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Reference:
Johannesen Katrine M., Liu Yuanyuan, Koko Mahmoud, Gjerulfsen Cathrine E., Sonnenberg Lukas, Schubert Julian, Fenger Christina D., Eltokhi Ahmed, Rannap Maert, Koch Nils A., ...- Genotype-phenotype correlations in SCN8A-related disorders reveal prognostic and therapeutic implications
Brain - ISSN 0006-8950 - (2021), awab321
Full text (Publisher's DOI): https://doi.org/10.1093/BRAIN/AWAB321

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Genotype-phenotype correlations in *SCN8A*-related disorders reveal prognostic and therapeutic implications

Katrine M. Johannesen,[1,2,†] Yuanyuan Liu,[3,†] Mahmoud Koko,[3] Cathrine E. Gjerulfsen,[1] Lukas Sonnenberg,[3,4] Julian Schubert,[3] Christina D. Fenger,[1] Ahmed Eltokhi,[3] Maert Rannap,[3] Nils A. Koch,[4] Stephan Lauxmann,[3,4] Johanna Krüger,[3] Josua Kegele,[3] Laura Canafoglia,[5] Silvana Franceschetti,[5] Thomas Mayer,[6] Johannes Rebstock,[6] Pia Zacher,[6] Susanne Ruf,[7] Michael Alber,[7] Katalin Sterbova,[8] Petra Lassuthová,[8] Marketa Vlckova,[8] Johannes R. Lemke,[9] Konrad Platzer,[9] Ilona Krey,[9] Constanze Heine,[9] Dagmar Wieczorek,[10] Judith Kroell-Seger,[11] Caroline Lund,[12] Karl Martin Klein,[13] P. Y. Billie Au,[14] Jong M. Rho,[15] Alice W. Ho,[15] Silvia Masnada,[16] Pierangelo Veggiotti,[16,17] Lucio Giordano,[18] Patrizia Accorsi,[18] Christina E. Hoei-Hansen,[19,20] Pasquale Striano,[21,22] Federico Zara,[22] Helene Verhelst,[23] Judith S. Verhoeven,[24] Bert van der Zwaag,[25] Aster V. E. Harder,[25] Eva Brilstra,[25] Manuela Pendziwiat,[26] Sebastian Lebon,[27,28] Maria Vaccarezza,[29] Ngoc Minh Le,[30] Jakob Christensen,[31] Sabine Grønborg,[32] Stephen W. Scherer,[33,34] Jennifer Howe,[34] Walid Fazeli,[35,36] Katherine B. Howell,[37,38,39] Richard Leventer,[37,38,39] Chloe Stutterd,[38,39] Sonja Walsh,[40] Marion Gerard,[41] Bénédicte Gerard,[42] Sara Matricardi,[43] Claudia M. Bonardi,[44] Stefano Sartori,[45] Andrea Berger,[46] Dorota Hoffman-Zacharska,[47] Massimo Mastrangelo,[48] Francesca Darra,[49] Arve Vøllo,[50] M. Mahdi Motazacker,[51] Phillis Lakeman,[52] Mathilde Nizon,[53] Cornelia Betzler,[54,55] Cecilia Altuzarra,[56] Roseline Caume,[57] Agathe Roubertie,[58] Philippe Gélisse,[58] Carla Marini,[59] Renzo Guerrini,[60] Frederic Bilan,[61] Daniel Tibussek,[62] Margarete Koch-Hogrebe,[63] M. Scott Perry,[64] Shoji Ichikawa,[65] Elena Dadali,[66,67] Artem Sharkov,[67,68] Irina Mishina,[66] Mikhail Abramov,[67] Ilya Kanivets,[69,70] Sergey Korostelev,[69,71] Sergey Kutsev,[66] Karen E. Wain,[72] Nancy Eisenhauer,[72] Monisa Wagner,[72] Juliann M. Savatt,[72] Karen Müller-Schlüter,[73] Haim Bassan,[74,75] Artem Borovikov,[66] Marie-Cecile Nassogne,[76] Anne Destrée,[77] An-Sofie Schoonjans,[78] Marije Meuwissen,[78] Marga Buzatu,[79] Anna Jansen,[80] Emmanuel Scalais,[81] Siddharth Srivastava,[82] Wen-Hann Tan,[83] Heather E. Olson,[82,84] Tobias Loddenkemper,[82] Annapurna Poduri,[82,84] Katherine L. Helbig,[85,86] Ingo Helbig,[85,86,87,88,89,90] Mark P. Fitzgerald,[85,86,87,89] Ethan M. Goldberg,[85,86] Timo Roser,[91] Ingo Borggraefe,[91,92] Tobias Brünger,[93] Patrick May,[94] Dennis Lal,[93,95,96,97] Damien Lederer,[77] Guido Rubboli,[1,98] Henrike O. Heyne,[9,99,100,101] Gaetan Lesca,[102,103] Ulrike B. S. Hedrich,[3] Jan Benda,[4] Elena Gardella,[1,2] Holger Lerche[3,‡] and Rikke S. Møller[1,2,‡]

[†,‡]**These authors contributed equally to this work.**

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Abstract:

We report detailed functional analyses and genotype-phenotype correlations in 392 individuals carrying disease-causing variants in *SCN8A*, encoding the voltage-gated Na^+ channel $Na_V1.6$, with the aim of describing clinical phenotypes related to functional effects. Six different clinical subgroups could be identified: 1) Benign familial infantile epilepsy (BFIE) (n=15, normal cognition, treatable seizures), 2) intermediate epilepsy (n=33, mild ID, partially pharmaco-responsive), 3) developmental and epileptic encephalopathy (DEE, n=177, severe ID, majority pharmaco-resistant), 4) generalized epilepsy (n=20, mild to moderate ID, frequently with absence seizures), 5) unclassifiable epilepsy (n=127), and 6) neurodevelopmental disorder without epilepsy (n=20, mild to moderate ID). Groups 1-3 presented with focal or multifocal seizures (median age of onset: four months) and focal epileptiform discharges, whereas the onset of seizures in group 4 was later (median: 42 months) with generalized epileptiform discharges. We performed functional studies expressing missense variants in ND7/23 neuroblastoma cells and primary neuronal cultures using recombinant tetrodotoxin-insensitive human $Na_V1.6$ channels and whole-cell patch-clamping. Two variants causing DEE showed a strong gain-of-function (GOF, hyperpolarising shift of steady-state activation, strongly increased neuronal firing rate), and one variant causing BFIE or intermediate epilepsy showed a mild GOF (defective fast inactivation, less increased firing). In contrast, all three variants causing generalized epilepsy induced a loss-of-function (LOF, reduced current amplitudes, depolarising shift of steady-state activation, reduced neuronal firing). Including previous studies, functional effects were known for 170 individuals. All 136 individuals carrying a functionally tested GOF variant had either focal (97, groups 1-3), or unclassifiable epilepsy (39), whereas 34 with a LOF variant had either generalized (14), no (11) or unclassifiable (6) epilepsy; only three had DEE. Computational modeling in the GOF group revealed a significant correlation between the severity of the electrophysiological and clinical phenotypes. GOF variant carriers responded significantly better to sodium channel blockers (SCBs) than to other anti-seizure medications, and the same applied for all individuals of groups 1-3.

