is slow. Eye contact is rarely made and hand contact with people or novel objects is avoided. Motor skills are usually slightly more advanced than social skills, though walking may be slightly unsteady and muscle tone reduced, though without overt signs of cerebellar pathology. Notably, pain and other aversive stimuli elicit a muted response in both degree and duration (Tavassoli et al., 2014). Co-morbidities are typical of neurodevelopmental disorders, including disrupted sleep, gastrointestinal disturbances, and uncoordinated oral and motor movements.

Linking disorders to changes in channel function

Several hotspots within the protein are enriched with disorder-associated mutations, including the 4th transmembrane voltage sensor, the intracellular N- and C-terminal tails, and areas that line the central pore. To date, only about 20 of the hundreds of disorder-associated variants have been electrophysiologically characterized (Ben-Shalom et al., 2017; Wolff et al., 2017). Nevertheless, some patterns are beginning to emerge, with mutations within particular subdomains often evoking similar changes to channel biophysical properties (Table 2).

Table 2. Electrophysiology and biophysics of *SCN2A* mutations (see attached excel file).

While each aspect of $Na_V1.2$ function is listed separately in Table 2, it is quite common for one point mutation to alter multiple channel properties. In some cases, the concerted changes to voltage dependence and kinetics are consistent, allowing for easy interpretation of whether the mutation renders the channel more or less excitable (termed gain- and loss-of-function, respectively). In other cases, a mix of what appear to be gain and loss-of-function effects are observed, and it is more difficult to understand how neuronal excitability is affected. Compartmental modeling can provide insight into the net effect on neuronal excitability in such conditions (Ben-Shalom et al., 2017). Even with these efforts, it can be difficult to place certain variants into “gain” or “loss” categories. For example, some EE-associated variants hyperpolarize the voltage dependence of activation to such a degree that neurons may end up being less excitable over time, as Na_Vs may be activated at rest, but the neuron never hyperpolarizes to allow the same channels to recover from inactivation. This variant is characterized as a “gain-of-function” variant when describing channel biophysics, but this description may not hold at the level of overall neuronal excitability. Similarly, a mutation at the ion selectivity filter (e.g., p.K1422E) can have mixed effects on excitability. This mutation converts the channel into a non-selective cation channel with increased persistent, Ca^{2+}-dominated current and reduced, non-selective conductance when activated (Boiko et al., 2003; Heinemann et al., 1992). Developing models that capture net neuronal excitability effects in such cases is quite difficult, especially considering potential downstream effects on Ca^{2+}-mediated cell signaling. More advanced experimental tools, including neuronal culture, induced pluripotent stem cell, and animal knockin models, should be leveraged.

***SCN2A* homologues across animal models and channels**

Voltage-gated sodium channels (Na_V) are derived from a calcium channel (Ca_V3) early in the evolution of animals (Anderson and Greenberg, 2001; Goldin, 2001; Yu and Catterall, 2003) with the DEKA ion selectivity filter in Na_V1 being derived from the ancestral DEEA filter (Figure 3, Table 3). Of note, the mutation p.K1422E, described earlier, (Table 2) reverts the $Na_V1.2$ filter to the ancestral state. The ten voltage-gated sodium channels in humans are derived from this Na_V1 channel (Figure 3) (Liebeskind et al., 2011; Moran et al., 2015; Widmark et al., 2011) with different model organisms reflecting stages in this differentiation (Table 3). $Na_V1.2$ retains a high degree of homology to both $Na_V1.1$ (disrupted in Dravet syndrome (Claes et al., 2001)) and $Na_V1.3$ and moderate homology to $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, and $Na_V1.7$ (Figure 3B). Disruption of $Na_V1.5$ function can lead to cardiac arrhythmias, including heart block, long QT, and Brugada Syndrome (Wang et al., 1995). Therapies aimed at modifying *SCN2A*/$Na_V1.2$ function will need to avoid cross reactivity with these other channels to prevent serious side effects.

Table 3. *SCN2A* homologues in model organisms.

*Zebrafish have two copies of each of these four Na_V channels due to relatively recent genome duplication.

