

COMMENTARY

The neonatal *SCN2A* mutant channel mimics adult channel properties

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The advancement of whole-genome and -exome sequencing has helped to identify de novo genetic alterations in patients with early-onset epilepsy encephalopathy (EOEE; Sugawara et al., 2001; Heron et al., 2002). However, the evidence that the mutations are indeed pathogenic or how they affect the protein biology and/or neuronal network has not always come hand-in-hand with the genetic discoveries. In a recent issue of *JGP*, Thompson et al. addressed this issue by determining the electrophysiological and neuronal effects of human mutations occurring in the voltage-activated $\text{Na}_v1.2$ (*SCN2A*) sodium channel (Thompson et al., 2020). They examined the effects of the mutations on the function of the channel itself and integrated these results into the context of neuronal firing or activity. They combined an interdisciplinary—empirical, and computational modeling—approach to conclude that these mutations result in a gain-of-function change, and that their effects are more pronounced when occurring in the neonatal channel isoform compared with the adult isoform of the channel. This work is of interest to physicians and scientists working in the epilepsy field as well as to those interested in $\text{Na}_v1.2$ channelopathies and to patients and family of those affected by the disease.

EOEEs affect one to two newborns out of every 1,000 births and are some of the most debilitating and consequential seizures to recur in human infants (Camfield et al., 1996). These patients have a grave prognosis with debilitating outcomes, particularly for intellectual and motor development (Engel, 2001). Therapeutics are available but vary in efficacy, in part because of the diverse etiology and complex physiology underlying different seizures. Clinicians have employed whole-genome and -exome sequencing to identify putatively pathogenic mutations occurring in those affected. *SCN2A* mutations have been identified in patients diagnosed with Ohtahara syndrome (OS; Nakamura et al., 2013; Wolff et al., 2017; Turkdogan et al., 2019), epilepsy of infancy with migrating focal seizures (EIMFS; Howell et al., 2015; Wolff et al., 2017; Su et al., 2018), and benign familial neonatal infantile syndrome (BFNIS; Misra et al., 2008; Scalmani et al., 2006; Liao et al., 2010b; Xu et al., 2007; Lauxmann et al., 2013), among other early-onset or childhood

epilepsies (Liao et al., 2010a; Schwarz et al., 2016; Ogiwara et al., 2009) and autism spectrum disorder (ASD; Ben-Shalom et al., 2017). Efforts over the last two decades have led to the understanding that *SCN2A* mutations are the second-most common genetic contributor to EIMFS and ASD, after the potassium channel *KCNT1* (Howell et al., 2015) and the fragile mental retardation protein *FMRP* (Ben-Shalom et al., 2017), respectively.

Thompson et al. (2020) examined the biophysical and cell functional ramifications of five mutants identified in patients with OS (Nakamura et al., 2013) or EIMFS (Howell et al., 2015), two of the most severe forms of EOEE. The mutants were expressed in the tsA201 heterologous expression system along with the $\beta 1$ (*SCN1B*) and $\beta 2$ (*SCN2B*) regulatory subunits, and channel electrophysiology studied using the whole-cell voltage clamp technique. The five mutants, which do not cluster to any distinct regions on the protein, conferred different changes to channel function (Fig. 1). The T236S mutant had increased sodium current without altering single channel conductance. Sodium channels are voltage-dependent in their activation, and T236S, E999K, and S1336Y mutants showed a heightened voltage sensitivity. Following activation, sodium channels enter into a state of inactivation, quieting channel activity for a time. This property is also voltage-dependent, and the mutants S1336Y, T1623N, and R1882Q required more depolarized membrane voltage before inactivating. Channel inactivation itself has a time-dependent component, and T1623N and R1882Q took longer to enter into the fast-inactivated state. When considered as a whole, these effects resulted in hyperactive channels. Computational modeling in a modified model of an immature neuron confirmed that these effects are likely to induce cell hyperexcitability.

