Elsevier Editorial System(tm) for Current Opinion in Physiology

Manuscript Draft

Manuscript Number:

Title: Using voltage-gated channelopathies as a paradigm for studying epilepsy-causing genes

Article Type: 02 Ion Channels

Keywords: epilepsy; channelopathy; sodium channels; Dravet; voltage-gated channels

Corresponding Author: Dr. Stephanie Schorge, PhD

Corresponding Author's Institution: UCL

First Author: Jenna C Carpenter

Order of Authors: Jenna C Carpenter; Stephanie Schorge, PhD

Nothing Declared

This piece of the submission is being sent via mail.

***Highlights (for review)**

- SCN1A now presents a framework for studying mechanisms of genetic epilepsies
- *De novo* mutations in potassium channels are defining two new channelopathies
- Studies are beginning to resolve the roles of calcium channels in epilepsies
- Studies of NMDA receptors set new standards for functional characterisations
- Mechanistic findings from channelopathies may provide insight into other epilepsies

Using voltage-gated channelopathies as a paradigm for studying epilepsy-causing genes

Short title: **Voltage-gated channels and epilepsy**

Jenna C Carpenter and Stephanie Schorge*
Department of Clinical and Experimental Epilepsy
UCL Institute of Neurology
Queen Square
London
WC1N 3BG
UK

*corresponding author: s.schorge@ucl.ac.uk

Abstract (120)

'Channelopathies', or mutations in ion channels, are long-established causes of epilepsy. Comprehensive genetic, mechanistic and clinical data for SCN1A, a voltage-gated sodium channel, has highlighted the differing contributions of neuronal sub-types in epilepsy and confirmed that genotype-phenotype relations, even for monogenic epilepsies, are strongly influenced by modifier genes and environmental factors. An emerging population of *de novo* mutations in voltage-gated potassium channels has defined two novel potassium channelopathies (KCNC1 and KCNA2), which may benefit from mechanistic insights from SCN1A. Meanwhile, increasing genetic evidence has strengthened the long-standing association of voltage-gated calcium channels with epilepsy. Finally, an integrative approach for the characterisation of genetic variation in NMDA receptors has created a new standard for predicting functional effects in new epilepsy genes.

1. Introduction

Voltage-gated ion channels (VGICs) are critical regulators of neuronal excitability and are well-established causes of genetic epilepsies [1,2]. Advances in sequencing technologies continue to identify novel mutations at rapid pace demanding a refinement in the strategies used to link mutations in VGICs to mechanisms triggering seizures. Extensive clinical, genetic and functional data for SCN1A, the best-studied epilepsy gene, has highlighted the phenotypic variability that can arise from a single mutation, the degree to which modifiers can influence disease severity and how a mutation may selectively exert its effects in different sub-sets of neurons. These findings are informing efforts to develop new treatments and are guiding studies of other monogenic epilepsies, including those caused by mutations in voltage-gated sodium, potassium and calcium channels (VGSCs, VGKCs and VGCCs). Recent studies in NMDA receptors (NMDARs) provide a framework for how to integrate functional data from multiple variants. Approaches integrating multiple variants including from multiple genes are likely to become increasingly important as sequencing allows characterisation of non-monogenic epilepsies.

2. Voltage-gated sodium channelopathies

Mutations in voltage-gated sodium channels (VGSCs) are some of the most frequent causes of genetic epilepsy in humans [3,4]. Whilst mutations have been identified in all four VGSC genes that are highly expressed in the central nervous system (CNS): (SCN1A, SCN2A, SCN3A, SCN8A), SCN1A remains the most clinically important epilepsy gene, with over 1000 mutations identified[4,5]. We focus first on progress made characterising the consequences of SCN1A mutations.