In conclusion, our data reveal clear genotype-phenotype correlations between age at seizure onset, type of epilepsy and gain- or loss-of-function effects of *SCN8A* variants. Generalized epilepsy with absence seizures is the main epilepsy phenotype of LOF variant carriers and the extent of the electrophysiological dysfunction of the GOF variants is a main determinant of the severity of the clinical phenotype in focal epilepsies. Our pharmacological data indicate that SCBs present a treatment option in *SCN8A*-related focal epilepsy with onset in the first year of life.

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Author affiliations:

1 Department of Epilepsy Genetics and Personalized Treatment, the Danish Epilepsy Center, 4293 Dianalund, Denmark

2 Institute for Regional Health Services, University of Southern Denmark, 5230 Odense, Denmark

3 Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tuebingen, 72072 Tuebingen, Germany

4 Institute for Neurobiology, University of Tuebingen, 72072 Tuebingen, Germany

5 Department of Diagnostics and Technology, Fondazione IRCCS Istituto Neurologio Carlo Besta, 20125 Milan, Italy

6 Epilepsy Center Kleinwachau, 01454 Dresden-Radeberg, Germany

7 Department of Pediatric Neurology and Developmental Medicine, University Children's Hospital, 72072 Tuebingen, Germany

8 Department of Child Neurology, Charles University, 2nd Faculty of Medicine and University Hospital Motol, 10000 Prague, Czech Republic

9 Institute of Human Genetics, University of Leipzig Hospitals and Clinics, 4275 Leipzig, Germany

10 Institute of Human Genetics, University Clinic, Heinrich-Heine-University, 40210 Düsseldorf, Germany

11 Children's department, Swiss Epilepsy Centre, Clinic Lengg, 8001 Zurich, Switzerland

12 National Centre for Rare Epilepsy-Related Disorders, Oslo University Hospital, 0001 Oslo, Norway.

13 Departments of Clinical Neurosciences, Medical Genetics and Community Health Sciences, Hotchkiss Brain Institute & Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, T2P 0A1 Calgary, Canada

14 Department of Medical Genetics, Alberta Children's Hospital Research Institute, University of Calgary, T6G 2T4 Alberta, Canada

15 Section of Pediatric Neurology, Alberta Children's Hospital, Cumming School of Medicine, University of Calgary, T2P 0A1 Calgary, Canada

16 Department of Child Neurology, V. Buzzi Children's Hospital, 20125 Milan, Italy

17 Department of Biomedical and Clinical Sciences, L. Sacco, University of Milan, 20125 Milan, 25100 Italy

18 Child Neuropsychiatric Unit, Civilian Hospital, 25100 Brescia, Italy

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19 Department of Pediatrics, Copenhagen University Hospital, Rigshospitalet, 2200 Copenhagen, Denmark
20 Department of Clinical Medicine, University of Copenhagen, 2200 Copenhagen, Denmark
21 Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, 16121 Genova, Italy
22 IRCCS "G. Gaslini" Institute, 16121 Genoa, Italy
23 Department of Pediatrics, Divisuion of Pediatric Neurology, Gent University Hospital, 9042 Gent, Belgium
24 Academic Center for Epileptology, Kempenhaeghe/Maastricht University Medical Center, 5591 Heeze, The Netherlands
25 Department of Genetics, University Medical Center Utrecht, Utrecht University, 3553 Utrecht, The Netherlands
26 Department of Neuropediatrics, Universitätsklinikum Schleswig Holstein Campus Kiel, 24106 Kiel, Germany
27 Pediatric Neurology and Neurorehabilitation Unit, Woman Mother Child Department, Lausanne University Hospital (CHUV), 1000 Lausanne, Switzerland1
28 University of Lausanne, 1000 Lausanne, Switzerland
29 Department of Pediatric Neurology, Hospital Italiano de Buenos Aires, C1428 Buenos Aires, Argentina
30 Center for Pediatric Neurology, Cleveland Clinic, 44102 Cleveland, Ohio, USA
31 Department of Neurology, Aarhus University Hospital, 8000 Aarhus, Denmark
32 Center for Rare Diseases, Department of Pediatrics and Department of Clinical Genetics, Copenhagen University Hospital Rigshospitalet, 2200 Copenhagen, Denmark
33 McLaughlin Centre and Dept. of Molecular Genetics, University of Toronto, 66777 Toronto, Ontario, Canada
34 The Centre for Applied Genomics and Dept. of Genetics and Genome Biology, The Hospital for Sick Children, 66777 Toronto, Ontario, Canada
35 Department of Neuropediatrics, University Hospital Bonn, 53229 Bonn, Germany
36 Institute for Molecular and Behavioral Neuroscience, University of Cologne, 50667 Cologne, Germany
37 Neurology Department, The Royal Children's Hospital Melbourne, 3002 Melbourne, Australia
38 Murdoch Children's Research Institute, 3052 Parkville, Australia

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39 Department of Pediatrics, University of Melbourne, Royal Children's Hospital, 3052 Parkville, Australia
40 Department of Neuropediatrics, Children's hospital, University Hospital Carl Gustav Carus, Technical University, 1099 Dresden, Germany
41 Genetic Department, CHU Côte de Nacre, 14118 Caen, France
42 Genetic Department, CHRU Strasbourg, 67000 Strasbourg, France
43 Child Neurology and Psychiatry Unit, Children's Hospital G. Salesi, 60121 Ancona, Italy
44 Department of Woman's and Child's Health, Padova University Hospital, 35100 Padova, Italy
45 Child Neurology and Clinical Neurophysiology Unit, Padova University Hospital, 35100 Padova, Italy
46 Department of Neuropediatrics, Klinikum Weiden, Kliniken Nordoberpfalz AG, 92637 Weiden, Germany
47 Department of Medical Genetics, Institute of Mother and Child, 00-034 Warsaw, Poland
48 Pediatric Neurology Unit, Vittore Buzzi Hospital, ASST Fatebenefratelli Sacco, 20100 Milan, Italy
49 Department of Surgical Sciences, Dentistry, Gynecology and Pediatrics, University of 37121 Verona, Italy
50 Department of Pediatrics, Oestfold Hospital, 1712 Graalum, Norway
51 Laboratory of Genome Diagnostics, Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, 1019 Amsterdam, Netherlands
52 Amsterdam UMC, University of Amsterdam, Department of Clinical Genetics, Amsterdam Reproduction and Development Research Institute, 1019 Amsterdam, Netherlands
53 CHU Nantes, Service de Génétique Médicale, 9 quai Moncousu, 44093 Nantes, France
54 Clinic for Neuropediatrics and Neurorehabilitation, Epilepsy Center for Children and Adolescents, Schön Klinik, 83569 Vogtareuth, Germany
55 Research Institute "Rehabilitation, Transition, Palliation", PMU Salzburg, 5020 Salzburg, Austria
56 Department of Pediatrics, St. Jacques Hospital, 25000 Besançon, France
57 CHU Lille, Clinique de Génétique, Guy Fontaine, 59000, Lille, France
58 NM, Univ Montpellier, INSERM, CHU Montpellier, Département de Neuropédiatrie, 34000 Montpellier, France