Similar changes have been reported by others examining different *SCN2A* mutations (for the most recent review, see Ben-Shalom et al., 2017). Thompson et al.'s findings also fit the paradigm that gain-of-function mutations are more likely to be associated with severe epilepsy while this holds true for loss-of-function mutations affecting those with less-severe epilepsy (Thompson et al., 2020; Begemann et al., 2019). Where

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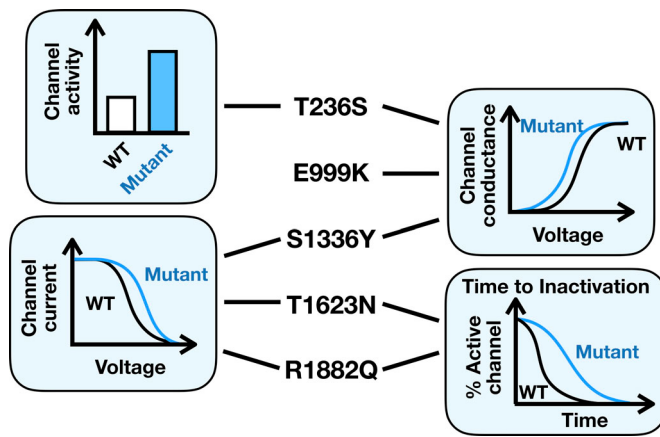


Figure 1. A pictorial summary of Thompson et al.'s work showing the types of gain-of-function changes the authors found for each of the five $Na_v1.2$ mutant channels.

Thompson et al.'s contribution is original, however, is that they investigated these five human mutations in the two splice isoforms that switch in abundance going from neonate to early childhood (Thompson et al., 2020). The two isoforms arise developmentally from the mutually exclusive insertion of exon 5: exon 5N designates the dominant neonatal form and exon 5A designates the post-neonatal adult form. These exons differ only in one amino acid at the 209 position: the neonatal isoform bears an asparagine (N), while the adult form incorporates the charged aspartic acid (D; Kasai et al., 2001). In mice, half the neonatal isoform is replaced by the adult form by P9, and from P15 on only about ~10% of the neonatal isoform remains (Gazina et al., 2010). Functionally, the adult isoform of the channel is more active than the neonatal one, and a model neuron containing the adult channel is hyperactive compared with the neonatal model neuron (Xu et al., 2007). A few other studies have considered both isoforms in studying the effects of other $SCN2A$ mutations (Xu et al., 2007; Liao et al., 2010b; Liao et al., 2010a), but no one has studied the ramifications of these particular mutations (T236S, E999K, S1336Y, T1623N, and R1882Q) in both the neonatal and adult isoforms.

Thompson et al. (2020) observed that some effects of the mutations were only manifest in the neonatal isoform. The twofold increase in sodium current of the neonatal T236S mutant compared with its wild type was no longer observed when the same comparison was made in the adult isoform. Likewise, only in the neonatal form did the mutant channels T236S, E999K, and S1336Y exhibit a heightened voltage-activation sensitivity. These findings imply that the brain of an infant harboring any of these mutations may experience hyperexcitability early in the infant's life, coinciding with the clinical observation of seizures in the first days of life in these patients (Nakamura et al., 2013; Howell et al., 2015).

It feels intuitive that one should reproduce the neonatal milieu, including the neonatal specific isoform of a channel, when studying the effects of mutations that are associated with early-onset epilepsies. But will the choice of isoform determine the mutational effects?

Let us start with the null hypothesis that mutant channels will behave alike whether it occurs in the neonatal or the adult isoform. We can start by taking a mutation that has been examined in both isoforms of the human channel, L1563V. This mutation, which occurs in the second transmembrane region of $SCN2A$ channel domain IV, was one of the first to be identified in a patient with BFNIS (Heron et al., 2002). This mutation has been studied in both human isoforms with and without the regulatory subunits (Xu et al., 2007; Misra et al., 2008; Berecki et al., 2018; Begemann et al., 2019). Xu et al. (2007) first reported that inactivation of the channel occurred more slowly in the mutant neonatal channel and that a greater, or more depolarized, membrane potential input was required to inactivate it. The neonatal L1563V was also quicker to recover from its inactivated state compared with the neonatal wild type. The adult wild type already recovers more quickly from inactivation compared with the neonatal wild type as described above, and this isoform was resistant to these mutational effects. Mathematical modeling confirmed that a model neuron with the mutant neonatal channel was more excitable compared with the wild type neonatal channel. As per model neurons with the adult channels, the mutant neuron was only slightly more excitable than the wild type counterpart. In summary: like a child playing make believe as a grownup, the mutant neonatal channel mimics the adult wild type channel properties. And once in the adult channel isoform, the mutant channel ceases its mimicry.