2.1 Identifying the complete clinical picture of SCN1A-associated epilepsies

Mutations in SCN1A are most frequently associated with Dravet syndrome (DS), a severe epileptic encephalopathy of childhood. Defects in SCN1A are also causative for generalised epilepsy with febrile seizures plus (GEFS+) and migraine; another paroxysmal disorder often comorbid with epilepsy[6]. The clinical spectrum of DS has broadened as the patient cohort has grown, such that non-epileptic features of DS, such as sleep disturbance, altered sensory processing and gate impairment are increasingly recognised[4]. The type of mutation that a patient has, e.g. truncating or missense, is not the only, or even the strongest predictor of severity[1]. Evidence from a cohort of children with DS caused by variable mutations (n=182) suggests that environmental factors,

particularly age of seizure onset, are stronger indicators of prognosis[7]. However, whilst vaccination may provoke the first seizures in a subset of children, this does not appear linked to clinical outcome[8]. With an increasing number of children with *de novo* mutations, the ability to reliably predict the pathogenicity of SCN1A variants, without resorting to *in vitro* models, has become more urgent. A recent *in silico* approach successfully predicted the pathogenicity of missense variants in VGSCs with an accuracy score above 0.9 [9]. Such predictive algorithms, combined with structural modelling, promise to be important diagnostic tools with relevance to other channelopathies[10].

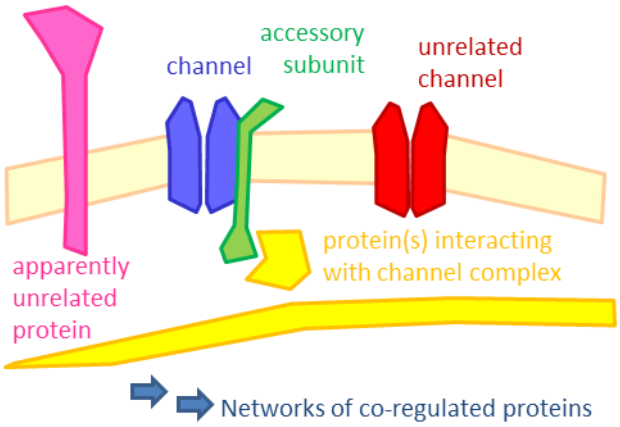

Figure 1: Non-channel contributions to channelopathies The putative causative channel (blue) may be modified by accessory proteins (green) that are required for normal function or localisation. Changes in the putative channel may be masked or exacerbated by changes in other, potentially unrelated channels (red), or by changes in proteins (yellow) that bind to the channel or its accessory subunits. Finally, in some cases mutations that change proteins (pink) with no obvious relation to, or impact upon the candidate channel may provide nearly identical clinical manifestations.

2.2 Disease mechanisms in DS implicate interneurons

VGSCs are critical for the activity of all neurons, and essential for network excitability. The first mouse model of DS suggested that loss of the Nav1.1 channels encoded by SCN1A might cause seizures by 'inhibition of an inhibitor', because SCN1A haploinsufficiency disproportionately affected interneurons[11]. An interneuronal pathology of DS has recently been supported by transcranial magnetic stimulation studies on patients [12]. Meanwhile, the contributions of different subsets of interneurons continue to be teased apart. One study has linked autistic behaviours to parvalbumin-positive interneurons, and hyperactivity to somatostatin-positive interneurons[13], suggesting different neuronal circuits may underlie different clinical features of DS.

2.3 Current treatments for DS

DS is difficult to manage because seizures are typically drug-resistant [*14]. Seizure control is important because the occurrence of early life seizures markedly increases disease severity in humans[7] as well as in mice carrying SCN1A mutations[15]. Adverse effects of early seizures may be general to epilepsies, as mice with mutations in Kv7 experience pronounced benefits from early life prophylactic treatment to prevent seizures [**16]. However, stopping seizures requires an effective treatment, and several strategies are underway to identify such a treatment for DS.

Cannabidiol, a non-intoxicating ingredient of marihuana, has recently received publicity as a game-changing treatment. In a randomised trial of patients with DS, cannabidiol was found to elicit a ~50% reduction in convulsive seizures, with 5% of patients achieving seizure freedom after 14 weeks [*14]. However, many patients experienced adverse effects on cannabidiol, highlighting the non-specificity of its pharmacology. Separately, surveys of physicians working with children with DS have identified elements of consensus for treatment with existing anti-epileptics. Benzodiazepines and

valproate are seen as effective[17,18], as well as some older drugs, including bromide, the first anti-epileptic drug used[4,19]. Overall, there is a strong consensus that sodium-channel blockers are contraindicated in DS because they worsen symptoms, presumably by exacerbating the inhibitory deficit associated SCN1A haploinsufficiency[11]. Mouse models of DS are now being used to screen treatments, with the first studies validating current clinical findings[18].

