Open access • Journal Article • DOI:10.1111/EPI.16438

Biological concepts in human sodium channel epilepsies and their relevance in clinical practice. — Source link

Andreas Brunklaus, Juanjiangmeng Du, Felix Steckler, Ismael I. Ghanty ...+31 more authors

Institutions: University of Glasgow, University of Cologne, University of Southern Denmark, University College London ...+10 more institutions

Published on: 23 Feb 2020 - Epilepsia (WILEY)

Topics: SCN3A, Epilepsy and Dravet syndrome

Biological concepts in human sodium channel epilepsies and their relevance in clinical practice

Andreas Brunklaus, Juanjiangmeng Du, Felix Steckler, Ismael Ghanty, Katrine Johannesen, Christina Fenger, Stephanie Schorge, David Baez-nieto, Hao-ran Wang, Andrew Allen, et al.

Biological concepts in human sodium channel epilepsies and their relevance in clinical practice

Brunklaus, Andreas; Du, Juanjiangmeng; Steckler, Felix; Ghanty, Ismael I.; Johannesen, Katrine M.; Fenger, Christina Dühring; Schorge, Stephanie; Baez-Nieto, David; Wang, Hao Ran; Allen, Andrew; Pan, Jen Q.; Lerche, Holger; Heyne, Henrike; Symonds, Joseph D.; Zuberi, Sameer M.; Sanders, Stephan; Sheidley, Beth R.; Craiu, Dana; Olson, Heather E.; Weckhuysen, Sarah; DeJonge, Peter; Helbig, Ingo; Van Esch, Hilde; Busa, Tiffany; Milh, Matthieu; Isidor, Bertrand; Depienne, Christel; Poduri, Annapurna; Campbell, Arthur J.; Dimidschstein, Jordane; Møller, Rikke S.; Lal, Dennis

Published in:
Epilepsia

DOI:
10.1111/epi.16438

Publication date:
2020

Document version
Accepted manuscript

Biological concepts in human sodium channel epilepsies and their relevance in clinical practice

Affiliations

Andreas Brunklaus[1,2,#†], Juanjiangmeng Du[3#], Felix Steckler[1,2], Ismael I Ghanty[1,2], Katrine M Johannesen[4,5], Christina Dühring Fenger[4,6], Stephanie Schorge[7,8], David Baez-Nieto[9], Hao-Ran Wang[9], Andrew Allen[9], Jen Q. Pan[9], Holger Lerche[10], Henrike Heyne[9,11,12], Joseph D Symonds[1,2], Sameer M Zuberi[1,2], Stephan Sanders[13], Beth R. Sheidley[14], Dana Craiu[15,16], Heather E. Olson[14], Sarah Weckhuysen[17,18,19], Peter DeJonge[17,18,19], Ingo Helbig[20,21,22,23,24], Hilde Van Esch[25], Tiffany Busa[26], Matthieu Milh[27,28], Bertrand Isidor[29], Christel Depienne[30,31], Annapurna Poduri[14,32], Arthur J Campbell[8], Jordane Dimidschstein[9], Rikke S. Møller[4,5†], and Dennis Lal[3,9,11,33,34,#†]

[1]The Paediatric Neurosciences Research Group, Royal Hospital for Children, Glasgow, G51 4TF, UK,

[2]School of Medicine, University of Glasgow, Glasgow, G12 8QQ, UK,

[3]Cologne Center for Genomics, University of Cologne, University Hospital Cologne, Cologne 50931, Germany,

[4]Deparment of Epilepsy Genetics and Personalized Medicine, The Danish Epilepsy Centre Filadelfia, Dianalund, Denmark,

[5]Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark,

[6]Amplexa Genetics A/S, Odense, Denmark

[7]Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London WC1N 1AX,UK,

[8]School of Pharmacy, University College London, London WC1N 1AX, UK,

[9]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA,

[10]Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Germany,

[11]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA 02142, USA,

[12]Institute for Molecular Medicine Finland: FIMM, University of Helsinki, Helsinki, Finland

[13]Department of Psychiatry, UCSF Weill Institute for Neurosciences, University of California, San Francisco, CA 94158, USA,

[14]Epilepsy Genetics Program, Department of Neurology, Division of Epilepsy and Clinical Neurophysiology, Boston Children's Hospital, Boston, MA 02115, USA,

[15]Carol Davila University of Medicine, Department of Clinical Neurosciences, Pediatric Neurology Discipline, Bucharest 050474, Romania,

[16]Pediatric Neurology Clinic, Alexandru Obregia Hospital, Bucharest 050474, Romania,

[17]Neurogenetics Group, Center for Molecular Neurology, VIB, Antwerp 2610, Belgium,

[18]Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp 2610, Belgium,

[19]Department of Neurology, University Hospital Antwerp, Antwerp 2610, Belgium,

[20]Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, USA

[21]The Epilepsy NeuroGenetics Initiative (ENGIN), Children's Hospital of Philadelphia, Philadelphia, USA

[22]Department of Biomedical and Health Informatics (DBHi), Children's Hospital of Philadelphia, Philadelphia, USA

[23]Department of Neurology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, USA

[24]Department of Neuropediatrics, University of Kiel, Kiel, Germany

[25]Department of Human Genetics and Center for Human Genetics, Laboratory for Genetics of Cognition, University Hospitals Leuven 3000, Belgium,

[26]Genetics department, CHU Timone Enfants, AP-HM, Marseille 13385, France,

[27]GMGF, INSERM UMR_S910, Aix-Marseille University, Marseille 13385, France,

[28]Laboratoire d'hématologie, Centre hospitalier Le Mans, Le Mans 72037, France,

[29]CHU Nantes, Medical genetics department, Nantes 44093, France,

[30]Institut für Humangenetik, Universitätsklinikum Essen, Germany,

[31]INSERM U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France.

[32]Harvard Medical School, Boston, MA 02115, USA,

[33]Epilepsy Center, Neurological Institute, Cleveland Clinic, Cleveland, OH 44106, USA,

[34]Genomic Medicine Institute, Lerner Research Institute Cleveland Clinic, Cleveland, OH 44106, USA

#A.B., J.D. and D.L. contributed equally to this work

†A.B. R.S.M and D.L are joint corresponding authors

Corresponding authors

Dr Andreas Brunklaus, MD(Res)
Consultant Paediatric Neurologist
Honorary Clinical Senior Lecturer University of Glasgow
Fraser of Allander Neurosciences Unit
Office Block, Ground Floor, Zone 2
Royal Hospital for Children
1345 Govan Road
Glasgow G51 4TF, United Kingdom
Tel: 0141 451 6487
Email: andreas.brunklaus@nhs.net

Dr Rikke S. Møller, PhD
Associate Professor,
Head of Department of Epilepsy Genetics and Personalized Medicine
Danish Epilepsy Centre/University of Southern Denmark
Dianalund, Denmark
Email: rimo@filadelfia.dk

Dr Dennis Lal, PhD
Assistant Professor and Assistant Staff at Cleveland Clinic Genomic Medicine Institute and Neurological Institute, Cleveland, OH, US
Visiting Scientist at Broad Institute of Harvard and M.I.T., Cambridge MA, US
Group Leader, University of Cologne, Köln, NRW, Germany
E-mail: Lald@ccf.org
E-mail: Dlal@broadinstitute.org

Key words: *SCN1A*, *SCN2A*, *SCN3A*, *SCN8A*, epilepsy, neurodevelopmental disorders

Number of text pages: 7

Number of words: 4256

Number of references: 55

Number of figures: 5

Number of tables: 1

Summary: 299

DR. ANDREAS BRUNKLAUS (Orcid ID : 0000-0002-7728-6903)

MRS. KATRINE JOHANNESEN (Orcid ID : 0000-0002-7356-3109)

DR. ANNAPURNA PODURI (Orcid ID : 0000-0002-7350-5136)

MRS. RIKKE STEENSBJERRE MØLLER (Orcid ID : 0000-0002-9664-1448)

Article type : Full length original research paper

Biological concepts in human sodium channel epilepsies and their relevance in clinical practice

Affiliations

Andreas Brunklaus[1,2,#†], Juanjiangmeng Du[3#], Felix Steckler[1,2], Ismael I Ghanty[1,2], Katrine M Johannesen[4,5], Christina Dühring Fenger[4,6], Stephanie Schorge[7,8], David Baez[9], Hao-Ran Wang[9], Andrew Allen[9], Jen Q. Pan[9], Holger Lerche[10], Henrike Heyne[9,11,12], Joseph D Symonds[1,2], Sameer M Zuberi[1,2], Stephan Sanders[13], Beth R. Sheidley[14], Dana Craiu[15,16], Heather E. Olson[14], Sarah Weckhuysen[17,18,19], Peter DeJonge[17,18,19], Ingo Helbig[20,21], Hilde Van Esch[22], Tiffany Busa[23], Matthieu Milh[24,25], Bertrand Isidor[26], Christel Depienne[27,28], Annapurna Poduri[14, 29], Arthur J Campbell[8], Jordane Dimidschstein[9], Rikke S. Møller[4,5†], and Dennis Lal[3,9,11,30,31,#†]