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59 Pediatric Neurology, Neurogenetics and Neurobiology Unit and Laboratories, Meyer Children's Hospital, University of Florence,50131 Florence, Italy
60 IRCCS Stella Maris, 56121 Pisa, Italy
61 Centre Hospitalier Universitaire de Poitiers, Service de Génétique, BP577, 86021 Poitiers, France
62 Center for Pediatric and Teenage Health Care, Child Neurology, 53757 Sankt Augustin, Germany
63 Vestische Kinder- und Jugendklinik, 45711 Datteln, Germany
64 Justin Neurosciences Center, Cook Children's Medical Center, 76101 Fort Worth, Texas, USA
65 Department of Clinical Diagnostics, Ambry Genetics, 92637 Aliso Viejo, California, USA
66 Research Centre for Medical Genetics, 100000 Moscow, Russian Federation
67 Veltischev Research and Clinical Institute for Pediatrics of the Pirogov Russian National Research Medical University, 100000 Moscow, Russian Federation
68 Genomed ltd, 100000 Moscow, Russian Federation
69 Svt. Luka's Institute of Child Neurology & Epilepsy, 100000 Moscow, Russian Federation
70 Russian Medical Academy Continuous Professional Education, 100000 Moscow, Russian Federation
71 I.M. Sechenov First Moscow State Medical University, 100000 Moscow, Russian Federation
72 Autism & Developmental Medicine Institute, Geisinger, 17837 Lewisburg, PA, USA
73 Epilepsy Center for Children, University Hospital Neuruppin, Brandenburg Medical School, 16816 Neuruppin, Germany
74 Pediatric Neurology & Development Center Shamir Medical Center (Assaf Harofe) Be'er Ya'akov, Tel Aviv, Israel
75 Tel Aviv University, Sackler faculty of Medicine, 5296001 Tel Aviv, Israel
76 Pediatric Neurology Unit, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, 1000 Brussels, Belgium.
77 Institute for Pathology and Genetics, 6040 Gosselies, Belgium
78 Department of Pediatrics and Pediatric Neurology, Antwerp University Hospital, University of Antwerp, 2650 Edegem, Belgium
79 Pediatric neurology, Marie Curie Hospital – CHU Charleroi, 6032 Charleroi, Belgium
80 Vrije Universiteit Brussel, UZ Brussel, Department of Pediatrics, Pediatric Neurology Unit, 1000 Brussels, Belgium

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81 Pediatric Neurology Unit, Department of Pediatrics, Centre Hospitalier de Luxembourg, 1313 Luxembourg, Luxembourg
82 Department of Neurology, Boston Children's Hospital and Harvard Medical School, 02108 Boston, USA
83 Department of Genetics, Boston Children's Hospital, 02108 Boston, USA
84 Epilepsy Genetics Program, Boston Children's Hospital, 02108 Boston, USA
85 Division of Neurology, Children's Hospital of Philadelphia, 19104 Philadelphia, PA, USA
86 The Epilepsy Neurogenetics Initiative (ENGIN), Children's Hospital of Philadelphia, 19104 Philadelphia, PA, USA
87 Department of Biomedical and Health Informatics (DBHi), Children's Hospital of Philadelphia, Philadelphia, PA, 19104 USA
88 Department of Neurology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, 19104 USA
89 Institute of Clinical Molecular Biology, Kiel University, Rosalind-Franklin-Strasse 12, 24105 Kiel, Germany
90 Department of Neuropediatrics, Kiel University, Rosalind-Franklin-Strasse 12, 24105 Kiel, Germany
91: Division of Pediatric Neurology, Developmental Medicine and Social Pediatrics, Department of Pediatrics, Dr. von Haunersches Children's Hospital, Ludwig-Maximilian-University of Munich, 80331 Munich, Germany
92 Comprehensive Epilepsy Center, Ludwig-Maximilian- University of Munich, 80331 Munich, Germany
93 Luxembourg Centre for Systems Biomedicine (LCSB), University Luxembourg, L-4243, Esch-sur-Alzette, Luxembourg
94 Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, 44102 Cleveland, OH, USA
95 Epilepsy Center, Neurological Institute, Cleveland Clinic, 44102 Cleveland, OH, USA
96 Stanley Center for Psychiatric Research, Broad Institute of Harvard and M.I.T., 02138 Cambridge, MA, USA
97 Cologne Center for Genomics (CCG), University of Cologne, 50667 Cologne, Germany
98 University of Copenhagen, 2200 Copenhagen, Denmark
99 Finnish Institute for Molecular Medicine (FIMM), University of Helsinki, 320 Helsinki, Finland

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100 Program for Medical and Population Genetics/Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, 02138 Cambridge, MA, USA
101 Analytic and Translational Genetics Unit, Massachusetts General Hospital, 02108 Boston, MA, USA
102 Department of Medical Genetics, Groupement Hospitalier Est, and ERN EpiCARE, University Hospitals of Lyon (HCL), 69001 Lyon, France
103 Institut Neuromyogène, CNRS UMR 5310 - INSERM U1217, Université de Lyon, Université Claude Bernard Lyon 1, 69001 Lyon, France

Correspondence to: Rikke S. Møller
Department of Epilepsy Genetics and Personalized Treatment
The Danish Epilepsy Center
Dianalund, Denmark
E-mail: rimo@filadelfia.dk

Correspondence may also be addressed to: Holger Lerche
Department of Neurology and Epileptology
Hertie Institute for Clinical Brain Research
University of Tuebingen
Tuebingen, Germany
E-mail: holger.lerche@uni-tuebingen.de

Running title: *SCN8A*-related genotype-phenotype correlations

Keywords: SCN8A; epilepsy; genetics; personalized medicine

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Introduction

Since the first pathogenic *SCN8A* variant was discovered in an affected individual with epilepsy[1], a wide clinical spectrum of neurodevelopmental phenotypes has been reported. The spectrum ranges from benign familial infantile epilepsy (BFIE) with self-limiting seizures and typical cognitive development[2-4], over an intermediate phenotype with variable seizure onset, treatable seizures and mild to moderate intellectual disability (ID)[5] to developmental and epileptic encephalopathies (DEE) with onset in the first year of life and moderate to severe ID[6-10], often with movement disorders, cortical visual impairment, severe gastrointestinal symptoms and increased risk of premature death[7, 10-15]. Furthermore, rare clinical presentations with ID, autism spectrum disorder (ASD) and movement disorders without epilepsy have been described[16-19].