2.4. Precision medicine for DS

DS is a prime candidate for a precision medicine approach, but many translational challenges exist. Theoretically, a selective agonist for Nav1.1 (encoded by SCN1A) would be ideal[20]. However, in practice, achieving specificity for Nav1.1 promises to be tremendously challenging given the degree of sequence identity shared between VGSCs – off-target interactions with, for example cardiac VGSCs, could have grave consequences. Only recently, and after several failed attempts, has the search for a specific inhibitor of SCN9A/NaV1.7, to treat pain, begun to find success[21].

An alternative therapeutic strategy for DS might be targeting disease-modifying genes rather than Nav1.1 itself. It is increasingly recognised that genetic modifiers influence disease severity, even in patients with highly penetrant, monogenic epilepsies such as DS[1]. In mouse models of DS the genetic background, (i.e. 129 vs C57BL/6) influences multiple readouts of disease severity, and the key modifying loci are being mapped[22]. Genetic factors are also likely to explain why genetically stratified patients exhibit varied responses to the same treatment. One study found that a patient with SCN1A deletion actually improved on carbamazepine, a drug that usually aggravates DS[23].

Genetic modifiers of VGSCs may not be the 'usual suspects' (e.g. binding partners, accessory subunits), but could represent distant or unrelated genes (Figure 1). In these cases, modifiers might be identified using systems genetic approaches, aimed at identifying networks of co-regulated genes that underlie key biological processes, such as memory [**24]. Recently, a gene co-expression network analysis approach identified a network of 320 genes (the M30 network) that was enriched for genetic variation in patients with epilepsy, and found the same network to be downregulated in mouse models of DS. Critically, valproate, which is one of the consensus treatments for DS[17,18], was found to upregulate the network [25]. Targeting new genetic modifiers will require efficient drug-screening platforms. The human gene network approach could be combined, for example, with a pharmacological screen in Drosophila to determine if compounds not yet used in DS could upregulate reporter genes in the M30 network[26]. Pharmacological screens in Drosphila have already been used to identify compounds that are capable of modulating a splice regulator known to modify sodium channel activity[26] – as one strategy for boosting SCN1A expression in DS.

2.5 Beyond SCN1A: other voltage-gated sodium channelopathies

SCN8A (Nav1.6) has emerged as an important cause of monogenetic epilepsy[27], and the mechanism is almost a mirror image of DS/SCN1A. Whilst SCNA1-associated seizures are typically caused by loss-of-function, mutations in SCN8A typically cause gain-of-function. Knock-in mouse models, created using pathogenic variants of SCN8A, support this molecular diagnosis, revealing different populations of neurons to be hyper-excitable[28,29]. Treatments are being developed that aim to rebalance neuronal activity, with efforts directed towards the development of selective antagonists of SCN8A[27]. Interestingly, GS967, an unconventional sodium channel blocker found to

alleviate symptoms in DS, appears to downregulate Nav1.6 channels in excitatory neurons, and may also have therapeutic benefit in SCN8A channelopathies[30].

The closely-related SCN2A is also an epilepsy gene where mutations tend to increase channel activity [2,31]. As with SCN1A, patients with mutations in SCN2A can have clinical phenotypes with widely varying severity and outcome[2,31,32], which has significant therapeutic implications for clinicians and families[31]. Meanwhile, whilst SCN3A is thought to be only expressed at low levels in the adult CNS, it has also been implicated as a rare cause of epilepsy and reduced channel availability is sufficient to cause seizures in mice [33]. No other VGSCs have been firmly linked to monogenic epilepsy (but see a case report of a single patient with bi-allelic mutations in SCN10A[34]).