[1]The Paediatric Neurosciences Research Group, Royal Hospital for Children, Glasgow, G51 4TF, UK,

[2]School of Medicine, University of Glasgow, Glasgow, G12 8QQ, UK,

[3]Cologne Center for Genomics, University of Cologne, University Hospital Cologne, Cologne 50931, Germany,

[4]Deparment of Epilepsy Genetics and Personalized Medicine, The Danish Epilepsy Centre Filadelfia, Dianalund, Denmark,

[5]Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark,

[6]Amplexa Genetics A/S, Odense, Denmark

[7]Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London WC1N 1AX,UK,

[8]School of Pharmacy, University College London, London WC1N 1AX, UK,

[9]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA,

[10]Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Germany,

[11]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA 02142, USA,

[12]Institute for Molecular Medicine Finland: FIMM, University of Helsinki, Helsinki, Finland

[13]Department of Psychiatry, UCSF Weill Institute for Neurosciences, University of California, San Francisco, CA 94158, USA,

[14]Epilepsy Genetics Program, Department of Neurology, Division of Epilepsy and Clinical Neurophysiology, Boston Children's Hospital, Boston, MA 02115, USA,

[15]Carol Davila University of Medicine, Department of Clinical Neurosciences, Pediatric Neurology Discipline, Bucharest 050474, Romania,

[16]Pediatric Neurology Clinic, Alexandru Obregia Hospital, Bucharest 050474, Romania,

[17]Neurogenetics Group, Center for Molecular Neurology, VIB, Antwerp 2610, Belgium,

[18]Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp 2610, Belgium,

[19]Department of Neurology, University Hospital Antwerp, Antwerp 2610, Belgium,

[20]Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA,

[21]Department of Neuropediatrics, Christian-Albrechts-University of Kiel, Kiel 24118, Germany,

[22]Department of Human Genetics and Center for Human Genetics, Laboratory for Genetics of Cognition, University Hospitals Leuven 3000, Belgium,

[23]Genetics department, CHU Timone Enfants, AP-HM, Marseille 13385, France,

[24]GMGF, INSERM UMR_S910, Aix-Marseille University, Marseille 13385, France,

[25]Laboratoire d'hématologie, Centre hospitalier Le Mans, Le Mans 72037, France,

[26]CHU Nantes, Medical genetics department, Nantes 44093, France,

[27]Institut für Humangenetik, Universitätsklinikum Essen, Germany,

[28]INSERM U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France.

[29]Harvard Medical School, Boston, MA 02115, USA,

[30]Epilepsy Center, Neurological Institute, Cleveland Clinic, Cleveland, OH 44106, USA,

[31]Genomic Medicine Institute, Lerner Research Institute Cleveland Clinic, Cleveland, OH 44106, USA

#A.B., J.D. and D.L. contributed equally to this work

†A.B. R.S.M and D.L are joint corresponding authors

Corresponding authors

Dr Andreas Brunklaus, MD(Res)

Consultant Paediatric Neurologist

Honorary Clinical Senior Lecturer University of Glasgow

Fraser of Allander Neurosciences Unit
Office Block, Ground Floor, Zone 2
Royal Hospital for Children
1345 Govan Road
Glasgow G51 4TF, United Kingdom
Tel: 0141 451 6487
Email: andreas.brunklaus@nhs.net

Dr Rikke S. Møller, PhD
Associate Professor,
Head of Department of Epilepsy Genetics and Personalized Medicine
Danish Epilepsy Centre/University of Southern Denmark
Dianalund, Denmark
Email: rimo@filadelfia.dk

Dr Dennis Lal, PhD
Assistant Professor and Assistant Staff at Cleveland Clinic Genomic Medicine Institute and Neurological Institute, Cleveland, OH, US
Visiting Scientist at Broad Institute of Harvard and M.I.T., Cambridge MA, US
Group Leader, University of Cologne, Köln, NRW, Germany
E-mail: Lald@ccf.org
E-mail: Dlal@broadinstitute.org

Key words: SCN1A, SCN2A, SCN3A, SCN8A, epilepsy, neurodevelopmental disorders

Number of text pages: 7

Number of words: 4256

Number of references: 55

Number of figures: 5

Number of tables: 1

Summary: 299

Summary

Objective: Voltage-gated sodium channels (SCNs) share similar amino acid sequence, structure, and function. Genetic variants in the four human brain-expressed SCN-genes SCN1A/2A/3A/8A have been associated with heterogeneous epilepsy phenotypes and neurodevelopmental disorders (NDD). To better understand the biology of seizure susceptibility in SCN-related epilepsies, our aim was to determine similarities and differences between sodium channel disorders, allowing us to develop a broader perspective on precision treatment than on an individual gene level alone.

Methods: We analysed genotype-phenotype correlations in large SCN-patient cohorts and applied variant constraint analysis to identify severe sodium channel disease. We examined temporal patterns of human SCN-expression and correlated functional data from in-vitro studies with clinical phenotypes across different sodium channel disorders.

Results: Comparing 865 epilepsy patients (504 SCN1A, 140 SCN2A, 171 SCN8A, 4 SCN3A, 46 copy number variation/CNV cases) and analysis of 114 functional studies allowed us to identify common patterns of presentation. All four epilepsy-associated SCN-genes demonstrated significant contstraint in both protein truncating and missense-variation when compared to other SCN-genes. We observed that age at seizure onset is related to SCN-gene expression over time. Individuals with gain-of-function SCN2A/3A/8A missense variants or CNV duplications share similar characteristics, most frequently present with early onset epilepsy (<3 months), and demonstrate good reponse to sodium channel blockers (SCBs). Direct comparison of corresponding SCN-variants across different SCN-subtypes illustrates that the functional effects of variants in corresponding channel locations are similar, however their clinical manifestation differs, depending on their role in different types of neurons in which they are expressed.

Significance: Variant function and location within one channel can serve as surrogate for variant effects across related sodium channels. Taking a broader view on precision treatment suggests that in those patients with a suspected underlying genetic epilepsy presenting with neonatal or early onset seizures (<3 months) SCBs should be considered.

Key words: SCN1A, SCN2A, SCN3A, SCN8A, epilepsy, neurodevelopmental disorders

Key points:

- Corresponding variants in SCN1A/2A/8A display similar function but result in different phenotypes depending on their role in different types of neurons.

- Variant function and location within one channel can serve as surrogate for variant effects across related sodium channels.

- Age at onset of sodium channel epilepsies correlates with SCN gene expression profiles.

- SCN1/2/3/8A show significant contstraint when compared to other sodium channel genes not linked to epilepsy.

- SCN2A/SCN8A GoF is commonest in early onset epilepsy (<3 months) and SCBs should be considered in affected individuals.

Introduction

Genetic variants in the genes SCN1A, SCN2A, SCN3A, and SCN8A, encoding the four neuronal voltage-gated sodium channels $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$, are responsible for a significant fraction of early onset genetic epilepsies and neurodevelopmental disorders (NDDs)[1]. Modern sequencing techniques have revolutionized the way we diagnose the genetic causes for these disorders, opening the door to precision medicine. However, it is often difficult to predict the impact of a variant without prior functional characterization. Different variants within the same gene may cause distinct clinical disorders (pleiotropy) with different drug responses, while variants in different channel genes may result in similar phenotypes (genetic heterogeneity). This complexity is well established for the epilepsy related sodium channel genes and is challenging for the development of medical therapies.

The clinical phenotypes associated with different sodium channel (SCN) disorders have characteristic presentations. Dravet Syndrome (DS), a severe developmental and epileptic encephalopathy, is caused by SCN1A missense and protein truncation variants as well as deletions[2,3]. Missense variants in SCN1A also account for approximately 10% of generalized epilepsy with febrile seizure plus ($GEFS^+$) cases[4]. Moreover, small copy number variations (CNVs) including microdeletions within SCN1A, as well as large CNVs that include the nearby genes SCN2A and SCN3A on chromosome 2, are found in a small percentage of DS patient[5–7]. In SCN1A, both loss-of-function (LoF) missense and protein truncating variants (PTVs) lead to reduced sodium current in GABAergic interneurons resulting in a classical DS phenotype presenting in the first year of life with prolonged, febrile and afebrile, generalised clonic or hemiclonic seizures. The epilepsy is usually resistant to standard anti-epileptic medication and affected individuals develop cognitive, behavioural, and motor impairment[8,9]. A minority of gain-of-function (GoF) SCN1A missense variants have been described, and these are associated with familial hemiplegic migraine (FHM)[10].