SCN8A encodes $Na_v1.6$, which is one of four voltage-gated sodium channels expressed in the mammalian brain. $Na_v1.6$ is found in the central and peripheral nervous system with a predominant expression in excitatory, but also in inhibitory neurons[20]. Previous studies have revealed that BFIE and DEEs are caused by missense variants with gain-of-function (GOF) effects, whereas truncating variants, deletions and certain missense variants causing loss-of-function (LOF) or both LOF and GOF effects have been associated with ID, ASD and movement disorders with or without seizures[3, 11, 17, 19, 21].

Treatment of *SCN8A*-related DEEs revealed frequent resistance to anti-seizure medications (ASMs), although treatment with sodium channel blockers (SCBs), especially high-dosage phenytoin, was beneficial in some affected individuals[22].

Here, we combined a detailed clinical analysis of the largest cohort of individuals with *SCN8A*-related neurodevelopmental disorders investigated to date with functional studies of newly detected variants in mammalian cells and primary neurons and explored the genotype-phenotype correlations in functional studies, computational modeling and treatment response in *SCN8A*.

Methods

Cohort ascertainment and phenotyping

Affected individuals were recruited through a network of collaborating clinicians, as well as GeneMatcher[23], by means of a standardized phenotyping sheet to assess clinical characteristics (medical history, seizure and physical characteristics, family history, neurodevelopment and

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cognition), EEG, neuroimaging and retrospective data on antiepileptic treatment. Seizures and epilepsy syndromes were classified according to the latest ILAE guidelines[24, 25].

Based on information on presence and severity of the epilepsy, seizure onset and cognitive status the affected individuals were categorized into the following a priori defined subgroups:

1) BFIE – infantile onset focal seizures with onset during infancy and normal cognitive development[3]

2) Intermediate epilepsy (IE) – individuals with a focal epilepsy (seizure types and EEG) of intermediate severity, reflecting neither BFIE nor DEE[5]

3) DEE[14] (with focal seizure types and EEG)

4) Generalized epilepsy (GE) – individuals with a generalized epilepsy (seizure types and EEG), thus not fitting groups 1, 2 or 3

5) Unclassifiable epilepsy (UE, insufficient data to be classified into one of the groups above, or rarely both focal and generalized seizure types or epileptiform discharges)

6) Neurodevelopmental disorder without epilepsy (NDDwoE)

See supplementary figure 2 for a flowchart describing the categorization of *SCN8A*-related phenotypes.

Treatment response was evaluated by the referring providers as seizure freedom (at least six months without seizures), seizure reduction (affected individual still on ASMs since provider and parents considered a beneficial effect), no change (ASMs terminated) or seizure aggravation noted by treating providers and parents. Phenytoin (PHT), carbamazepine (CBZ), oxcarbazepine (OXC), lacosamide (LCM), lamotrigine (LTG) and zonisamide (ZNS) were all classified as SCBs.

Variant pathogenicity was assessed according to the ACMG guidelines[26]. All variants were absent from gnomAD r2.1.1 (https://gnomad.broadinstitute.org/) and BRAVO (https://bravo.sph.umich.edu/freeze8/hg38/). Pathogenic or likely pathogenic variants were included. *SCN8A* transcript NM_014191.3 was used for coding variant nomenclature.

Previously published cases

A PubMed search on "*SCN8A*" was performed, and all publications with affected individuals were included in the present study. The latest search was performed on May 15th 2020. Papers not available in English were excluded. Only original cases and only probands were included. Pathogenic or likely pathogenic variants were included and the variant locations were remapped on

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NM_014191.3 when necessary to harmonize their presentation in this article. Data on functional studies were also collected. If affected individuals were published more than once, all papers are included in the list. Duplications of affected individuals were avoided by follow-up with the referring clinician/corresponding author.

Ethics

The study was approved by the local ethical committees or followed other local guidelines. Previously unpublished individuals (or parents, in case of minors) signed informed consent.

Frequency

The Danish Epilepsy Centre is the only tertiary hospital in Denmark specialized in treating epilepsy, and the majority of affected individuals with non-acquired epilepsy are referred to the center for genetic testing. Furthermore, inquiries were sent to all national clinical genetic departments. Thus, we were able to estimate the frequency of *SCN8A* variants in the Danish population by using the birth cohort from 2006 to 2017 in the electronical population database of the Danish National Statistics.

Functional studies

Variants were chosen for functional studies according to the clinical phenotype of variant carriers and the location of variants in the $Na_V1.6$ channel protein to cover the phenotypic spectrum of *SCN8A*-related disorders (see Results for details). All methods have been previously described[19] and are summarized in the Supplementary methods.

Neuronal simulations

To investigate how changes in Na^+ current properties affect neuronal firing behavior, we simulated a model neuron with sodium, potassium and leak currents (details provided in supplementary methods). We simulated firing by injecting step currents of 0 to 0.75 nA for 2s and analyzed the voltage traces for the firing rate after the neuron adapted to the injected current. The resulting input-output curve was then analyzed for its area under the curve (AUC). We modified the following six parameters of the Na^+ current. The half activation voltages $V_{1/2}$ of the activation and inactivation curves were shifted within ± 10 mV of the original values. The slopes k of the activation and inactivation curves as well as the maximum conductance g_{Na} were multiplied with a scaling factor

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ranging from 0.5 to 2. The persistent current *z* of inactivation ranged from 0 to 5% of the maximum inactivation. Only a single parameter was modified at a time. The effect of alteration *i* on the AUC was quantified relative to the AUC of the unchanged model AUC_0 by

$$AUC\ contrast_i = \frac{AUC_i - AUC_0}{AUC_0} \quad (1)$$

For each altered parameter, the slope m_j of the regression line through the origin between the parameter changes x and its resulting $AUC\ contrasts$ was calculated. To compare magnitudes of effects across different alteration scales, parameter changes *x* were set to $\Delta V_{1/2} = V_{1/2,i} - V_{1/2,\,0}$, $\Delta z = z_i - z_0$ and $log(scaling\ factor_i)$ for *k* and g_{Na}.