3. Novel potassium channelopathies

Compared to VGSCs, mutations in voltage-gated potassium channels (VGKCs) are still relatively rare causes of epilepsy. However, whilst the pace of gene discovery may have slowed for VGSCs, two novel (voltage-gated) potassium channelopathies been identified in the past two years. A number of *de novo* mutations have been discovered in KCNA2 in epileptic encephalopathies. Functionally characterised variants have been found to be loss-of-function or (surprisingly for a potassium channel) gain-of-function [35]. The phenotypic spectrum of patients with mutations in KCNA2, even within families, is broad, although gain-of-function mutations are associated with more severe phenotypes [36,37].

A recurring *de novo* mutation has also been identified in KCNC1 as a novel cause of progressive myoclonic epilepsy[38]. An impressive cohort of 20 patients, all sharing the same R320H mutation in the S4 voltage sensor, recently allowed highly detailed genotype-phenotype investigations. One outcome from this study was the realisation that symptoms are alleviated for a number of patients during fever and that this could be explained by altered biophysical properties of the channel at higher temperatures[39]. Meanwhile a *de novo* mutation in KCND3 has been described in a complex patient who has seizures[40], although it is unclear whether the mutation directly causes seizures.

4. Voltage-gated calcium channels (VGCCs) and epilepsy: an equivocal relationship

Like VGSCS, VGCCs are critical for supporting excitability in neuronal networks. In humans, mutations in CACNA1A have been most strongly associated with ataxia or migraine, but some CACNA1A mutations are associated with a significant epileptic comorbidity[6]. In addition, compound heterozygous mutations[41], and *de novo* mutations[42,43] in CACNA1A have been linked epileptic encephalopathies. Evidence from mouse models suggest CACNA1A plays a key role in regulating thalamocortical connections and suppresses absence-like seizures under normal conditions[44].

However, it is the T-type calcium channels that are most closely associated with absence epilepsy[45]. Rodent models of absence epilepsy have been mapped to both mutations in in T-type channels and to non-genetic changes in T-type currents, but causative mutations in humans have not been clearly identified. Antagonists specific for T-type channels are highly effective in models of absence epilepsy[46], and genetic variation in these channels has been linked with differing responses to treatment in patients [47]. These findings may hold relevance for other epilepsies because variation in in a T-type channel gene (CACNA1G) has been shown to modify severity in a mouse model of SCN2A-associated epilepsy [48]. Thus, whilst it remains uncertain whether

mutations in T-type calcium channels cause epilepsy (in humans), these channels appear to be important modifiers of severity and treatment outcome in other types of epilepsy.

5. NMDA receptors (NMDARs) leap to the front of the epilepsy genetics field

NMDARs are not intrinsically voltage gated, but they do require depolarisation to open, and recent studies of rare variants in these channels, have catapulted these genes to the centre of the epilepsy field. The NMDAR genes GRIN2A (more associated with epilepsy) and GRIN2B (more associated with intellectual disability) are some of the most intolerant to functional variation in the human genome[**49]. A novel strategy to estimate the 'overall impact' of a panel of disease associated rare variants on receptor function (Figure 2) has established a new gold-standard for probing genotype-phenotype relationships [**49]. This approach has already led to potential new treatments for a cohort of patients with NMDAR mutations[50].

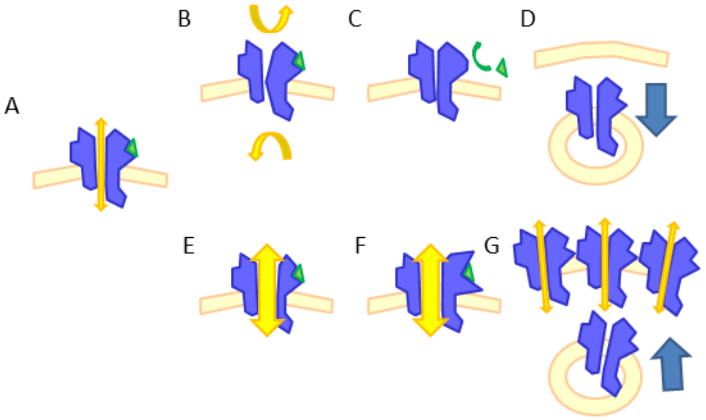

Figure 2: Assembling the 'overall impact' of factors affecting channel function. In order to fully characterise the effects of a mutation, the behaviour of the 'ancestor' or wild-type channel (A) is compared to mutants. Mutations may have conflicting effects on (B) gating – where the ligand binds or voltage-sensors move, but the pore does not open properly or (E) opens too easily. Binding, (C) when the ligand cannot bind, or the voltage sensor no longer detects or responds to voltage changes or responds too easily (F). Trafficking when channels fail to establish in the membrane (D), or too many channels are sent to the membrane (G). Ideally all factors are assessed in the target cell type and the consequences are modelled to provide an integrated output.