It appears then that we should reject the null hypothesis. But it is more nuanced than that, as others have observed differences in an adult form of the mutant channel compared with the adult wild type channel. The adult L1563V mutant channels continue to show a more depolarized voltage-dependent inactivation compared with the adult wild type (Misra et al., 2008; Berecki et al., 2018; Begemann et al., 2019). A similar pattern has also been observed in the C-terminal R1882Q mutation found in EIMFS patients (Table 1). Whether expressed in the neonatal or adult isoforms, the R1882Q channel has a more depolarized voltage-dependent inactivation pattern. The mutant channel is also slower in entering into the fast-inactivated state irrespective of the isoform type (Berecki et al., 2018). Hence, it may be that some effects of the mutations observable in the neonatal isoform will persist into the adult isoform.

Nevertheless, differences in experimental design (Table 1) and the small sample size reveal major limitations for the analysis at hand. The inclusion of the $\beta 1$ and $\beta 2$ regulatory subunits, or the cell type used may alter the effects of the mutation on channel electrophysiology, and the specific conditions vary among these studies. Interestingly, there are disease-causing mutations within the developmentally regulated exon 5. They occur immediately downstream of the neonatal/adult N209D and lead to the substitution of a charged aspartic acid: G211D (Kodera et al., 2013), N212D, and V213D (Nakamura et al., 2013). Given their proximity and specific substitution, any differential effects of these mutations in the neonatal versus adult isoforms may help further weigh in on the relevance of using either isoform in future studies of EOE-related $Na_v1.2$ mutations.

What do Thompson et al.'s findings mean when considered clinically? With more data points, these results could be translated

Table 1. Comparison of Nav1.2 L1563V and R1882Q electrophysiology studies

Disease	SCN2A α subunit		Experimental conditions		Channel electrophysiological properties						Reference	
	Mutation	Species	Isoform	Exogenous β 1/ β 2 subunit	Cell type	Current density	V-dependent activation	V-dependent inactivation	Fast inactivation time constant τ	Persistent inward current		Recovery from inactivation
BFNIS	L1563V	Human	Neonatal	Neither	tsA201	Unchanged	Unchanged	Depolarized	Greater	Unchanged	Quicker	Xu et al., 2007
			Adult	Neither	tsA201	Unchanged						
			Adult	β 1 and β 2	tsA201	Decreased	Unchanged	Depolarized	Greater	Unchanged	Quicker	
			Adult	Neither	Chinese hamster ovarian cells	Unchanged	Depolarized	Depolarized	Like the adult WT	Unchanged	Quicker	
			Adult	β 1 and β 2	HEK293T	Unchanged	Unchanged	Depolarized	Like the adult WT	Unchanged	Unchanged	
EIMFS	R1882Q	Human	Adult	Neither	Chinese hamster ovarian cells	Increased	Hyperpolarized	Depolarized	Greater	Increased	Unchanged	Begemann et al., 2019 Berecki et al., 2018
			Neonatal	β 1 and β 2	tsA201	Unchanged	Unchanged	Depolarized	Greater	Unchanged	Unchanged	
			Adult	β 1 and β 2	tsA201	Unchanged	Depolarized	Depolarized	Greater	Unchanged	Unchanged	
			Adult	β 1 and β 2	tsA201	Unchanged	Depolarized	Depolarized	Greater	Unchanged	Unchanged	

into a roadmap for those clinicians advising a patient with a particular mutation on what the prognosis may be and what treatment regimens might be most favorable. Nevertheless, it remains unknown how mutations affecting the same gene could lead to a spectrum of diseases of varying severity. *SCN2A* mutations are found in those with severe as well as benign forms of epilepsies, a conundrum also observed among those who have mutations in the sodium-activated potassium channel Slack (*KCNT1*; Kim and Kaczmarek, 2014). *KCNT1* and *SCN2A* patients do share similarities in that their cognitive development and function are affected, and mutations in either are the most likely genetic alterations to be found in patients with ASD or the fragile x syndrome (Kim and Kaczmarek, 2014; Ben-Shalom et al., 2017). It may be that there is a third common protein regulator or neuronal modulator at the systems level that ultimately determines the severity of disease, though this remains just a speculation. Uncovering the answer will require further mechanistic insights into individual channel mutations, more refined and complex mathematical modeling, and animal models of these disease types. This feels immense in scope, but just 20 years ago such vision was inconceivable. And as the field advances, I hope that we will all benefit from its collective wisdom.

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