Variants in SCN2A have been identified in different forms of infantile epilepsy including benign infantile seizures, developmental and epileptic encephalopathies (DEEs), Ohtahara or West syndrome[11–13]. Recent studies propose that GoF missense variants in SCN2A are associated with neonatal or early infantile seizures presenting at less than 3 months of age, whereas LoF missense and PTVs are associated with later onset epilepsy and

ASD/NDDs[14–18]. SCN8A encephalopathy presents in infancy with multiple seizure types including focal, tonic, clonic, myoclonic absence seizures, and epileptic spasms[19–22]. The developmental outcome is poor and many patients have motor manifestations including hypotonia and movement disorders. A small number of patients have also been reported with milder phenotypes such as benign infantile seizures, paroxysmal dyskinesia, and isolated intellectual disability (ID)[23,24]. GoF missense variants appear to be associated with epileptic encephalopathy, whereas LoF variants are seen in NDDs without epilepsy[25,26]. SCN3A-associated epilepsies are clinically heterogeneous presenting with mainly GoF missense variants, early-onset seizures, epileptic encephalopathy, polymicrogyria and developmental impairment[27,28].

In order to better understand the biology of seizure susceptibility in SCN-related epilepsies our aim was to determine similarities and differences between sodium channel disorders and apply variant constraint analysis to identify severe sodium channel disease. This approach allowed us to develop a broader perspective on precision treatment than on an individual gene or variant level and to recognise common patterns among SCN-related disorders informing clinical practice.

Methods

Ethics statement

Retrospective review of anonymized clinical referral data and variant findings were approved by the relevant institutional review boards.

Study design and participants

We identified epilepsy patients carrying single nucleotide variants affecting SCN1A/2A/8A from two sites: the Danish Epilepsy Centre Filadelfia (Dianalund, Denmark) including case series by Møller et al.[29], Wolff et al.[15], Gardella et al.[30] (in print) and unpublished cases (supplementary table 1) and the Royal Hospital for Children (Glasgow, UK) including case series by Zuberi et al.[2] and unpublished cases (supplementary table 1). Diagnostic criteria have been published previously[2,15,31]. Additional SCN1A patients were included from the published case series by Depienne et al.[32]. In order to identify SCN3A variants, we performed a PubMed search (up to October 2019) using the terms "epilepsy" and "SCN3A". To enrich for high confidence disease-associated variants with large effect, we excluded SCN variants present in individuals from the general population. Specifically, we removed patients with variants observed in the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org).

We identified patients carrying copy number variants (CNVs) covering SCN1A/2A/3A/8A from three sites: The Boston Children's Hospital (Boston, USA); the Danish Epilepsy Centre Filadelfia (Dianalund, Denmark), and University Hospital Antwerp (Belgium). All local ethics boards approved the enrollment. We performed a literature review (using PubMed up to October 2019) and a DECIPHER database (v9.14)[33] search for individuals carrying a CNV covering SCN1A/2A/3A/8A. The following search terms were used: "CNV" in combination with one of the target SCN-genes ("SCN1A", "SCN2A", "SCN3A" or "SCN8A). Only patients with

SCN-CNVs (<15 Mb) were included. The clinical phenotype information, including seizure onset and medication response, was collected (supplementary table 2).

Review of SCN functional missense variants

To collect functionally tested missense variants, we performed a PubMed screen (up to October 2019) with the terms "clamp" and "SCN1A", "SCN2A", "SCN3A" or "SCN8A" using R package RISmed 2.17. We included missense variants of the classic isoforms of SCNs from patients presenting with epilepsy and/or neurodevelopmental disorders, which have been functionally tested by whole-cell patch-clamp experiments. Variants observed in the general population, thus present in the Genome Aggregation Database (GnomAD, http://gnomad.broadinstitute.org), were removed from the analyses. We only included variants characterized in mammalian cell lines to improve biophysical comparisons. Variants were categorized either as gain-of-function (GoF), loss-of-function (LoF) or 'mixed' function regarding their biophysical properties. We define any biophysical change entailing an increase in the Na+ permeability as GoF, and the opposite for LoF. A few cases showed a paradoxical change i.e. decrease in the peak current and increase in the persistent current. Where one effect was not clearly dominant, these cases were classified as 'mixed' effect on function. Key electrophysiological features and patient phenotypes are detailed in supplementary table 3.

Variant constraint classification

Genes that have statistically fewer variants than expected are considered to be under evolutionary selection and thus associated with disease when mutated. The missense and PTV constraint scores were derived from the Exome Aggregation Consortium (ExAC). We considered SCN-genes with missense Z scores (intolerance to missense variation) ≥ 3.09 or the probability of being loss-of-function intolerant (pLI) scores ≥ 0.9 as intolerant of missense or PTV variants[34].

Statistical analysis

Non-normally distributed data, such as age at seizure onset, are given as median with semi-interquartile ranges (semi-IQR) and the Mann-Whitney U test was used to compute differences in age distribution by variant type and between genes. Variant enrichment and sodium channel blocker response was calculated using Fisher's exact test. Significance was tested at the 5% level and analysis performed using SPSS version 24.0.

Results

Phenotypes vs. SCN variant types analysis

We ascertained a total of 865 epilepsy patients that fulfilled the study criteria (supplementary table 4). These consisted of 504 SCN1A patients (Glasgow: 261, Denmark: 44, Depienne: 199), 140 SCN2A patients (Denmark), 171 SCN8A patients (Denmark), four SCN3A patients (literature) and 46 CNVs (Boston/Denmark/Belgium/literature).

SCN1A: Among the 504 patients with SCN1A variants 490 had DS and 14 GEFS+. Nearly all PTV carriers (99.6%) had DS, compared to 94% of missense carriers. Moreover, we observed a higher proportion of PTVs in SCN1A (53%) compared to SCN2A (9%, $p<0.001$) and SCN8A (4%, $p<0.001$).

SCN2A: Of patients presenting with SCN2A variants 50% (70/140) had developmental and epileptic encephalopathies (DEEs, including EOEE, EIMFS, OS, WS, LGS), 19% (26/140) benign epilepsies, 14% (20/140) other unclassified epilepsies and 17% (24/140) primary ASD features with later occurrence of epilepsy. A significantly higher proportion of PTV carriers had autistic features (9 out of 13; 69%) compared to the SCN2A missense variant carriers (15 out of 127; 12%; $p<0.001$).

SCN3A: Literature review identified a total of 14 patients with SCN3A variants. Six of these were found in gnomAD, three had no detailed age at onset data available and one was inherited from an unaffected father. Of the remaining four patients, three were de novo, all presenting within the first days of life with an epileptic encephalopathy and various features including focal seizures, microcephaly, polymicrogyria and developmental delay. The fourth patient presented much later at five years of age with a GEFS+ phenotype.

SCN8A: Among the 171 patients with SCN8A variants, 64% (110/171) had DEEs, 25% (42/171) intermediate phenotypes, 6% (11/171) benign epilepsies and 5% (8/171) other unclassified epilepsies.

CNVs: We identified 46 patients with seizures carrying SCN-CNVs (10 reported for the first time in this study and 36 from the literature and DECIPHER database[33]). The most commonly observed CNVs affected three genes, SCN1-2-3A, due to their clustered genomic locations within 1.4 Mb on chromosome 2q24.3 (supplementary table 2). Apart from SCN1A deletions associated with DS phenotypes, all other CNV cases exhibited a heterogeneous epilepsy phenotype with mild to severe neurological disorders such as ID, developmental delay (DD), dysmorphism, and coordination problems. We noted a difference in the reported response to sodium channel blockers depending on CNV type. Of the 13 patients with documented SCB use, a *"*positive response*"* to SCBs was exclusively seen in those with CNV duplications (9/13), whereas *"*no response*"* to SCBs was only seen in patients with CNV deletions (4/13, $p=0.001$, supplementary table 2).

Seizure onset vs. SCN variant types

Among SCN-missense variant carriers, we observed a significant pattern in the emergence of seizures over time: SCN2A patients were the earliest to present with seizures (median 13 days), followed by SCN8A patients (median 4 months; $p_{SCN2A\ vs.\ SCN8A}<0.001$) and finally SCN1A missense patients (median 6 months; $p_{SCN1A\ vs.\ SCN2A}<0.001$; figure 1 and supplementary table 4). All three patients with de novo SCN3A variants included in this report presented in the first days of life.

In SCN2A patients, missense variant carriers showed a significantly earlier age of onset (median 13 days) compared to PTV carriers (median 36 months; $p<0.001$), with two distinct peaks occurring in the neonatal and later infantile period. A similar pattern was observed between SCN8A PTV (median 11 months) and missense patients (median 4 months; $p=0.04$). There was no difference in age of seizure onset among SCN1A missense and PTV patients and 96.4% (486/504) of SCN1A patients presented at ≥3 months.

Patients carrying SCN-CNV duplications presented with seizures as early as SCN2/3/8A missense variant carriers (medians 3-17 days) and significantly earlier than those with CNV deletions whose seizure onset occurred much later (medians 3-10 months, $p_{del\ vs.\ dup}<0.001$), similar to SCN1/2/8A PTV patients (figure 1).