6. Concluding remarks

VGICs have revealed a tremendous amount about the genetics and mechanisms of epilepsy. As initially demonstrated for DS and increasingly and emerging as a trend in channelopathies, genotype-phenotype relationships can be highly variable even with identical or equivalent mutations. Studies are probing beyond the channels themselves to identify genetic modifiers, which may allow for a much needed paradigm-shift in epilepsy drug development. In addition mutations in genes other than ion channels are increasingly being identified in epilepsy. Future work exploring how these mutations cause seizures may benefit from lessons learnt from channelopathies. For example, the discovery that loss-of-function mutations in SCN1A disproportionately affect interneurons was a mechanistic breakthrough that radically changed the consensus on treatments for DS. Modelling the effects of non-ion channel mutations in different types of neuron may produce similar breakthroughs for many genes. After a spell of unbiased gene sequencing the field should re-assess how much the preponderance of channelopathies in epilepsy was a consequence of where we looked.

7. Acknowledgements

S Schorge is supported by a fellowship from the Royal Society. J Carpenter is supported by a PhD Studentship from the Wellcome Trust.

1. Symonds JD, Zuberi SM: **Genetics update: Monogenetics, polygene disorders and the quest for modifying genes**. *Neuropharmacology* 2017, doi:10.1016/j.neuropharm.2017.07.016.

2. Helbig I, Tayoun AAN: **Understanding Genotypes and Phenotypes in Epileptic Encephalopathies**. *Mol Syndromol* 2016, **7**:172–181.

3. Brunklaus A, Zuberi SM: **Dravet syndrome--from epileptic encephalopathy to channelopathy**. *Epilepsia* 2014, **55**:979–984.

4. Gataullina S, Dulac O: **From genotype to phenotype in Dravet disease**. *Seizure* 2017, **44**:58–64.

5. Djémié T, Weckhuysen S, von Spiczak S, Carvill GL, Jaehn J, Anttonen A-K, Brilstra E, Caglayan HS, de Kovel CG, Depienne C, et al.: **Pitfalls in genetic testing: the story of missed SCN1A mutations**. *Mol Genet Genomic Med* 2016, **4**:457–464.

6. Prontera P, Sarchielli P, Caproni S, Bedetti C, Cupini LM, Calabresi P, Costa C: **Epilepsy in hemiplegic migraine: Genetic mutations and clinical implications**. *Cephalalgia Int J Headache* 2017, doi:10.1177/0333102416686347.

7. Cetica V, Chiari S, Mei D, Parrini E, Grisotto L, Marini C, Pucatti D, Ferrari A, Sicca F, Specchio N, et al.: **Clinical and genetic factors predicting Dravet syndrome in infants with SCN1A mutations**. *Neurology* 2017, **88**:1037–1044.

8. Verbeek NE, van der Maas NAT, Sonsma ACM, Ippel E, Vermeer-de Bondt PE, Hagebeuk E, Jansen FE, Geesink HH, Braun KP, de Louw A, et al.: **Effect of vaccinations on seizure risk and disease course in Dravet syndrome**. *Neurology* 2015, **85**:596–603.

9. Holland KD, Bouley TM, Horn PS: **Comparison and optimization of in silico algorithms for predicting the pathogenicity of sodium channel variants in epilepsy**. *Epilepsia* 2017, **58**:1190–1198.

10. Huang W, Liu M, Yan SF, Yan N: **Structure-based assessment of disease-related mutations in human voltage-gated sodium channels**. *Protein Cell* 2017, **8**:401–438.

11. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA: **Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy**. *Nat Neurosci* 2006, **9**:1142–9.

12. Stern WM, Sander JW, Rothwell JC, Sisodiya SM: **Impaired intracortical inhibition demonstrated in vivo in people with Dravet syndrome**. *Neurology* 2017, **88**:1659–1665.

