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# Epilepsy channelopathies go neddy: stabilizing Na<sub>v</sub>1.1 channels by neddylation

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**Loss-of-function mutations of *SCN1A* encoding the pore-forming  $\alpha$  subunit of the Na<sub>v</sub>1.1 neuronal sodium channel cause a severe developmental epileptic encephalopathy, Dravet syndrome (DS). In this issue of the *JCI*, Chen, Luo, Gao, et al. describe a phenocopy for DS in mice deficient for posttranslational conjugation with neural precursor cell expressed, developmentally downregulated 8 (NEDD8) (neddylation), selectively engineered in inhibitory interneurons. Pursuing the possibility that this phenotype is also caused by loss of Na<sub>v</sub>1.1, Chen, Luo, Gao, and colleagues show that interneuron excitability and GABA release are impaired, Na<sub>v</sub>1.1 degradation rate is increased with a commensurate decrease of Na<sub>v</sub>1.1 protein, and Na<sub>v</sub>1.1 is a substrate for neddylation. These findings establish neddylation as a mechanism for stabilizing Na<sub>v</sub>1.1 subunits and suggest another pathomechanism for epileptic sodium channelopathy.**

## The Na<sub>v</sub>1.1 sodium channel in epilepsy

Epilepsy is a disorder of brain function of which the cardinal feature is an enduring propensity for generating seizures. These anomalous paroxysms of hypersynchronous network activity may arise from a variety of brain insults, including developmental defects, acquired lesions (trauma, stroke, infection), and a myriad of associated gene defects. Mutations that disrupt ion channel genes, so-called channelopathies, are often identified in familial epilepsy syndromes or as de novo lesions in developmental epileptic encephalopathy.

Not surprisingly, epilepsy mutations often occur in genes coding for the pore-forming  $\alpha$  subunits of voltage-gated sodium channels that generate action potentials in excitable cells (1–3). Mutant  $\alpha$  subunits may have altered function or may fail to form a functional channel, and these discoveries have served as the foundation for understanding epileptogenesis

at a molecular level. Accessory subunits of the channel complex and channel-interacting proteins have also emerged as culprits in epileptic channelopathies (4). In this issue of the *JCI*, Chen, Luo, Gao, et al. (5) report on uncovering a new potential mechanism for sodium-channel epilepsy, wherein disrupted neddylation of the channel  $\alpha$  subunit increases the protein degradation rate and results in lower Na<sup>+</sup> current density with reduced GABAergic inhibition by interneurons.

The human genome contains nine distinct  $\alpha$  subunit genes (*SCN1A*, *SCN2A*, etc.) coding for the main pore-forming subunits Na<sub>v</sub>1.1, Na<sub>v</sub>1.2, etc. and four accessory  $\beta$  subunit genes (*SCN1B*, etc.). Epilepsy syndromes have been associated with mutations of Na<sub>v</sub>1.1, Na<sub>v</sub>1.2, Na<sub>v</sub>1.3, Na<sub>v</sub>1.6, and the  $\beta_1$  subunit. The number of identified epilepsy mutations for Na<sub>v</sub>1.1 greatly exceeds that for all the other sodium-channel subunits, and mutations of Na<sub>v</sub>1.1 cause a wide variety of clinical phenotypes

(Figure 1) ranging from mild to severe epilepsy with seizures refractory to drug treatment, developmental delay, and cognitive impairment (i.e., epileptic encephalopathy). A genotype/phenotype pattern is emerging wherein missense mutations of Na<sub>v</sub>1.1 with modest alterations of channel function are found in mild syndromes (generalized epilepsy with febrile seizures [GEFS] and generalized epilepsy with febrile seizures plus [GEFS<sup>+</sup>]). In contrast, haploinsufficiency from a single null allele (e.g., frameshift, nonsense with premature truncation) causes a severe developmental epileptic encephalopathy with onset at six months of age (Dravet syndrome [DS]) (3). Over 700 pathogenic or likely pathogenic variants of *SCN1A* are listed for DS on ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>) as of March 2021.