Phenotypes vs. functional SCN variant effects

We reviewed functional properties of 114 SCN-variants fulfilling our inclusion criteria. We identified 53 electrophysiologically tested SCN1A variants, 31 SCN2A, five SCN3A and 25 SCN8A variants. The majority of SCN1A epilepsy-associated variants (75%) showed a LoF of the $Na_V1.1$ channel and a minority showed mixed effects (25%). In contrast, the majority of functionally tested epilepsy-associated variants in SCN2A/3A/8A exhibited GoF features, 67%, 75% , and 76% respectively, suggesting that increased channel function is a common biophysical defect in SCN2A/3A/8A-associated epilepsy (figure 2A, supplementary tables 3 & 5).

Investigating the seizure onset of patients carrying different types of functional variants in the same gene, we observed no difference in seizure onset between SCN1A LoF and mixed variants (figure 2B). By contrast, all SCN2A GoF missense variants (N=16) were identified in early-onset epilepsy-ascertained patients (median 17 days), and 14 of those (88%) presented at <3 months of age, whereas SCN2A LoF variants (N=5) were identified in patients with later onset childhood seizures and NDDs (median 11 months, p<0.001). A similar trend not reaching significance was noticed in the SCN8A cohort, where GoF missense variants (N=13) were associated with early-onset epilepsy (median 3 months) compared to LoF (N=3, median 18 months, p=0.07). All seven SCN8A variants presenting at <3 months were GoF. The size of the SCN3A cohort was very small, however three out of four (75%) were GoF presenting with early onset epilepsy.

Comparison of missense variants across SCN1A, SCN2A and SCN8A

We detected 8 pairs of missense variants in which there was a corresponding disease-associated variant in a different SCN-gene: there were three SCN1A/2A pairs, four SCN1A/8A pairs and one SCN2A/8A pair (table 1; figure 3). The missense variants in each of those pairs appear to have similar functional consequences (3 GoF and 5 LoF effects). SCN1A LoF is associated with DS/GEFS+, while GoF variants are associated with FHM. However, the corresponding LoF SCN2A and SCN8A variants lead to primary neurodevelopmental disorders/ASD whereas GoF variants result in severe early onset epilepsy (DEE).

To illustrate the distribution of missense variants and their function between the three different channel subtypes, we plotted the position of 185 SCN1A variants (Glasgow 132/functional studies 53), 158 SCN2A variants (Denmark 127/functional studies 31) and 189 SCN8A variants (Denmark 164/functional studies 25) across the SCN-protein, showing that variants are mainly clustered in homologous domains (figure 3). Whilst SCN1A missense variants are distributed across the entire homologous domain, only very few SCN2A/8A variants are found in the S5-6 pore loop regions. Variants that occurred in the S5-6 pore loop regions appeared to be predominantly LoF, regardless of the channel subtype (89%, 16 out of 18), whereas variants that occurred for example in the voltage sensing S3-4, S4 and S4-5 regions harboured a mixture of GoF (17%), mixed (29%) and LoF (54%) effects (figure 3; supplementary table 3).

Phenotype vs. SCNs variant intolerance

Using constraint analysis we aimed to determine if there were common features between epilepsy-associated sodium channel genes and non-epilepsy-associated sodium channel genes. The SCN-family (SCN1-11A, 10 genes) shows a high degree of protein sequence conservation, especially in the transmembrane domains[35]. To understand why SCN1A/2A/3A/8A are particularly associated with severe early-onset de novo epilepsies and NDDs, we first evaluated variant intolerance of each SCN-gene. Among 60,000 individuals from the general population annotated in the ExAC database, SCN1A, SCN2A, SCN3A and SCN8A all show strong depletion for PTV (pLI score >0.9) and missense variants (missense Z-score >3.09; figure 4). This suggests strong evolutionary constraints on epilepsy associated SCN-genes in contrast to variants in SCN4/9/10/11A that are tolerated for both truncating and missense variants and mainly associated with familial (less severe) SCN-disease.

Discussion

Genotype-phenotype correlations across the four brain-expressed SCNs reveal distinct patterns of functional effects. The majority of SCN1A-related epilepsies are caused by LoF missense variants, full gene deletions, and PTVs. The clinical features of DS patients are consistent, presenting at similar ages regardless of variant type. GEFS+ patients tend to present later and carry mainly missense variants[2,36]. Only a small minority of SCN1A variants present with an epilepsy phenotype different from the GEFS+/DS spectrum. The variant T226M was recently reported in patients presenting with a more severe early infantile epileptic encephalopathy than typical SCN1A Dravet syndrome[37]. This variant has been shown to have some gain-of-function effects, resulting in cells that are no longer able to fire action potentials due to accumulation of channels in inactivated states. Subsequently a mixed effect is observed where in some conditions the currents can be larger, however ultimately leading to a loss of neuronal activity[38,39].

By contrast, the majority of SCN2A/3A/8A-associated early-onset epilepsies including benign epilepsies and epileptic encephalopathies are caused by GoF missense variants and full gene duplications. The PTVs in SCN2A/3A/8A do not lead to a clinically defined epilepsy syndrome but to heterogeneous NDDs including autism with or without later onset seizures[15,17,22,26,40]. Moreover, in the SCNs CNV cohort, we observed that patients with duplications presented with significantly earlier seizure onset and responded better to sodium channel blockers compared to patients with deletions. This early seizure onset is likely caused by duplication of the SCN2A/SCN3A genes, which are the earliest SCNs expressed during development, resulting in GoF effects due to SCN2A/SCN3A protein overexpression[41].

Variant effects across different channel subtypes

Our direct comparison of corresponding SCN-variants across different sodium channel subtypes illustrates that the functional effects of variants at conserved channel locations are similar, however their clinical manifestation differs, which is consistent with the channels playing different roles in different types of neurons. For example, a similar functional effect, such as LoF due to a variant in SCN1A at a specific location will lead to DS, likely due to disruption of inhibitory neurons. However, a variant in SCN2A at the same location, displaying the same LoF function effect, leads to NDD/ASD, likely due to changes in excitatory neurons. Only very few GoF variants are seen in SCN1A presenting with milder FHM phenotypes[10] suggesting that GoF may be better tolerated in inhibitory networks compared to excitatory networks, where they lead to severe DEE. Our findings suggest that functional measurements that are recorded in a specific SCN-variant may serve as a valuable surrogate for the function of a corresponding variant at the same position across different SCN-subtypes where subtype-specific functional data are not available.

Comparing the distribution of disease-associated missense variants among the different SCN-subtypes revealed that whilst variants are mainly clustered in homologous domains (particularly the voltage sensing and pore regions), there is a difference in distribution between SCN1A and SCN2A/8A. Epilepsy-associated SCN1A variants are frequently seen in the S5-6 intervening pore loop that is vital for channel function, whereas only very few SCN2/8A variants are observed in this region. Voltage gated sodium channels have a central pore surrounded by four pore-forming modules composed of S5 and S6 segments and an intervening S5-6 pore loop. This loop forms a large extracellular funnel with an ion selectivity filter vital to control ion selectivity[42]. Almost all variants reported in this region lead to LoF, underscoring its functional significance. Previously we were able to show that Dravet syndrome-associated missense variants in SCN1A cluster in the S5-6 pore loop region in keeping with LoF being the key mechanism in SCN1A variants[2]. This is different for SCN2A and SCN8A variants, which frequently present with both GoF and LoF properties. This split between GoF and LoF effects is also seen in the cardiac sodium channel SCN5A where GoF variants cause LQT3 and LoF variants Brugada syndrome. Loss-of-function Brugada syndrome variants are mainly observed in the S5-6 pore loop, whilst no pore loop variants are seen in gain-of-function LQT3 carriers[43]. We observe the same effect in SCN2A/8A, where variants in the S5-6 pore loop region appear to be mainly LoF, implying that variants in this region often lead to LoF across different SCN[44] .Sodium channel blockers are unlikely to be effective in patients with LoF variants in this region. Contrary to previous work, we observe that variants in the S4 region are not associated with one predominant effect, but a range of LoF, mixed and GoF effects, suggesting that function is determined by the individual variant change, rather than a particular S4 region effect[44].

Age-specific expression of sodium channels

In human fetal brains, SCN1A is expressed at a lower level compared to SCN2A/3A/8A, and steadily increases throughout childhood into adult life[45,46]. This differential gene expression profile is mirrored in the phenotypical seizure presentation, as the earliest seizure onset is observed in patients carrying variants in SCN2A (and SCN3A), followed by SCN8A and SCN1A respectively (figure 1). SCN1A is predominantly expressed in inhibitory neurons, whereas, SCN2A/3A/8A are predominantly expressed in excitatory neurons. However, iPSC-work has shown that increased excitability of principal neurons equally contributes to network hyperexcitability in DS[47]. The distinct developmental- and neuronal type-specific expression of SCN1A may explain the

phenotypic differences and variations in drug response with exacerbation of seizures in DS patients due to SCB therapy[8,15,18,48].