Species	Number of Na_V channel isoforms	Closest human $Na_V1.2$ homologue	Percent homology to human $Na_V1.2$
Yeast (*Saccharomyces cerevisiae*)	0	N/A	N/A
Worm (*Caenorhabditis elegans*)	0	N/A	N/A
Fruit Fly (*Drosophila melanogaster*)	2	*Para* / Para	70%
Zebrafish (*Danio rerio*)	4x2*	*Scn1lab* / $Na_V1.1$Lb	86%
Frog (*Xenopus Tropicalis*)	6	*Scn2a* / nav1.2	85%
Chicken (*Gallus gallus*)	9	*SCN2A* / $Na_V1.2$	93%
Mouse (*Mus musculus*)	10	*Scn2a* / $Na_V1.2$	98%
Rat (*Rattus norvegicus*)	10	*Scn2a* / $Na_V1.2$	98%
Rhesus (*Macaca mulatta*)	10	*Scn2a* / $Na_V1.2$	99.9%
Human (*Homo sapiens*)	10	*SCN2A* / $Na_V1.2$	100%

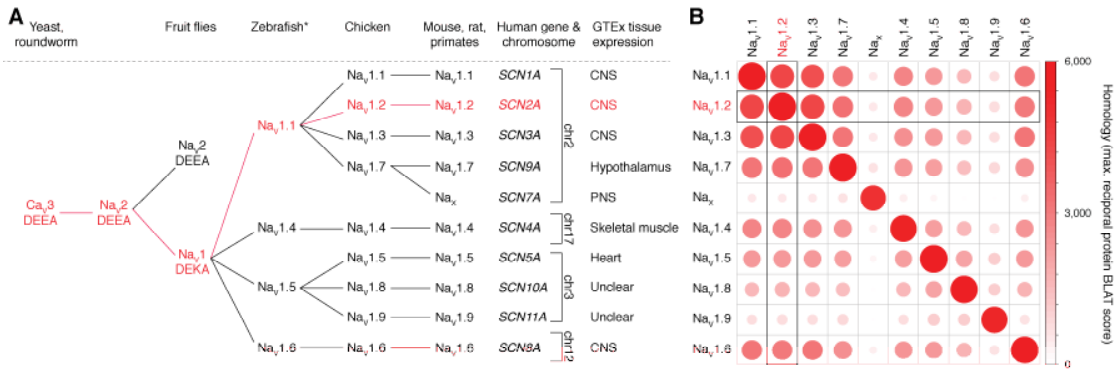

Figure 3. Homology of *SCN2A* across model organisms and voltage-gated sodium channels. A) Voltage-gated sodium channels (Na_V) are derived from T-type voltage-gated calcium channels (Ca_V3) (Anderson and Greenberg, 2001; Yu and Catterall, 2003). In fruit flies the ancestral Na_V2 channel, with the ion selectivity filter composed of the four amino acids DEEA, co-exists with the Na_V1 channel with the DEKA filter. All Na_V channels in humans are derived from this Na_V1 DEKA filter, while the Na_V2 filter has been lost (Liebeskind et al., 2011; Widmark et al., 2011). A series of gene duplication and differentiation events give rise to the ten channels seen in humans and reflected in the proximity of similar channels in the genome (Liebeskind et al., 2011; Widmark et al., 2011). **B)** Homology between the 10 Na_V channels in humans, a large circle and intense red color indicate high homology. *Zebrafish have two copies of each of these four Na_V channels due to relatively recent genome duplication. CNS: Central nervous system; PNS: Peripheral nervous system; GTEx: Genotype-Tissue Expression Project (www.gtexportal.org).