13. Rubinstein M, Han S, Tai C, Westenbroek RE, Hunker A, Scheuer T, Catterall WA: **Dissecting the phenotypes of Dravet syndrome by gene deletion**. *Brain J Neurol* 2015, **138**:2219–2233.

*14. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, Scheffer IE, Thiele EA, Wright S: **Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome**. *N Engl J Med* 2017, **376**:2011–2020.

This important clinical trial robustly quantifies the efficacy of cannabidiol for Dravet syndrome, helping to resolve and clarify anecdotal reports that have received extensive publicity.

15. Dutton SBB, Dutt K, Papale LA, Helmers S, Goldin AL, Escayg A: **Early-life febrile seizures worsen adult phenotypes in Scn1a mutants**. *Exp Neurol* 2017, **293**:159–171.

16. Marguet SL, Le-Schulte VTQ, Merseburg A, Neu A, Eichler R, Jakovcevski I, Ivanov A, Hanganu-Opatz IL, Bernard C, Morellini F, et al.: **Treatment during a vulnerable developmental period rescues a genetic epilepsy. *Nat Med* 2015, **21**:1436–1444.

Marguet and colleagues show that even in the presence of a causative mutation in a voltage gated channel, 'seizures beget seizures' and more importantly, preventing seizures has a significant impact on long term severity of the genetic disease.

17. Wirrell EC, Laux L, Donner E, Jette N, Knupp K, Meskis MA, Miller I, Sullivan J, Welborn M, Berg AT: **Optimizing the Diagnosis and Management of Dravet Syndrome: Recommendations From a North American Consensus Panel**. *Pediatr Neurol* 2017, **68**:18–34.e3.

18. Hawkins NA, Anderson LL, Gertler TS, Laux L, George AL, Kearney JA: **Screening of conventional anticonvulsants in a genetic mouse model of epilepsy**. *Ann Clin Transl Neurol* 2017, **4**:326–339.

19. Ishii A, Watkins JC, Chen D, Hirose S, Hammer MF: **Clinical implications of SCN1A missense and truncation variants in a large Japanese cohort with Dravet syndrome**. *Epilepsia* 2017, **58**:282–290.

20. Frederiksen K, Lu D, Yang J, Jensen HS, Bastlund JF, Larsen PH, Liu H, Crestey F, Dekermendjian K, Badolo L, et al.: **A small molecule activator of Nav 1.1 channels increases fast-spiking interneuron excitability and GABAergic transmission in vitro and has anti-convulsive effects in vivo**. *Eur J Neurosci* 2017, **46**:1887–1896.

21. Flinspach M, Xu Q, Piekarz AD, Fellows R, Hagan R, Gibbs A, Liu Y, Neff RA, Freedman J, Eckert WA, et al.: **Insensitivity to pain induced by a potent selective closed-state Nav1.7 inhibitor**. *Sci Rep* 2017, **7**:39662.

22. Hawkins NA, Zachwieja NJ, Miller AR, Anderson LL, Kearney JA: **Fine Mapping of a Dravet Syndrome Modifier Locus on Mouse Chromosome 5 and Candidate Gene Analysis by RNA-Seq**. *PLoS Genet* 2016, **12**:e1006398.

23. Takaori T, Kumakura A, Ishii A, Hirose S, Hata D: **Two mild cases of Dravet syndrome with truncating mutation of SCN1A**. *Brain Dev* 2017, **39**:72–74.

24. Johnson MR, Shkura K, Langley SR, Delahaye-Duriez A, Srivastava P, Hill WD, Rackham OJL, Davies G, Harris SE, Moreno-Moral A, et al.: **Systems genetics identifies a convergent gene network for cognition and neurodevelopmental disease. *Nat Neurosci* 2016, **19**:223–232.

This study develops a new approach to linking genetic variation to disease, using systems of co-regulated genes rather than individual genes as a readout of genetic load.

25. Delahaye-Duriez A, Srivastava P, Shkura K, Langley SR, Laaniste L, Moreno-Moral A, Danis B, Mazzuferi M, Foerch P, Gazina EV, et al.: **Rare and common epilepsies converge on a shared gene regulatory network providing opportunities for novel antiepileptic drug discovery**. *Genome Biol* 2016, **17**:245.