Epilepsy patients with distinct types of SCN2A variants present with seizures at different ages: those with GoF missense variants usually present within the first two months after birth, whereas those with LoF missense variants present on average nine months later. Those with PTVs exhibit seizures typically after 3 years[15,16]. Furthermore, CNV duplications covering SCN2A are associated with neonatal onset seizures. This mirrors Allen Brain Atlas data illustrating that SCN2A is highly expressed in the prenatal stage, in particular at mid/late fetal-neonatal stage. We observed 2 distinct peaks of presentation among patients with SCN2A missense variants: those presenting early-on (<3 months) with GoF variants and those presenting later with LoF variants. Contributing to the different ages of onset and clinical symptoms may be the two different developmental expression patterns of $Na_V1.2$ channels in myelinated and unmyelinated nerve fibers[15,49,50]. Recent work showed that early infantile epilepsy patients carrying SCN2A GoF missense variants responded well to SCBs, compared to late-onset patients carrying LoF variants[15,18]. Thus, taken together, the association between SCN2A and early seizure onset can be mostly explained by the early developmental expression of SCN2A and elevated channel function due to GoF variants and duplications.

SCN2A/8A expression correlations

Patients with SCN8A missense variants have later onset seizures compared to SCN2A carriers in keeping with work by Liao et al. demonstrating that $Na_V1.2$ is expressed early in axon initial segments of excitatory neurons while $Na_V1.6$ is not expressed early on but becomes the predominant excitatory channel during development[49]. Moreover, an in vivo study identified that $Na_V1.2$ channels could replace missing $Na_V1.6$ channels at nodes of Ranvier and axon initial segments of neurons in SCN8A knockout mice[51]. This SCN2A/8A co-expression might offer a reciprocal rescue mechanism for both, SCN2A and SCN8A variants and is clinically reflected in the good response of both epilepsies to SCBs, particularly for those presenting with early onset GoF[8]. Taken together, the correlated expression profiles and phenotypic similarities suggest that $Na_V1.2$ and $Na_V1.6$ appear to compensate partially upon the disruption in either SCN2A or SCN8A function.

SCN constraint analysis aids variant interpretation

Our results show that the marked evolutionary constraint among SCN-genes suggests variants identified in SCN1A/2A/3A/8A are intolerant of both truncating and missense variants and more likely to be associated with dominant early-onset de novo disorders such as severe epilepsy and NDDs. SCN5A is intolerant of LoF variants, and is associated with life threatening Brugada syndrome[47]. By comparison, variants in familial SCN disease such as SCN4A periodic paralysis/myotonia or SCN9/10/11A related pain disorders are better tolerated for both truncating and missense variants (figure 4)[43]. Our analysis further supports the emerging evidence that SCN3A, which shows strong depletion for PTV and missense variants, is a good candidate gene for epilepsy even though only a few patients have been reported to date[27,28].

Additionally, the variant constraint results indicate that, besides SCN1A/2A/3A/8A, other members of the SCN-gene family are unlikely to be associated with severe epilepsy/NDDs. For example, SCN9A has an established role in familial pain disorders[43], however, its pathogenicity in severe forms of epilepsy has never been confirmed. Using variants in >60,000 individuals from the general population, we observed that SCN9A variant numbers were similar to variant numbers expected by chance. This suggests variants in SCN9A are less likely to contribute to severe epilepsy compared to variants in SCN1A/2A/3A/8A. Therefore, in clinical practice, constraint analysis could aid interpretation of SCN-variants in diseases, which are under negative natural selection.

Clinical relevance and implications for precision medicine

We observe common patterns across different SCN-related disorders revealing a framework for genotype-phenotype correlations that is applicable across channel types. This allows us to develop a broader perspective on precision treatment than is available when each individual gene or variant is considered separately, supporting specific recommendations. Patients with SCN1A-positive DS whose epilepsy usually starts with febrile seizures after 3 months, is caused by loss of inhibitory neuronal function and responds well to benzodiazepines but worsens with SCBs[8,52]. Among SCN2A variant carriers the responsivess to medication appears to be more complex and directly linked to variant function. Those with early onset seizures (<3 months) due to GoF effects appear to respond well to SCBs whereas those with later onset epilepsy and NDDs due to LoF variants often remain treatment resistant[15–18,40]. There are only limited reports on pathogenic SCN3A variants, however most of these present within the first days of life due to GoF effects and there is evidence to show that mutant channels may respond to SCBs[28]. Recent case series of patients with SCN8A variants clearly demonstrate how variants associated with NDDs showed LoF effects, whereas those associated with epilepsy showed GoF effects with good response to SCBs[19,24,26,53].

This study presents clinical and experimental evidence that GoF SCN2/3/8A variants and copy number duplications respond well to sodium channel blockage. We can show that the likelihood of an SCN2A or SCN8A variant being GoF is particularly high in very young children <3 months of age (88% and 100% respectively) and SCB treatment is recommended in infants where an SCN2A or SCN8A variant has been confirmed.

We would argue that our data support that once emergency AED management and imaging/metabolic tests have been completed in a young child presenting with seizures in the first 3 months of life, and a genetic diagnosis seems likely, there is a rationale to consider SCB treatment. At this early stage genetic testing results are often not yet available and may take weeks and months to conclude. However, there is robust population and cohort based evidence showing that the genetic epilepsies commonly presenting at this early age (<3 months) are KCNQ2, KCNQ3, CDKL5, SCN2A and STXBP1, but not SCN1A[54,55]. These young infants will in the majority of cases respond to SCBs without the expectation for seizures to worsen when SCBs are given. The theoretical risk of seizure exacerbation due to SCBs is comparatively low, because we show how unlikely SCN1A variants are to present at this young age. Neverthesless, clinicians should remain vigilant and switch drugs at the first signs of seizure aggravation following SCB administration.

We suggest that in those patients with a suspected underlying genetic cause presenting with neonatal or very early onset seizures (<3 months) SCBs should be considered, whereas in later onset epilepsy SCBs appear mainly effective in SCN8A related disease and are contraindicated in Dravet syndrome.

Abbreviations

DS: Dravet Syndrome
GoF: Gain-of-function
LoF: Loss-of-function
CNV: Copy Number Variation
NDDs: Neurodevelopmental Disorders
PTVs: Protein Truncating Variants
SCBs: Sodium Channel Blockers
SCN/Na_V: Sodium Channel
EOEE: Early onset epileptic encephalopathy
EIMFS: Epilepsy of infancy with migrating focal seizures
OS: Ohtahara syndrome
WS: West syndrome
LGS: Lennox-Gastaut syndrome
AED: Antiepileptic drug

Acknowledgements

We thank all the clinicians, patients and their families. This study makes use of data generated by the DECIPHER community. A full list of centers who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. The Wellcome Trust provided funding for the project.

Confilicts of Interest

Nothing to report.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Study Funding

J.Du was supported by the Koeln Fortune grant number 241/2017.

References

1. Lal D, May P, Samocha KE, Kosmicki JA, Robinson EB, Møller RS, et al. Gene family information facilitates variant interpretation and identification of disease-associated genes. bioRxiv. 2017;159780.
2. Zuberi SM, Brunklaus A, Birch R, Reavey E, Duncan J, Forbes GH. Genotype-phenotype associations in SCN1A-related epilepsies. Neurology. 2011;76:594–600.
3. Claes LR, Deprez L, Suls A, Baets J, Smets K, Van Dyck T, et al. The SCN1A variant database: a novel research and diagnostic tool. Hum Mutat. 2009;30:E904–20.
4. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. Nat Genet. 2000;24:343–5.
5. Marini C, Scheffer IE, Nabbout R, Mei D, Cox K, Dibbens LM, et al. SCN1A duplications and deletions detected in Dravet syndrome: Implications for molecular diagnosis. Epilepsia. 2009;50:1670–8.
6. Davidsson J, Collin A, Olsson ME, Lundgren J, Soller M. Deletion of the SCN gene cluster on 2q24.4 is associated with severe epilepsy: An array-based genotype–phenotype correlation and a comprehensive review of previously published cases. Epilepsy Res. 2008;81:69–79.
7. Wang J, Kurahashi H, Ishii A, Kojima T, Ohfu M, Inoue T, et al. Microchromosomal deletions involving SCN1A and adjacent genes in severe myoclonic epilepsy in infancy. Epilepsia. 2008;49:1528–34.
8. Brunklaus A, Ellis R, Reavey E, Forbes GH, Zuberi SM. Prognostic, clinical and demographic features in SCN1A mutation-positive Dravet syndrome. Brain. 2012;135:2329–36.
9. Dravet C. Les epilepsies graves de l'enfant. Vie Med. 1978;8:543–8.
10. Mantegazza M, Cestèle S. Pathophysiological mechanisms of migraine and epilepsy: Similarities and differences. Neurosci Lett. 2018;667:92–102.
11. Ogiwara I, Ito K, Sawaishi Y, Osaka H, Mazaki E, Inoue I, et al. De novo mutations of voltage-gated sodium channel αII gene SCN2A in intractable epilepsies. Neurology. 2009;73:1046–53.
12. Heron SE, Crossland KM, Andermann E, Phillips HA, Hall AJ, Bleasel A, et al. Sodium-channel defects in benign familial neonatal-infantile seizures. Lancet. 2002;360:851–2.
13. Nakamura K, Kato M, Osaka H, Yamashita S, Nakagawa E, Haginoya K, et al. Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome. Neurology. 2013;81:992–8.
14. Ben-Shalom R, Keeshen CM, Berrios KN, An JY, Sanders SJ, Bender KJ. Opposing Effects on NaV 1.2 Function Underlie Differences Between SCN2A Variants Observed in Individuals With Autism Spectrum Disorder or Infantile Seizures. Biol Psychiatry. 2017;82:224–32.
15. Wolff M, Johannesen KM, Hedrich UBS, Masnada S, Rubboli G, Gardella E, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. Brain. 2017;140:1316–36.
16. Lauxmann S, Verbeek NE, Liu Y, Zaichuk M, Müller S, Lemke JR, et al. Relationship of

electrophysiological dysfunction and clinical severity in SCN2A-related epilepsies. Hum Mutat. 2018;39:1942–56.