These slopes were then used to score biophysical changes *j* of ionic current variants to estimate their effect on the firing behavior and the severity of their neurological impairments with

$$score_j = x_j \cdot m_j \quad (2)$$

where $score_j$ is the scoring for one alteration of a biophysical parameter. This resulted in the following equations for the biophysical scores of SCN8A variants:

$$score_{conductance} = log\frac{g_{mut}}{g_{wt}} \cdot 0.091 \quad (3)$$

$$score_{activation,V_{1/2}} = (V_{1/2,mut} - V_{1/2,wt}) \cdot (-0.12\ mV^{-1}) \quad (4)$$

$$score_{activation,k} = log\frac{k_{mut}}{k_{wt}} \cdot 1.2 \quad (5)$$

$$score_{inactivation,V_{1/2}} = (V_{1/2,mut} - V_{1/2,wt}) \cdot 0.0016\ mV^{-1} \quad (6)$$

$$score_{inactivation,k} = log\frac{k_{mut}}{k_{wt}} \cdot 0.011 \quad (7)$$

$$score_{inactivation,persistent} = (z_{mut} - z_{wt}) \cdot (-0.004) \quad (8)$$

Finally, these scores are summed up to predict the severity of the respective variants

$$Electrophysiological\ score = \sum score_j \quad (9)$$

Classification of phenotype severity

For the correlation analysis between electrophysiological score and severity of the disease in *SCN8A* variant carriers, we classified the individuals in four severity groups: BFIE, IE, DEE with mild or moderate ID, and DEE with severe ID. We assigned weights to the four grades of severity

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(BFIE=1, IE=2, DEE with mild or moderate ID=3, DEE with severe ID=4). In case the same variant was associated with different phenotypes, we calculated a weighted average score. As this analysis was performed for the GOF variants only, the GE, UE and NDDwoE groups were not included.

Statistical analysis

Clinical data was analysed using Stata version 15.1 for Mac (StataCorp, College Station, Texas, USA). For categorical data, Fisher's Exact Test was used, and for continuous data the Kruskal-Wallis test and Mann-Whitney test were used. Significance was evaluated using a two-tailed test of proportions and significance was reached if $p<0.05$. The do-file used to perform the analyses is available upon request.

Electrophysiological data were analysed using Clampfit software of pClamp 10.6 (Axon Instruments), Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), or Igor Pro (Wavemetrics, Portland, OR, USA). Statistics were performed using one-way ANOVA with Dunnett´s posthoc test, ANOVA on ranks with Dunn´s posthoc test or Fisher´s exact test in GraphPad prism (GraphPad software, San Diego, CA, USA). For all statistical tests, significance is indicated in the figures (using the following symbols: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), or directly indicated in the text.

Data availability

Data will be made available upon request.

Results

Phenotypes within the whole cohort

We assessed a cohort of 91 unpublished and 301 previously published affected individuals (392 affected individuals in total). One hundred and twenty-seven affected individuals had unclassifiable epilepsy due to a lack of information and have not been included in the analyses below. Age at follow-up/inclusion ranged from two months to 44 years (median 4.3 years). We differentiated the following phenotypes:

- BFIE (n=15): Median age at seizure onset in this subgroup was six months (range: two weeks to one year). The most prevalent seizure types were focal (40%), focal to bilateral tonic-clonic (27%) and bilateral tonic-clonic without identifiable focal onset (27%). Cognition was normal in all affected individuals. Treatment response was known in 10, all

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of whom were seizure free: Six with a SCB, one with a non-SCB and three with a combination. Seven/15 (47%) variants were inherited from an affected parent. Two variants were recurrent: p.(Glu1483Lys) and p.(Asn1877Ser)[2, 3].

- IE (n=33): IE was defined as a focal epilepsy fitting neither BFIE nor DEE categories (see supplementary Fig. 2 for a flow chart and distinction criteria). Median age at seizure onset was five months (range: two months to seven years). The most prevalent seizure types were focal (64%), tonic (33%), and bilateral tonic-clonic (55%). Cognition was normal in 33%. Mild ID was present in 52% and moderate ID in 15%. Additional features included speech delay (36%), behavioral disorders (ADHD, autistic features, aggression) (19%) or ataxia (11%). Treatment response was known in 31 affected individuals; 14 affected individuals were seizure free: eight with an SCB, three with a non-SCB and three with a combination. Three variants were inherited from an affected parent, while four were unknown and the remainder were *de novo*. Two variants were recurrent: p.(Gly1475Arg) and p.(Asn1877Ser)[5].
- DEE (n=177): Median age at seizure onset was three months (range: first day of life to 36 months). The most frequent seizure types were focal (70%), tonic (76%) and bilateral tonic-clonic (89%) seizures. Cognition ranged from moderate (22%) to severe (73%) ID and was unknown in the remainder (5%). Additional features included hypotonia in 50% and cortical vision impairment (CVI) in 32%. Treatment response was known in 128 affected individuals: 26 affected individuals (20%) were seizure free, 11 of them with SCBs (42%), seven with non-SCBs (27%) and eight with a combination (31%). The vast majority of variants occurred *de novo* (90%), three were inherited from an affected parent, and the inheritance was unknown in 11 individuals. Twenty-five variants were recurrent: p.(Arg850Gln/Gly) and p.(Arg1872Trp/Gln/Leu) were the most common[10, 14, 27] (suppl. Table 1).
- GE (n=20): The median age at seizure onset was 42 months (range: nine months to 14 years). The most prevalent seizure types were absence (80%), generalized tonic-clonic (20%) and febrile seizures (15%). Cognition was normal in 20%, and ranged from mild (30%) or moderate (35%) to severe ID (10%). Cognition was unknown in 5%. Additional features included ataxia (5%), behavioral disorders (autism, aggression, anxiety; 28%), and speech delay (19%). Treatment response was known in 16 affected individuals; six were seizure free: one with a SCB and five with a non-SCB. Ten variants occurred *de novo*, five

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were inherited from an affected parent, and five were of unknown inheritance. None of the variants were recurrent.

- NDDwoE (n=20): Individuals with NDDwoE did not have epilepsy at time of inclusion (median age nine years, range three to 35 years). Cognition ranged from normal (10%) to mild (45%), moderate (20%) or severe ID (15%), and was unknown in 10%. Additional features included behavioral disorders (ASD, ADHD, 43%), delayed speech (24%) and microcephaly (19%). Six variants occurred *de novo*, whereas nine were inherited from affected or mosaic parents and the remainder was unknown. Three variants were recurrent: p.(Gly384Arg), p.(Arg931Gln) and p.(Ala1622Asp).