26. Lin W-H, Giachello CNG, Baines RA: **Seizure control through genetic and pharmacological manipulation of Pumilio in Drosophila: a key component of neuronal homeostasis**. *Dis Model Mech* 2017, **10**:141–150.

27. Meisler MH, Helman G, Hammer MF, Fureman BE, Gaillard WD, Goldin AL, Hirose S, Ishii A, Kroner BL, Lossin C, et al.: **SCN8A encephalopathy: Research progress and prospects**. *Epilepsia* 2016, **57**:1027–1035.

28. Lopez-Santiago LF, Yuan Y, Wagnon JL, Hull JM, Frasier CR, O'Malley HA, Meisler MH, Isom LL: **Neuronal hyperexcitability in a mouse model of SCN8A epileptic encephalopathy**. *Proc Natl Acad Sci U S A* 2017, **114**:2383–2388.

29. Ottolini M, Barker BS, Gaykema RP, Meisler MH, Patel MK: **Aberrant sodium channel currents and hyperexcitability of medial entorhinal cortex neurons in a mouse model of SCN8A encephalopathy**. *J Neurosci Off J Soc Neurosci* 2017, doi:10.1523/JNEUROSCI.2709-16.2017.

30. Anderson LL, Hawkins NA, Thompson CH, Kearney JA, George AL: **Unexpected Efficacy of a Novel Sodium Channel Modulator in Dravet Syndrome**. *Sci Rep* 2017, **7**:1682.

31. Wolff M, Johannesen KM, Hedrich UBS, Masnada S, Rubboli G, Gardella E, Lesca G, Ville D, Milh M, Villard L, et al.: **Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders**. *Brain J Neurol* 2017, doi:10.1093/brain/awx054.

32. Howell KB, McMahon JM, Carvill GL, Tambunan D, Mackay MT, Rodriguez-Casero V, Webster R, Clark D, Freeman JL, Calvert S, et al.: **SCN2A encephalopathy: A major cause of epilepsy of infancy with migrating focal seizures**. *Neurology* 2015, **85**:958–966.

33. Lamar T, Vanoye CG, Calhoun J, Wong JC, Dutton SBB, Jorge BS, Velinov M, Escayg A, Kearney JA: **SCN3A deficiency associated with increased seizure susceptibility**. *Neurobiol Dis* 2017, **102**:38–48.

34. Kambouris M, Thevenon J, Soldatos A, Cox A, Stephen J, Ben-Omran T, Al-Sarraj Y, Boulos H, Bone W, Mullikin JC, et al.: **Biallelic SCN10A mutations in neuromuscular disease and epileptic encephalopathy**. *Ann Clin Transl Neurol* 2017, **4**:26–35.

35. Syrbe S, Hedrich UBS, Riesch E, Djémié T, Müller S, Møller RS, Maher B, Hernandez-Hernandez L, Synofzik M, Caglayan HS, et al.: **De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy**. *Nat Genet* 2015, **47**:393–399.

36. Corbett MA, Bellows ST, Li M, Carroll R, Micallef S, Carvill GL, Myers CT, Howell KB, Maljevic S, Lerche H, et al.: **Dominant KCNA2 mutation causes episodic ataxia and pharmacoresponsive epilepsy**. *Neurology* 2016, **87**:1975–1984.

37. Masnada S, Hedrich UBS, Gardella E, Schubert J, Kaiwar C, Klee EW, Lanpher BC, Gavrilova RH, Synofzik M, Bast T, et al.: **Clinical spectrum and genotype–phenotype associations of KCNA2-related encephalopathies**. *Brain* 2017, doi:10.1093/brain/awx184.

38. Muona M, Berkovic SF, Dibbens LM, Oliver KL, Maljevic S, Bayly MA, Joensuu T, Canafoglia L, Franceschetti S, Michelucci R, et al.: **A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy**. *Nat Genet* 2015, **47**:39–46.