17. Begemann A, Acuña MA, Zweier M, Vincent M, Steindl K, Bachmann-Gagescu R, et al. Further corroboration of distinct functional features in SCN2A variants causing intellectual disability or epileptic phenotypes. Mol Med. 2019;25:6.
18. Berecki G, Howell KB, Deerasooriya YH, Cilio MR, Oliva MK, Kaplan D, et al. Dynamic action potential clamp predicts functional separation in mild familial and severe de novo forms of SCN2A epilepsy. Proc Natl Acad Sci. 2018;115:E5516–25.
19. Larsen J, Carvill GL, Gardella E, Kluger G, Schmiedel G, Barisic N, et al. The phenotypic spectrum of SCN8A encephalopathy. Neurology. 2015;84:480–9.
20. Meisler MH, Helman G, Hammer MF, Fureman BE, Gaillard WD, Goldin AL, et al. SCN8A encephalopathy: Research progress and prospects. Epilepsia. 2016;57:1027–35.
21. Veeramah KR, O'Brien JE, Meisler MH, Cheng X, Dib-Hajj SD, Waxman SG, et al. De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. Am J Hum Genet. 2012;90:502–10.
22. Gardella E, Marini C, Trivisano M, Fitzgerald MP, Alber M, Howell KB, et al. The phenotype of SCN8A developmental and epileptic encephalopathy. Neurology. 2018;91:e1112–24.
23. Gardella E, Becker F, Møller RS, Schubert J, Lemke JR, Larsen LHG, et al. Benign infantile seizures and paroxysmal dyskinesia caused by an SCN8A mutation. Ann Neurol. 2016;79:428–36.
24. Wagnon JL, Barker BS, Ottolini M, Park Y, Volkheimer A, Valdez P, et al. Loss-of-function variants of SCN8A in intellectual disability without seizures. Neurol Genet. 2017;3:1–5.
25. Wagnon JL, Barker BS, Hounshell JA, Haaxma CA, Shealy A, Moss T, et al. Pathogenic mechanism of recurrent mutations of SCN8A in epileptic encephalopathy. Ann Clin Transl Neurol. 2015;3:114–23.
26. Liu Y, Schubert J, Sonnenberg L, Helbig KL, Hoei-Hansen CE, Koko M, et al. Neuronal mechanisms of mutations in SCN8A causing epilepsy or intellectual disability. Brain. 2019;142:376–90.
27. Lamar T, Vanoye CG, Calhoun J, Wong JC, Dutton SBB, Jorge BS, et al. SCN3A deficiency associated with increased seizure susceptibility. Neurobiol Dis. 2017;102:38–48.
28. Zaman T, Helbig I, Božović IB, DeBrosse SD, Bergqvist AC, Wallis K, et al. Mutations in SCN3A cause early infantile epileptic encephalopathy. Ann Neurol. 2018;83:703–17.
29. Møller RS, Larsen LHG, Johannesen KM, Talvik I, Talvik T, Vaher U, et al. Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies. Mol Syndromol. 2016;7:210–9.
30. Gardella E, Møller R. Phenotypic and genetic spectrum of SCN8A- related disorders, treatment options, and outcomes. Epilepsia (in print).
31. Johannesen KM, Gardella E, Encinas AC, Lehesjoki A, Linnankivi T, Petersen MB, et al. The spectrum of intermediate SCN8A- related epilepsy. Epilepsia. 2019;60:epi.14705.
32. Depienne C, Trouillard O, Saint-Martin C, Gourfinkel-An I, Bouteiller D, Carpentier W, et al. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333

patients. J Med Genet. 2009;46:183–91.

33. Firth H V., Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, et al. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet. 2009;84:524–33.
34. Lek M, Karczewski KJ, Minikel E V., Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285–91.
35. Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and Structure-Function Relationships of Voltage-Gated Sodium Channels. Pharmacol Rev. 2005;57:397–409.
36. Cetica V, Chiari S, Mei D, Parrini E, Grisotto L, Marini C, et al. Clinical and genetic factors predicting Dravet syndrome in infants with SCN1A mutations. Neurology. 2017;88:1037–44.
37. Sadleir LG, Mountier EI, Gill D, Davis S, Joshi C, DeVile C, et al. Not all SCN1A epileptic encephalopathies are Dravet syndrome. Neurology. 2017;89:1035–42.
38. Berecki G, Bryson A, Terhag J, Maljevic S, Gazina E V., Hill SL, et al. SCN1A gain of function in early infantile encephalopathy. Ann Neurol. 2019;85:514–25.
39. Beck VC, Hull JM, Isom LL. Beyond Dravet Syndrome: Characterization of a Novel, More Severe SCN1A-Linked Epileptic Encephalopathy. Epilepsy Curr. 2019;19:266–8.
40. Sanders SJ, Campbell AJ, Cottrell JR, Moller RS, Wagner FF, Auldridge AL, et al. Progress in Understanding and Treating SCN2A -Mediated Disorders. Trends Neurosci. 2018;41:442–56.
41. Thuresson A-C, Van Buggenhout G, Sheth F, Kamate M, Andrieux J, Clayton Smith J, et al. Whole gene duplication of SCN2A and SCN3A is associated with neonatal seizures and a normal intellectual development. Clin Genet. 2017;91:106–10.
42. Catterall WA. Voltage-gated sodium channels at 60: structure, function and pathophysiology. J Physiol. 2012;590:2577–89.
43. Brunklaus A, Ellis R, Reavey E, Semsarian C, Zuberi SM. Genotype phenotype associations across the voltage-gated sodium channel family. J Med Genet. 2014;51:650–8.
44. Holland KD, Bouley TM, Horn PS. Location: A surrogate for personalized treatment of sodium channelopathies. Ann Neurol. 2018;84:1–9.
45. Cheah CS, Westenbroek RE, Roden WH, Kalume F, Oakley JC, Jansen LA, et al. Correlations in timing of sodium channel expression, epilepsy, and sudden death in Dravet syndrome. Channels. 2013;7:468–72.
46. Biella G, Di Febo F, Goffredo D, Moiana A, Taglietti V, Conti L, et al. Differentiating embryonic stem–derived neural stem cells show a maturation-dependent pattern of voltage-gated sodium current expression and graded action potentials. Neuroscience. 2007;149:38–52.
47. Liu Y, Lopez-Santiago LF, Yuan Y, Jones JM, Zhang H, O'Malley HA, et al. Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism. Ann Neurol. 2013;74:128–39.
48. Escayg A, Goldin AL. Sodium channel SCN1A and epilepsy: Mutations and mechanisms. Epilepsia. 2010;51:1650–8.
49. Liao Y, Deprez L, Maljevic S, Pitsch J, Claes L, Hristova D, et al. Molecular correlates of age-dependent seizures in an inherited neonatal-infantile epilepsy. Brain. 2010;133:1403–14.

50. Liao Y, Anttonen A-K, Liukkonen E, Gaily E, Maljevic S, Schubert S, et al. SCN2A mutation associated with neonatal epilepsy, late-onset episodic ataxia, myoclonus, and pain. Neurology. 2010;75:1454–8.
51. Van Wart A, Matthews G. Impaired Firing and Cell-Specific Compensation in Neurons Lacking Nav1.6 Sodium Channels. J Neurosci. 2006;26:7172–80.
52. Guerrini R, Dravet C, Genton P, Belmonte A, Kaminska A, Dulac O. Lamotrigine and seizure aggravation in severe myoclonic epilepsy. Epilepsia. 1998;39:508–12.
53. Møller RS, Johannesen KM. Precision Medicine: SCN8A Encephalopathy Treated with Sodium Channel Blockers. Neurotherapeutics. 2016;13:190–1.
54. Symonds JD, Zuberi SM, Stewart K, McLellan A, O'Regan M, MacLeod S, et al. Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort. Brain. 2019;142:2303–18.
55. Olson HE, Kelly M, LaCoursiere CM, Pinsky R, Tambunan D, Shain C, et al. Genetics and genotype-phenotype correlations in early onset epileptic encephalopathy with burst suppression. Ann Neurol. 2017;81:419–29.