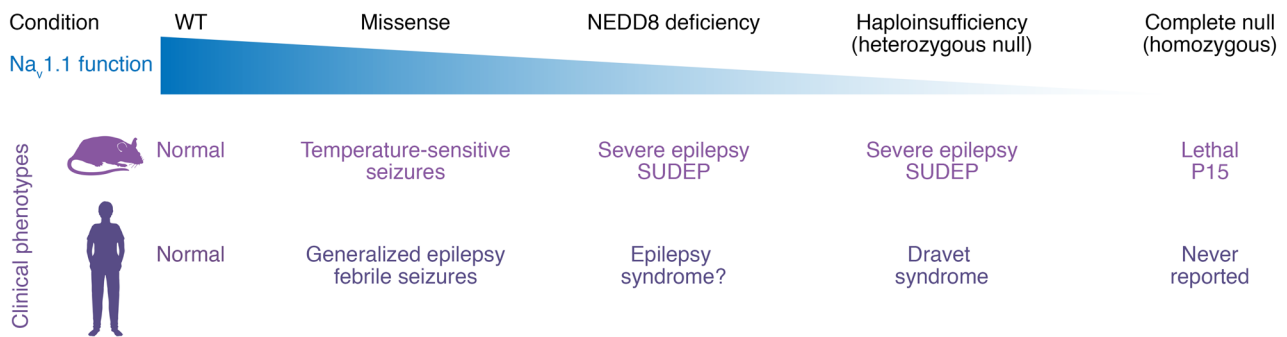
The association of a severe epilepsy phenotype with a sodium-channel loss of function was initially puzzling because epilepsy is a disorder of anomalously enhanced excitability of neuronal activity. A major clue was provided by the Na<sub>v</sub>1.1 knockout mouse (6). Global haploinsufficiency from heterozygous deletion of exon 26 (*Scn1a*<sup>+/−</sup>) leads to early onset seizures (spontaneous and temperature induced), mild ataxia, and sudden unexplained death in epilepsy (SUDEP), which is similar to what occurs in individuals with DS. Na<sub>v</sub>1.1-deficient mice had a reduction of Na<sup>+</sup> current density and decreased firing rates of parvalbumin expressing inhibitory GABAergic neurons (PVINs), but not excitatory neurons, in the cerebral cortex, hippocampus, cerebellar Purkinje cells, and reticular nucleus of the thalamus. A similar epilepsy phenotype occurred with selective haploinsufficiency (PV-*Scn1a*<sup>+/−</sup>) restricted to PV-positive interneurons, while the epilepsy phenotype was milder with *Scn1a*<sup>+/−</sup> selective for somatostatin-positive inhibitory interneurons (7, 8). Conversely, haploinsufficiency of *Scn1a* in excitatory neurons ameliorates the DS phenotype (9). Exceptions to this geno-

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**Figure 1. Spectrum of epilepsy syndromes from Na<sub>v</sub>1.1 loss of function.** Na<sub>v</sub>1.1 loss of function caused by *SCN1A* mutations or NEDD8 deficiency induces a wide variety of clinical phenotypes with varying severity.

type/phenotype paradigm indicate more work needs to be done. For example, about half of the *SCN1A* variants reported in DS cases are missense mutations, some of which caused gain-of-function changes in expression studies (10); the reduced excitability of PVINs in *Scn1a*<sup>+/-</sup> mice at P14 later resolved at P18–P21, whereas seizures persisted (11). Others reported hyperexcitability and increased Na<sup>+</sup> currents in P21–P24 hippocampal pyramidal neurons, suggesting overexpression of other sodium-channel genes (12). Nevertheless, the effects of murine Na<sub>v</sub>1.1 loss of function coupled to severe early onset seizures and SUDEP are robust and were the basis for implicating Na<sub>v</sub>1.1 in the neddylation studies by Chen, Luo, Gao, et al. reported in this issue (5).

### Epilepsy phenotype from disrupted neddylation in mouse interneurons

Posttranslational modification by the ubiquitin-like protein neural precursor cell expressed, developmentally downregulated 8 (NEDD8) regulates many cellular functions (transcription, proliferation, differentiation, apoptosis), is essential for dendritic spine maturation and stability (13), and has been implicated in disorders of the central nervous system (14). To interrogate the role of NEDD8 in parvalbumin-positive inhibitory interneurons (PVINs), Chen, Luo, Gao, et al. (5) generated a conditional knockout of an obligatory subunit for the NEDD8-specific E1 (NAE1). Unexpectedly, the mice had severe epilepsy, ataxia, and a median survival of only 60 days. The abundance of PVINs was unchanged in *PV-Nae1*<sup>-/-</sup> mice, but inhibitory GABAergic neurotransmission was reduced and intrinsic excitability

was reduced for PVINs, but not pyramidal neurons. Further investigation for the cause of reduced excitability in PVINs revealed a Na<sup>+</sup> current density of 62% of WT. When measured by immunoblot, Na<sub>v</sub>1.1 protein amounts were decreased, whereas the abundance of the other major  $\alpha$  subunit (Na<sub>v</sub>1.6) remained unchanged. By comparison, Na<sup>+</sup> current density for PVINs in the DS *Scn1a*<sup>+/-</sup> mice was 47% of WT and 38% of WT for homozygous *Scn1a*<sup>-/-</sup> (6). Epileptic seizures began around P30 for *PV-Nae1*<sup>-/-</sup> mice, whereas for DS *Scn1a*<sup>+/-</sup> mice, seizure onset was earlier (P21), consistent with a more severe loss of Na<sup>+</sup> current for *Scn1a*<sup>+/-</sup>.