39. Oliver KL, Franceschetti S, Milligan CJ, Muona M, Mandelstam SA, Canafoglia L, Boguszewska-Chachulska AM, Korczyn AD, Bisulli F, Di Bonaventura C, et al.: **Myoclonus epilepsy and ataxia due to KCNC1 mutation: Analysis of 20 cases and K(+) channel properties**. *Ann Neurol* 2017, **81**:677–689.

40. Smets K, Duarri A, Deconinck T, Ceulemans B, van de Warrenburg BP, Züchner S, Gonzalez MA, Schüle R, Synofzik M, Van der Aa N, et al.: **First de novo KCND3 mutation causes severe Kv4.3 channel dysfunction leading to early onset cerebellar ataxia, intellectual disability, oral apraxia and epilepsy**. *BMC Med Genet* 2015, **16**:51.

41. Reinson K, Õiglane-Shlik E, Talvik I, Vaher U, Õunapuu A, Ennok M, Teek R, Pajusalu S, Murumets Ü, Tomberg T, et al.: **Biallelic CACNA1A mutations cause early onset epileptic encephalopathy with progressive cerebral, cerebellar, and optic nerve atrophy**. *Am J Med Genet A* 2016, **170**:2173–2176.

42. Epi4K Consortium. Electronic address: epi4k@columbia.edu, Epi4K Consortium: **De Novo Mutations in SLC1A2 and CACNA1A Are Important Causes of Epileptic Encephalopathies**. *Am J Hum Genet* 2016, **99**:287–298.

43. Byers HM, Beatty CW, Hahn SH, Gospe SM: **Dramatic Response After Lamotrigine in a Patient With Epileptic Encephalopathy and a De NovoCACNA1A Variant**. *Pediatr Neurol* 2016, **60**:79–82.

44. Bomben VC, Aiba I, Qian J, Mark MD, Herlitze S, Noebels JL: **Isolated P/Q Calcium Channel Deletion in Layer VI Corticothalamic Neurons Generates Absence Epilepsy**. *J Neurosci Off J Soc Neurosci* 2016, **36**:405–418.

45. Cain SM, Snutch TP: **Voltage-Gated Calcium Channels in Epilepsy**. In *Jasper's Basic Mechanisms of the Epilepsies*. Edited by Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV. National Center for Biotechnology Information (US); 2012.

46. Tringham E, Powell KL, Cain SM, Kuplast K, Mezeyova J, Weerapura M, Eduljee C, Jiang X, Smith P, Morrison J-L, et al.: **T-type calcium channel blockers that attenuate thalamic burst firing and suppress absence seizures**. *Sci Transl Med* 2012, **4**:121ra19.

47. Glauser TA, Holland K, O'Brien VP, Keddache M, Martin LJ, Clark PO, Cnaan A, Dlugos D, Hirtz DG, Shinnar S, et al.: **Pharmacogenetics of antiepileptic drug efficacy in childhood absence epilepsy**. *Ann Neurol* 2017, **81**:444–453.

48. Calhoun JD, Hawkins NA, Zachwieja NJ, Kearney JA: **Cacna1g is a genetic modifier of epilepsy caused by mutation of voltage-gated sodium channel Scn2a**. *Epilepsia* 2016, **57**:e103-107.

** 49. Swanger SA, Chen W, Wells G, Burger PB, Tankovic A, Bhattacharya S, Strong KL, Hu C, Kusumoto H, Zhang J, et al.: **Mechanistic Insight into NMDA Receptor Dysregulation by Rare Variants in the GluN2A and GluN2B Agonist Binding Domains**. *Am J Hum Genet* 2016, **99**:1261–1280.

This impressive study systematically characterises a series of mutations and rare variants in NMDA receptor genes, building an integrative approach that considers receptor trafficking, gating, and ligand binding to create a synthetic overview of the effects of mutations on NMDA receptor activity.

50. Ogden KK, Chen W, Swanger SA, McDaniel MJ, Fan LZ, Hu C, Tankovic A, Kusumoto H, Kosobucki GJ, Schulien AJ, et al.: **Molecular Mechanism of Disease-Associated Mutations in the Pre-M1 Helix of NMDA Receptors and Potential Rescue Pharmacology**. *PLoS Genet* 2017, **13**:e1006536.