Figure Legends

Figure 1 | Age at seizure onset of SCN-variant carriers and associated gene expression strength.

Legend: Seizure onset age scale (y-axis is log10 transformed), PTV = protein truncating variant carriers, Missense = missense variant carriers, CNV del = copy number variant deletion carriers, CNV dup = copy number variant duplication carriers, Number of patients: SCN1A = 504, SCN2A = 140, SCN3A = 4, SCN8A = 171, CNV = 46. Gene expression strength shown by age (timepoints: Preterm, 0-4 months, 10 months-1year, 2-3 years, 4-8 years, >8 years). The larger the circle the stronger the gene expression (Epilepsy-associated SCNs exhibit specific development-dependent gene expression patterns; RNA-seq expression data obtained from Allen Brain Atlas; http://www.brainspan.org/static/download.html).

Figures 2A & B | Summary of electrophysiologically tested SCN1A/2A/3A/8A variants in the literature.

Figure 2A | Frequency of phenotypes according to SCN variants and function.

Legend: EPI = epilepsy, FHM = familial hemiplegic migraine, ASD = autism spectrum disorder, NDD = neurodevelopmental disorder, LoF = loss-of-function, GoF = gain-of-function, Mixed = mixed function (Supplementary table 3 and 5).

Figure 2B | Differential age at seizure onset according to SCN variants and function

Legend: Seizure onset age scale (y-axis is log10 transformed), LoF = loss-of-function, GoF = gain-of-function, Mixed = mixed function. Number of patients: SCN1A = 40, SCN2A = 24, SCN8A = 18 (Supplementary table 3 and 5).

Figure 3 | Comparison of missense variants and function effects across SCN1A/2A/8A.

Legend: Identical/corresponding variant pairs across different SCNs are highlighted (as per table 1; the corresponding sequence numbers are not identical as the amino acid sequence between SCN1A/2A/8A variants differs slightly), LoF = loss-of-function, GoF = gain-of-function, DS = Dravet syndrome, FHM3 = familial hemiplegic migraine type 3, GEFS+ = genetic epilepsy with febrile seizures plus, DEE = developmental and epileptic encephalopathy, ASD = autism spectrum disorder, NDD = neurodevelopmental disorder. Variants marked in black represent missense variants from Glasgow (SCN1A, n=132) and Danish cohorts (SCN2A, n=127 and SCN8A, n=164) respectively. Functionally tested variants are presented in coulour: red = loss-of-function (LoF), green = gain-of-function (GoF), orange = mixed function.

Figure 4 | Variant constraints of SCNs.

Constraint missense Z-scores and pLI scores for SCN genes in the general population (60, 000 individuals in ExAC database). High missense Z-scores (>3.09, x-axis) suggest that genes are intolerant of missense variants. High pLI scores (> 0.9, y-axis) suggest that genes are intolerant for protein-truncating variants. The missense and PTV constrained group contains four epilepsy-associated genes, SCN1A/2A/3A/8A.

Table 1: Corresponding variants, phenotypes and function across different brain sodium channels

Pair	Gene/Variant	Function	Phenotype	Reference	Corresponding Gene/Variant	Function	Phenotype	Reference
1	***SCN1A***; L263V; D1 S5	GoF; WCC: Y, $\uparrow I_{NaP}$, $\leftarrow V_{1/2\ Act.}$, $\rightarrow V_{1/2\ FI}$	FHM3	Kahlig (2008)	***SCN8A***; L267V; D1 S5	Likely* GoF (Phenotype suggestive of GoF)	DEE; Sz onset 2.5 months, Sz reduction with SCBs	Denis (2019)
2	***SCN1A***; R946C; D2 S5-6	LoF; WCC: None	Dravet syndrome	Volkers (2011)	***SCN2A***; R937C; D2 S5-6	LoF; WCC: None	ASD	Begemann (2019)
3	***SCN1A***; R946H; D2 S5-6	LoF; WCC: None	Dravet syndrome	Volkers (2011)	***SCN2A***; R937H; D2 S5-6	LoF; WCC: None	ASD	Ben-Shalom (2017)
4	***SCN1A***; G979R; D2 S6	LoF; WCC: None	Dravet syndrome	Rhodes (2005)	***SCN8A***; G964R; D2 S6	LoF; WCC: None	NDD without epilepsy	Wagnon (2017)
5	***SCN1A***; Q1489K; D3-4 linker	GoF; WCC: Y, $\uparrow I_{NaP}$, $\leftarrow V_{1/2\ Act.}$, no change $V_{1/2\ FI}$	FHM3	Kahlig (2008) Cestèle (2013)	***SCN8A***; Q1470K; D3-4 linker	Likely* GoF (Phenotype suggestive of GoF)	DEE, Sz onset 1 day, Sz free with SCBs	Denis (2019)
6	***SCN1A***; P1632S; D4 S3-4	LoF; WCC: Y, $\leftarrow V_{1/2\ Act.}$, $\leftarrow V_{1/2\ FI}$,	Dravet syndrome	Rhodes (2005)	***SCN2A***; P1622S; D4 S3-4	LoF; WCC: Y, $\leftarrow V_{1/2\ FI}$,	ASD and Sz onset 21 months	Wolff (2017)
7	***SCN1A***; R1657C; D4 S4-5	LoF; WCC: Y, $\downarrow$CD, $\downarrow I_{NaP}$, $\rightarrow V_{1/2\ Act.}$, $\leftarrow V_{1/2\ FI}$	GEFS+	Lossin (2003)	***SCN8A***; R1638C; D4 S4-5	LoF; WCC: Y, $\rightarrow V_{1/2\ Act.}$, no change $V_{1/2\ FI}$	NDD without epilepsy	Wengert (2019)
8	***SCN2A***; R1882Q; C-Term	GoF; WCC: Y, $\uparrow$CD, $\uparrow I_{NaP}$, $\leftarrow V_{1/2\ Act.}$, $\rightarrow V_{1/2\ FI}$	DEE, Sz onset 1 day	Wolff (2017)	***SCN8A***; R1872Q; C-Term	GoF; WCC: Y, $\uparrow$CD, $\leftarrow V_{1/2\ Act.}$, $\rightarrow V_{1/2\ FI}$	DEE, Sz onset 4 months	Wagnon (2015)

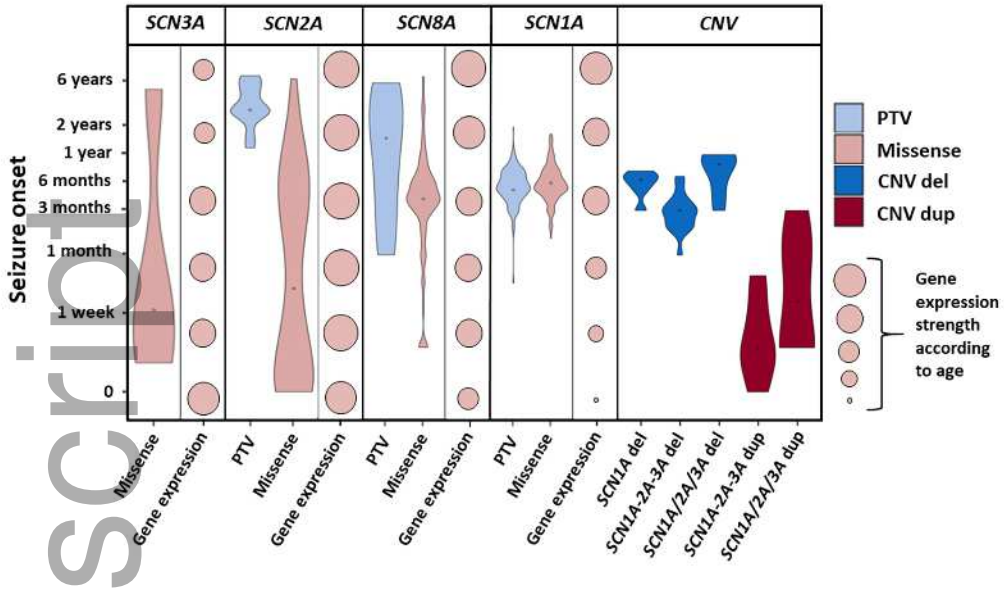
Phenotypical features: FHM3 = familial hemiplegic migraine type 3, GEFS+ = genetic epilepsy with febrile seizures plus, DEE = developmental and epileptic encephalopathy, ASD = autism spectrum disorder, NDD = neurodevelopmental disorder, Sz = seizure, SCB = sodium channel blocker

Corresponding variant = variant among different *SCN* at the same position/location in the *SCN* protein. The corresponding sequence numbers are not identical as the amino acid sequence between *SCN1A/2A/8A* variants differs slightly.