The observed reduction in Na<sup>+</sup> current density is sufficient to explain the reduced excitability of PVINs, but why does impaired neddylation lead to lower amounts of Na<sub>v</sub>1.1? There is no previously established role for regulating the abundance of voltage-gated ion channel proteins by neddylation, although for the epithelial sodium channel ( $\alpha$ -ENaC), NEDD8 conjugated to cullin-1 increases ubiquitination and proteolytic degradation of the channel (15). Chen, Luo, Gao, and colleagues excluded a NEDD8 effect on *Na<sub>v</sub>1.1* transcript levels in *PV-Nae1*<sup>-/-</sup> mice (5). Instead, expression studies in a human cell line (tsA-201 cells) showed an increased rate of proteasome-dependent Na<sub>v</sub>1.1 degradation when neddylation was inhibited using MLN4924. The interpretation is that neddylation stabilizes Na<sub>v</sub>1.1 by preventing ubiquitination, just the inverse of how NEDD8/cullin-1 regulates  $\alpha$ -ENaC.

What is the evidence Na<sub>v</sub>1.1 is a substrate for neddylation? NEDD8 immunoreactivity was detected after immunoprecipitation of tagged Na<sub>v</sub>1.1 expressed in a

human cell line (HEK cells) or for native Na<sub>v</sub>1.1 from brain. Moreover, the NEDD8 signal was reduced in the presence of the NAE inhibitor MLN-4924 and for brain homogenates from *PV-Nae1*<sup>-/-</sup> mice, consistent with neddylation of Na<sub>v</sub>1.1. To search for potential neddylation sites at lysines in Na<sub>v</sub>1.1, the authors screened for variants of Lys residues in ClinVar and an epilepsy database. Thirteen epilepsy variants were identified, two of which were studied further because they are located in cytoplasmic loops. The Na<sub>v</sub>1.1 K1936E variant, 74 residues upstream from the C-terminal Lys, was expressed in HEK cells and found to have increased rates of Na<sub>v</sub>1.1 degradation, reduced NEDD8 immunostaining, and lower Na<sup>+</sup> current density, all of which are consistent with impaired neddylation and destabilized Na<sub>v</sub>1.1 protein. Na<sub>v</sub>1.1 K1936 is not conserved in Na<sub>v</sub>1.6 (established using the multiple sequence alignment program CLUSTAL 1.2.4), which may account for the lack of a detectable change for Na<sub>v</sub>1.6 expression in *Nae1*<sup>-/-</sup> mice. Unfortunately, the clinical annotation for Na<sub>v</sub>1.1 K1936E was insufficient to ascertain the confidence level showing that this is indeed a pathogenic mutation for epilepsy.

Many substrates for neddylation are surely present in PVINs, and the downstream effects of this posttranslational modification may be diverse and extensive. The authors acknowledge that effects beyond the reduction of Na<sub>v</sub>1.1 may contribute to the ataxia and severe epilepsy in the *PV-Nae1*<sup>-/-</sup> mouse. A proteome screen identified 5167 proteins with 169 downregulated and 279 upregulated for *PV-Nae1*<sup>-/-</sup> mice compared with WT. The former group is implicated in neural development, synaptic plasticity, and glutamatergic neu-

rotransmission, whereas the upregulated set is involved with ubiquitin-regulated catabolism and the metabolic pathway. Interestingly, of the 53 ion channel genes identified in this analysis, only the reduction of Na<sub>v</sub>1.1 could readily account for the decreased excitability observed in PVINs.

## Open questions and clinical implications

The study by Chen, Luo, Gao, et al. in this issue of the *JCI* identifies a mechanism for fine-tuning neuronal excitability by neddylation-dependent stabilization of ion channel proteins and also reveals that selective disruption of this regulatory pathway in PVINs leads to ataxia, severe epilepsy, and SUDEP in mice (5). This phenotype highly resembles that of DS models in which the coding potential of *Scn1a* is destroyed and lends credence to the notion that loss-of-function defects for Na<sub>v</sub>1.1 in PVINs produces susceptibility to severe epilepsy. It remains to be established that epilepsy in humans is attributable to impaired neddylation of Na<sub>v</sub>1.1. One question is whether haploinsufficiency of Nae1 or some other critical component for NEDD8 conjugation will be sufficient to cause epilepsy. A global null of *Nae1* is embryonic lethal (16). If neddylation is also deficient in excitatory pyramidal neurons, will the balance between excitation and inhibition be restored to ameliorate the risk of seizures as occurs in *Scn1a*<sup>-/-</sup> mice (9)? Similarly, it is unknown whether a single Na<sub>v</sub>1.1 K1936E allele is sufficient

to cause an epilepsy phenotype in mice or humans. The probability of homozygous Na<sub>v</sub>1.1 K1936E would be exceedingly small, except for cases of consanguinity. More studies in murine models may shed light on whether the heterozygous cases are sufficient to cause epilepsy.

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