Clinical details for all previously unpublished and published individuals (as data were available) are summarized in supplementary table 1.

Functional studies

We examined seven *SCN8A* variants functionally, chosen to represent the most important aspects of the clinical spectrum, particularly the newly identified phenotype with GE, for which functional studies had not been performed up to now. Three variants (p.(Leu840Pro), p.(Phe846Ser), p.(Asn1877Ser)) were seen in affected individuals with onset of seizures in the first year of life; the first two in affected individuals with DEE who were resistant to all ASMs including high-dose SCBs[7], the third in affected individuals with BFIE or intermediate epilepsy who were responsive to SCBs[2, 5]. Three variants (p.(Ile1654Asn), p.(Val1758Ala), p.(Thr1787Pro)) were seen in affected individuals with GE with late onset absence seizures, in two cases preceded by febrile seizures[5, 28]. The last variant (p.(Asn374Lys)) was found in an affected individual with a focal epilepsy with moderate ID and late onset at seven years of age; so an unusual phenotype for which the pathogenicity of the *SCN8A* variant had to be shown[5].

The biophysical consequences of these seven variants were first studied in ND7/23 cells. p.(Phe846Ser) and p.(Leu840Pro) variants induced hyperpolarising shifts of the activation curves, indicating clear GOF effects (Figs. 1B ,1C, Supplementary Table 2). The p.(Asn1877Ser) variant caused a significant depolarising shift of steady-state fast inactivation and slightly slowed the time course of fast inactivation (Fig. 1D, 1F, Table S2). In contrast, p.(Ile1654Asn), p.(Val1758Ala) and p.(Thr1787Pro) variants dramatically reduced the peak current density; especially cells transfected with p.(Ile1654Asn) barely exhibited any Na^+ current, hence no gating parameters were obtained for this variant (Figs. 2A, 2B, Supplementary Table 2). Furthermore, the p.(Val1758Ala) and

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p.(Thr1787Pro) variants shifted activation curves toward more depolarised potentials suggesting LOF effects, although the former variant impaired, and the latter enhanced fast inactivation (Figs. 2B, 2C, 2D, 2F, Supplementary Table 2). Additionally, these two variants, as well as p.(Asn374Lys) to a minor extent, accelerated recovery from fast inactivation (Fig. 2E, Supplementary Table 2).

Next, intrinsic and firing properties were examined in transfected cultured hippocampal mouse neurons in the absence or presence of tetrodotoxin (TTX). In the absence of TTX, action potential (AP) firing was jointly determined by endogenous and transfected Na^+ channels. Only the p.(Thr1787Pro) variant significantly decreased the AP firing rate compared to neurons transfected with WT channels, as revealed by the area under the curve of input-output relationships (Figs. 2B, 2C, Supplementary Table 3). The p.(Phe846Ser) variant decreased, whereas the p.(Thr1787Pro) variant increased both rheobase and AP threshold compared to the WT (Figs. 2D, 2E, Supplementary Table 3), indicating the former enhanced, whereas the latter impaired neuronal excitability.

In the presence of TTX, AP firing was dependent on the TTX-insensitive transfected Na^+ channels. Under these conditions, p.(Phe846Ser) and p.(Asn1877Ser) variants significantly increased AP firing, whereas p.(Thr1787Pro) and p.(Ile1654Asn) decreased AP firing (Figs. 3B, 3C, Supplementary Table 4). Additionally, the p.(Phe846Ser), p.(Leu840Pro) and p.(Asn1877Ser) variants decreased rheobase and the former two variants further reduced the threshold for AP firing indicating increased neuronal excitability. In contrast, neurons transfected with p.(Val1758Ala), p.(Thr1787Pro) or p.(Ile1654Asn) variants exhibited significantly decreased peak Na^+ currents resulting in very few action potentials firing in the presence of TTX, hence no parameters of single APs were obtained. Neurons expressing p.(Asn374Lys) showed comparable Na^+ peak currents and AP firing to WT channels (Fig. 3, Supplementary Table 4).

In summary, p.(Phe846Ser), p.(Leu840Pro) and p.(Asn1877Ser) showed a GOF effect or increased neuronal firing; p.(Asn374Lys) showed mild GOF effects and did not alter neuronal firing; p.(Val1758Ala), p.(Thr1787Pro) and p.(Ile1654Asn) showed LOF effects or decreased neuronal firing.

Genotype-phenotype correlations in GOF and LOF variant carriers and extrapolation to the whole cohort of affected individuals

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Clinical data combined with functional results of this and earlier studies enabled us to explore genotype-phenotype correlations; 170 affected individuals carrying a known LOF (n=34) or GOF (n=136) variant. Detailed phenotypic analysis in those affected individuals and a comparison to the whole cohort may allow an extrapolation of genotype-phenotype correlations that could be valid for all individuals in which causative *SCN8A* variants have been detected. These phenotypic comparisons are provided in Figure 4 and the next two paragraphs.

Phenotypes of affected individuals with loss-of-function variants. Thirty-four affected individuals (12 previously unpublished) carried clear LOF variants, either deletions, splice-site, frameshift or stop-variants, or missense variants with confirmed LOF effect either in this or previous studies[17-19, 21]. Twenty-three individuals (68%) had epilepsy. The median age at seizure onset was 24 months (range 1 month to 14 years). Seizure types included absence seizures (11/23), bilateral TCs (7/23) and myoclonic seizures (5/23). Additionally, 4/23 had febrile seizures within the first year of life, all with later onset (>two years of age) of afebrile seizure types. Fourteen/34 had a phenotype of generalized epilepsy, 11/34 had a NDDwoE, three/34 had DEE and the remaining six/34 had an unclassifiable epilepsy. EEGs showed background slowing, generalized spike-wave activity, slow rhythmic activity or remained normal.

Nine/23 affected individuals with epilepsy were seizure-free: one with a SCB (LTG), seven with non-SCBs (VPA monotherapy in four affected individuals) and one on a combination (LTG and TPM). Out of four additional individuals who tried SCBs, one had a worsening of seizures with ZNS, two had no effect, and one had a reduction of seizures with LTG.

None of the individuals with LOF variants died prematurely.

When we compared this presentation of clear LOF variant carriers to the phenotypes of the whole cohort of affected individuals, there was a clear correlation to two groups: one with generalized epilepsy, and one with NDDwoE. The only exception was three affected individuals with DEE in the LOF group. The main phenotypic features of these groups including analyses of the age at seizure onset (except febrile seizures) and seizure types are summarized and contrasted to the groups with GOF variants in Figure 4 and Table 1.