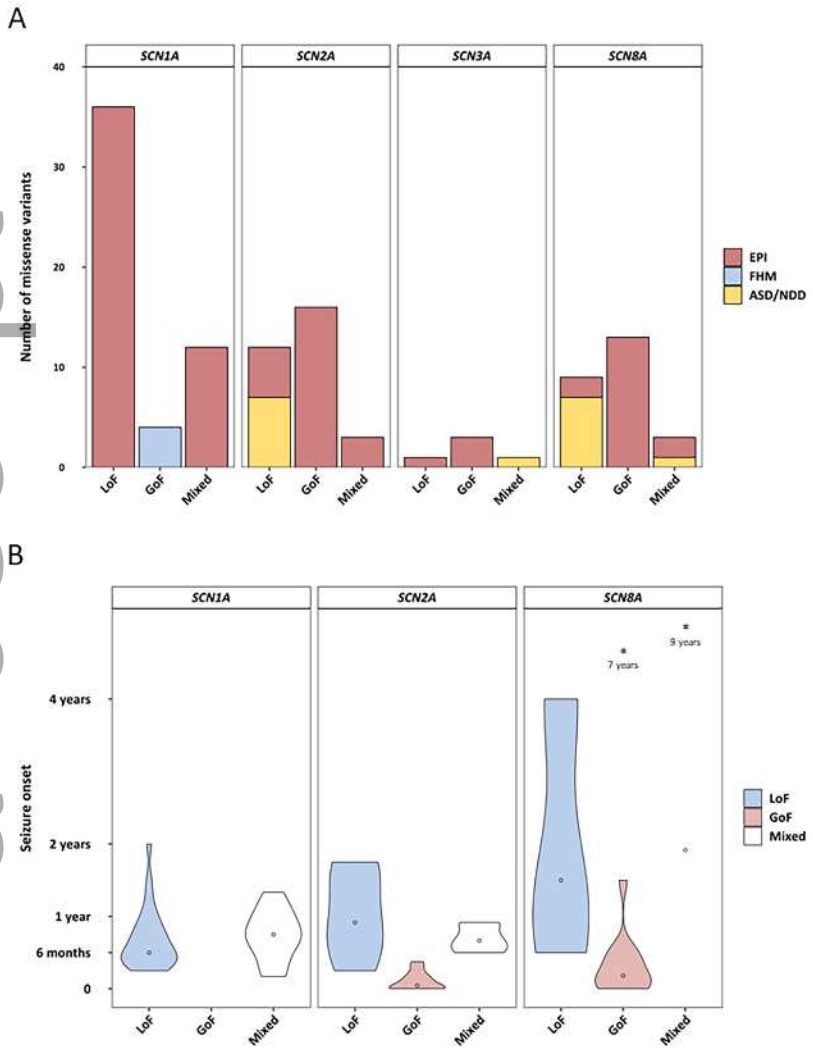
Electrophysiological key features: Arrows (→) are used for electrophysiological parameters. The direction of the arrows indicate hyperpolarizing (←) or depolarizing shifts (→), as well as an increase (↑) or decrease (↓) of parameters, (↓↓ = >50% decrease)

Electrophysiological abbreviations: GoF: gain-of-function, LoF: loss-of-function, WCC: whole cell current (Y = measurable, N = not measurable), Act: activation, CD: current density, FI: fast inactivation, I_{NaP}: persistent sodium current, $V_{1/2\ Act.}$: half-activation of steady-state activation curve, $V_{1/2\ FI}$: half-inactivation of steady-state fast inactivation curve

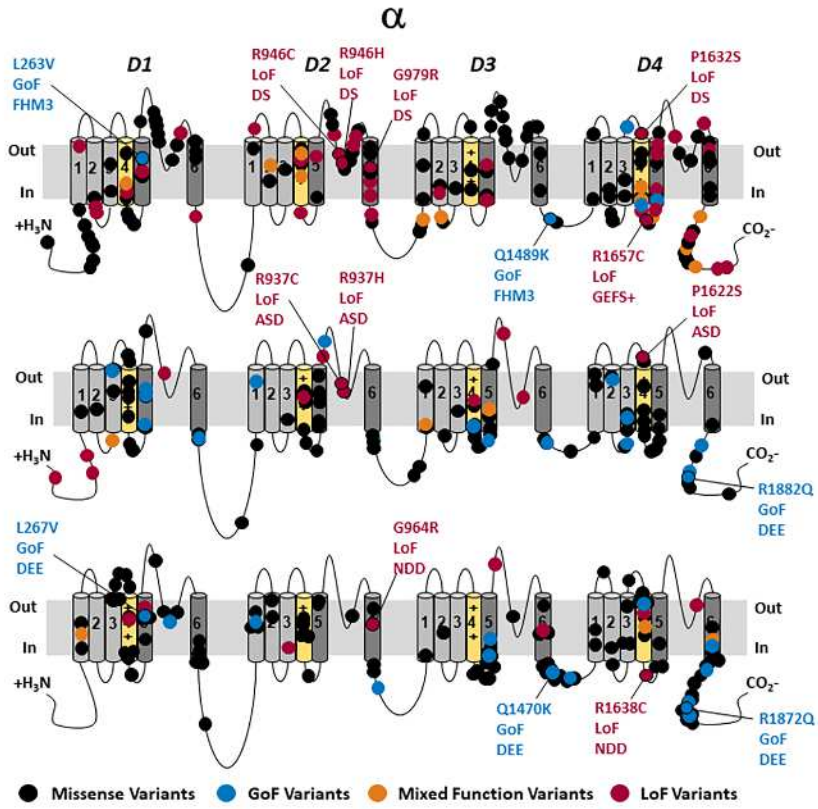
*No functional data were available for the 2 corresponding *SCN8A* variants in pairs 1 and 5, however the described *SCN8A* phenotypes and medication response data are highly suggestive of GoF variants.



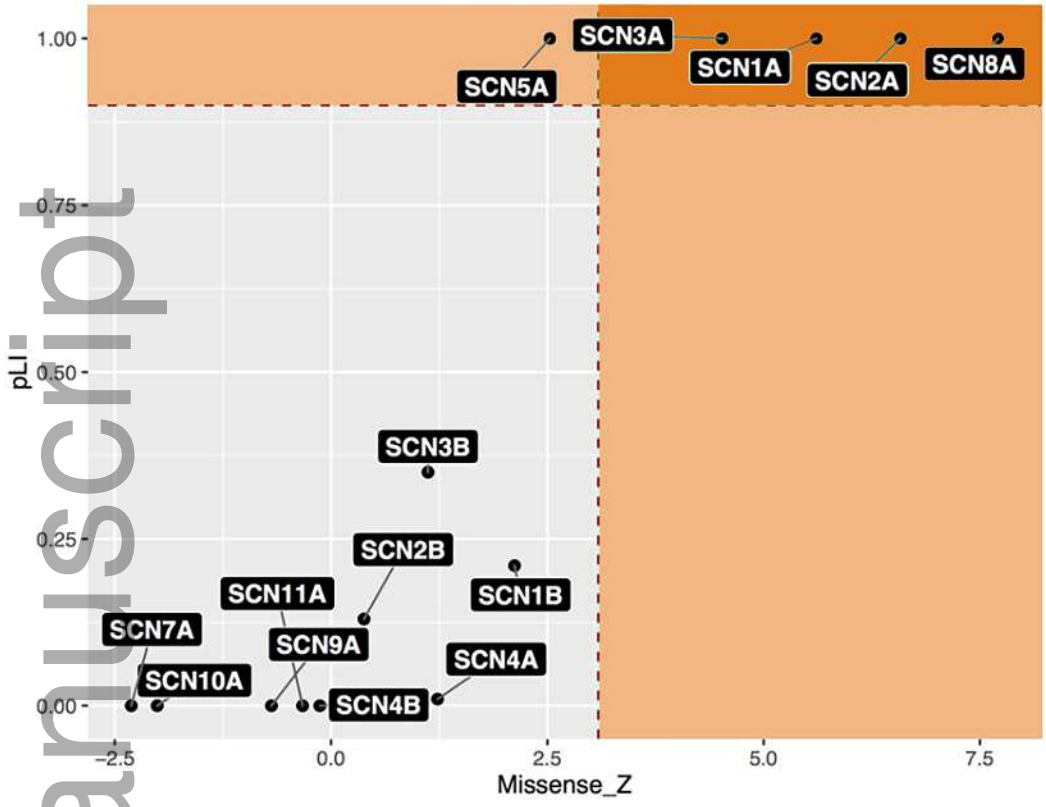
epi_16438_f1.tif



epi_16438_f2.tif



epi_16438_f3.tif



epi_16438_f4.tif