Existing treatments

Seizures associated with *SCN2A* mutations often cannot be controlled despite the use of multiple anti-epileptic drugs (AEDs). However, for children with early onset seizures (≤3 months), sodium channel blockers, such as phenytoin and carbamazepine, are more effective (Wolff et al., 2017), as would be expected based on a gain of function effect (Ben-Shalom et al., 2017). Of note, this is the opposite of best practice guidelines for neonatal seizures and is a compelling reason for rapid genetic testing in this condition (Wirrell et al., 2015). For children with ASD/DD and late-onset seizures (≥12mths) the opposite is true, with other AEDs offering the best options, including levetiracetam, benzodiazepines, valproate, and keppra. Few data exist for guiding AED use in seizures starting between 3mths and 12mths, however the gain of function nature of these variants would suggest sodium channel blockers would be more efficacious. Some reports have described clinical improvement with less mainstream medications, including lidocaine (Foster et al., 2017; Ogiwara et al., 2009), ethosuximide (Wolff et al., 2017), and acetazolamide (Leach et al., 2016; Y Liao et al., 2010), though data on the generalizability of these effects are limited.

At present, no *SCN2A*-specific recommendations can be made for the treatment of other symptoms and best practices for developmental delay, ASD, and co-morbid disorders should be followed.

Future treatments

Would modification of $Na_V1.2$ function improve symptoms in the *SCN2A* mediated disorders? If so, several approaches, including small molecules or gene therapy, could provide desperately needed treatment and potentially modify the underlying pathology (Maljevic et al., 2017; Thompson et al., 2017). As with the development of any therapy, we must balance patient safety against the desire for rapid progress.

Based on the current model of *SCN2A* pathology, there is a risk of overshooting with such a treatment and converting EE into ASD/DD and vice versa. This might suggest a narrow therapeutic window of activity. Careful characterization of the functional variant will be necessary to avoid exacerbating the symptoms in a misclassified variant. The high degree of similarity between *SCN2A*/$Na_V1.2$ and other voltage-gated sodium channels, including *SCN5A*/$Na_V1.5$ that can induce cardiac arrhythmias if disrupted, suggests that cross reactivity is potential concern.

We also need to assess the reversibility of the symptoms. *SCN2A* expression begins early in gestation (Kang et al., 2011) and disordered function may alter brain development. In keeping with this concern, computational modeling of loss of function mutations suggests that neuronal excitability is restored in the mature brain as $Na_V1.6$ replaces $Na_V1.2$'s role in action potential generation at two years of age, however symptoms of ASD/DD persist. It will therefore be critical to understand the physiological changes that persist within the brain in LoF conditions. Similarly, the encephalopathy in gain of function mutations may be secondary to seizures and tractable to treatment, alternatively seizures may simply be one feature of a more complex neurodevelopmental disorder that begins *in utero*. Experiments equivalent to the reversal of symptoms in *MECP2* mice (Guy et al., 2007) are critical for both loss and gain of function phenotypes.

Data collection

Analysis of data from publications and clinical cohorts has already provided insights into genotype-phenotype relationships and appropriate therapies (Ben-Shalom et al., 2017; Wolff et al., 2017). Further progress will require collaboration between scientists and families, including the participation of families in the FamilieSCN2A Foundation (www.scn2a.org) and the *SCN2A* patient registry (https://simonsvipconnect.org). There is considerable scope for further development of the tools for collecting and analyzing

SCN2A patient data including greater data consistency, longitudinal, rather than cross sectional, phenotypes (Bernier et al., 2017), and detailed records of the medications used and their efficacy and side effects. Alongside these phenotype data, it will be necessary to perform functional analyses and develop a database to record these data and outcomes (Table 2), possibly in concert with homologous sodium channels (Figure 3). The development of high-throughout functional analysis would accelerate this considerably.

Conclusion

SCN2A mutations result in at least two severe disorders, with gain of function leading to early-onset seizures and encephalopathy and loss of function leading to ASD and/or developmental delay. Normalization of $Na_V1.2$ function may yield therapeutic benefit in both conditions, though the issues of therapeutic window and cross reactivity to other channels must be addressed. Concurrent development of such therapies, alongside basic science to understand the neurological impact and reversibility of these mutations, is essential. The results of these endeavors will have important implications, in families affected by *SCN2A* mutations, but also for scientists and families considering the wider phenomena of epileptic encephalopathy, ASD, and developmental delay.

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