Phenotypes of affected individuals with gain-of-function variants. One hundred and thirty-six affected individuals from the total cohort (29 unpublished) carried missense variants that were shown to cause GOF[1, 6, 17, 19, 29, 30]. All 136 suffered from epilepsy. The most prevalent seizure types

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were focal (32%), focal tonic (31%) or bilateral TC seizures (43%). Febrile seizures were seen in 4%. Median age at seizure onset was four months (range: first day of life to 45 months). Half of the affected individuals (54%) were diagnosed with DEE, followed by unclassifiable epilepsy in 29%, intermediate epilepsy in 10% and BFIE in 7%. Individuals with BFIE had either no interictal epileptiform abnormalities or rare diffuse spike and wave complexes. Individuals with intermediate epilepsy had heterogeneous EEG features with trains of beta and delta activity, and focal spike and slow waves, bilaterally in the parieto-occipital regions, with or without diffuse spreading. The majority of individuals with severe DEE had background slowing, polymorphic delta and beta activity, and multifocal spike and slow waves, predominant in the posterior quadrants.

Treatment data was available in 84 affected individuals. Twenty-six/84 affected individuals were seizure free; 16 with SCBs. Seizure reduction was seen in 47 affected individuals, 18 with SCBs. Two affected individuals showed worsening of seizures with SCBs; both affected individuals were resistant to several ASMs and had increased seizure frequency with OXC and LEV[31]. Eight affected individuals did not try SCB treatment: two had a mild phenotype and were seizure free with VPA or VPA+LEV[5, 32] and one affected individual also had a mild phenotype and seizure reduction with VPA[33]. The remaining five affected individuals all had pharmacoresistant seizures, it is unknown why SCBs were not tried.

Twelve affected individuals were prematurely deceased (9%), all with a DEE phenotype; ten due to a general worsening in their overall and neurological condition followed by organ failure and two due to definite or probable SUDEP (1.5%).

As in the LOF group, there was a clear correlation in the GOF group to specific phenotypes in the whole cohort, namely BFIE, intermediate epilepsy, and DEE. None of the GOF variant carriers had a generalized epilepsy or a NDDwoE.

A summary of these data is provided in Figure 4, Table 1 and Supplementary Table 5. The obvious difference in the age of onset was statistically significant between GOF and LOF variant carriers (Mann-Whitney test, $p < 0.001$), and between BFIE/IE/DEE vs. GE (Kruskal-Wallis test, $p < 0.001$). Statistical analysis also confirmed that affected individuals with LOF variants mostly had a generalized epilepsy, whereas affected individuals with GOF had one of the focal epilepsy phenotypes (BFIE, intermediate epilepsy or DEE) (Fisher's Exact Test, $p < 0.001$).

Correlation of clinical and electrophysiological phenotypes in the GOF group. We scored all GOF variants according to their functional characterizations from this and previous studies,

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including a total of 16 variants. The LOF variants were not included due to a large proportion of deleterious variants, which lead to non-functional protein that cannot be well differentiated. The electrophysiological score was determined by the effect of biophysical changes of variants on neuronal firing simulated by a single-compartment conductance based model (see methods and supplementary methods). This analysis revealed that (i) alterations of activation properties severely affect neuronal firing; (ii) changes in the sodium conductivity have a moderate effect; (iii) changes of inactivation properties and the persistent current affect neuronal firing mildly (Fig. 5A-F, Supplementary Table 5).

The electrophysiological scores of GOF variants significantly correlated with the clinical severity of affected individuals carrying these variants (Fig. 5G, $p = 0.0079$, $r = 0.64$), which were previously classified into BFIE, IE, DEE with mild/moderate ID and DEE with severe ID (scored as 1, 2, 3 and 4 respectively). Variants causing different phenotypes were averaged over this score (see methods). In a separate analysis, we also compared the individual severity of each affected individual with the electrophysiological score of the variants. The scores in the groups with DEE are significantly higher than the ones in BFIE and IE, but there is no significant difference between BFIE and IE or DEE with mild/moderate ID and DEE with severe ID (Fig. 5H).

Genetic landscape of *SCN8A* variants

We analyzed 256 different variants in 392 individuals. Missense variants (n=233) accounted for the majority of disease-causing variants (91%). Nineteen variants were deleterious frameshift, stop-gain or canonical splice-site variants. Seven were inframe indels or deletions. Three patients had biallelic missense variants[34]. Sixty-one recurring missense variants were found in 244 affected individuals. Two hundred and ninety-seven variants occurred *de novo*, 31 were inherited from an affected or unaffected mosaic parent, and for 64 segregation was unknown.

The distribution of variants across the $Na_V1.6$ channel protein is shown in Figure 6. Most GOF variants (shown in red) and also variants causing focal epilepsy (BFIE/IE/DEE) without functional data (shown in light red), are concentrated at the cytoplasmic side and in the voltage sensors (protein regions given as codon positions: 212-263, 398-418, 838-883, 966-986, 1283-1336, 1450-1523, 1602-1660, 1753-1775), whereas most LOF variants were found in the pore region (protein regions: 274-408, 893-976, 1347-1460, 1669-1765). The region-specific distribution of GOF and BFIE/IE/DEE variants in comparison to the distribution of LOF and GE/NDDwoE variants is highly significant (Fisher's Exact test p-value <0.00001). This is also illustrated in a 3D structural

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model which additionally reveals that population variants taken from the gnomAD database are located in different regions. Most disease-causing variants were located in functionally important and evolutionary conserved regions of the channel, whereas variants from the gnomAD database are in less conserved regions [Supplementary. Fig. 1 and Supplementary video 1 (the video is available to view at figshare: https://doi.org/10.6084/m9.figshare.15141018).

Frequency of *SCN8A*-related disorders in the Danish population

From the year 2006 to 2017, an average of 60,934 children were born per year. During the same period, 13 affected individuals were diagnosed with an *SCN8A*-related disorder in Denmark, yielding an estimated frequency of 1/56,247.

Evaluation of treatment effects

Treatment responses to specific ASMs were grouped into four categories - seizure-free, seizure reduction, no change and worsening - as described in the methods section. We differentiated between SCBs and non-SCBs, in LOF and GOF variant carriers, GE and focal epilepsies (BFIE/IE/DEE). The results are shown in Figure 7. GOF variant carriers responded better to SCBs than to non-SCBs (Fisher's Exact Test, $p < 0.001$), whereas there was no difference between the treatment of SCBs and non-SCBs for LOF variant carriers (numbers very small, Fisher's Exact Test, $p = 0.48$). Similar results were obtained for the treatment of SCBs versus non-SCBs in affected individuals with focal epilepsy vs. those with GE.

Discussion

Our study provides a detailed analysis of the correlation between clinical phenotypes, genotypes and electrophysiological characterizations, suggesting the following four main conclusions:

1. There are five main phenotypes in *SCN8A*-related disorders in this largest series of 392 individuals collected to date: BFIE, IE, DEE, GE and NDDwoE.
2. There is a significant correlation between GOF variants causing focal epilepsy of different/increasing severity (BFIE, IE, DEE) and LOF variants causing GE or NDDwoE, allowing an extrapolation from the clinical phenotypes of clear GOF and LOF variant carriers to the whole cohort. There was only one exception from this rule: three DEE affected individuals with onset between five and ten months carrying stop-gain or canonical splice-site (hence, predicted LOF) variants (see discussion below for exact phenotypes).

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3. The clinical severity of epilepsies associated with GOF variants is at least partially determined by the degree of the electrophysiological dysfunction, as there was a significant correlation between the clinical and electrophysiological phenotypes using a new system to estimate the influence of different gating parameters on neuronal firing.
4. As previously suggested in smaller studies, seizures in individuals with disease-causing GOF variants respond better to sodium channel blockers (SCBs) than to other ASMs.

Functional studies

We examined altogether 14 variants (this study and previously [19]) covering the whole clinical spectrum of *SCN8A*-related phenotypes. Using both neuroblastoma cells to study the effects of the mutant channels on gating properties and murine primary neuronal cultures to study effects on intrinsic neuronal properties and firing, we found mild GOF effects or an increase in neuronal firing to be associated with BFIE or IE (p.(Gly1475Arg), p.(Glu1483Lys) and p.(Asn1877Ser)), whereas more severe gating defects, particularly strong hyperpolarizing shifts of steady-state activation that also on average led to stronger increase in neuronal firing, were associated with DEE (p.(Arg223Gly), p.(Leu840Pro), p.(Phe846Ser), p.(Met1760Ile) and p.(Arg1872Trp)). One previously published variant, p.(Arg223Gly), was initially suggested to cause a predominant LOF[11] and had a phenotype of spasms with onset at six months, severe ID and an epileptic encephalopathy. However, there were also GOF features identified, and when we retested this variant in our system, GOF effects predominated and it increased neuronal firing (Liu, Koko, Lerche, unpublished). When we used a one compartment neuronal model system, we were able to weight the gating defects of all GOF variants that were functionally investigated so far according to their effect on neuronal firing, revealing a significant correlation between the severity of the clinical and electrophysiological phenotypes. Such a correlation was not observed when we used a different scoring system (data not shown) based only on the degree of the electrophysiological dysfunction (i.e. without weighting the effects on neuronal firing), which, however, served well previously to correlate clinical and electrophysiological phenotypic severity in *SCN2A*-related epilepsy[35].
In contrast, the p.(Ile1654Asn), p.(Thr1787Pro) and p.(Val1758Ala) variants, all causing GE with absence seizures as a new recurring phenotype, showed a LOF in neuroblastoma cells or decreased neuronal firing. Also, variants not causing epilepsy either caused a clear LOF and decreased neuronal firing (p.(Gly964Arg) and p.(Arg1620Leu)), or a strong GOF on channel gating leading to a depolarisation block, i.e. LOF on a neuronal level (p.(Ala1622Asp))[19]. Additional variants

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characterized by other groups confirm these results. Many other variants causing GOF effects mainly on channel gating (only few studies with limited results on neuronal firing) were clearly associated with focal epilepsies (Supplementary Table 1).

The p.(Ala1319Thr) variant caused a depolarising shift of the activation curve of Na^+ current in *x. laevis* oocytes[36, 37] and decreased firing of cerebellar Purkinje cells[38, 39] indicating a LOF. The *Scn8a Med^{jo}* mouse carrying the corresponding variant in the mouse channel exhibited absence epilepsy revealed by slow-wave discharges (SWD) in EEG recordings associated with absence-like seizures[40], reproducing the clinical phenotype found in the affected individual carrying the p.(Ala1319Thr) variant. Other epilepsy-related LOF variants were also associated with absence epilepsy, such as p.(Asn544fs*39) and p.(Glu587*)[5, 31]. In another, conditional mouse model, a complete *Scn8a* LOF restricted to inhibitory neurons of the thalamic reticular nucleus also caused absence-like epilepsy with generalized SWD[40, 41]. It is therefore intriguing to speculate that *SCN8A* variants cause absences and other generalized seizures due to a LOF in inhibitory neurons, whereas the GOF effects are more important in excitatory neurons, as discussed in more detail further below including the clinical consequences.

The p.(Asn374Lys) variant showed only very mild GOF gating defects but did not change parameters in neurons. The phenotype with late onset focal epilepsy at seven years without other neuropsychiatric symptoms also does not fit the five main categories. Thus, while we cannot exclude a mild contribution of this variant to seizures in this affected individual, we consider it to be a variant of unknown significance

Phenotype correlations in GOF vs. LOF variant carriers

When we compared the clinical phenotypes of the confirmed 136 GOF and 34 LOF variant carriers with the whole group of 392 individuals, there were strikingly congruent phenotypes, which strongly suggest that BFIE, IE and DEE are generally caused by GOF, while GE and NDDwoE are caused by LOF variants. Not only did seizure types resemble each other in those groups, but also an analysis of the age of onset of seizures strongly confirmed this hypothesis (Table 1, Fig. 4). Only three cases with presumed DEE and a LOF variant did not fit into this pattern. One patient carried a p.(Met1481Ilefs*12) variant with onset of pharmaco-resistant tonic and bilateral tonic-clonic seizures and episodes of status epilepticus at five months of age, severe ID, no speech and poor eye contact. Two patients with seizure onset at five or ten months carried a splice-site variant predicting exon skipping to cause a large in-frame deletion cutting out the whole pore region of domain III,

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p.(Pro1428_Lys1473del) (Fig. 6). They had severe ID, no speech, poor eye contact and hypotonia. EEG showed multifocal epileptiform discharges. Thus, these single cases could not be differentiated from the DEE cohort caused by GOF variants. The epilepsy phenotype of two further patients with similar splice-site variants was unclassifiable. Alternative consequences on the $Na_V1.6$ protein other than those predicted here (p.(Pro1428_Lys1473del) and p.(Met148Ilefs*12)), e.g., resulting from activation of cryptic splice sites, cannot be ruled out completely.

In a previous study on *SCN2A*[42], we demonstrated that different neonatal and infantile epilepsy syndromes were caused by GOF variants, whereas later onset seizures were due to a LOF of $Na_V1.2$. In *SCN2A,* the boundary for the onset of seizures between GOF and LOF was very early – approximately three months of age[42]. In the present study, the onset in both groups was later (median age at onset of four months in the GOF subgroup, and 24 months in the LOF subgroup), and the ranges were more overlapping. This difference may be a reflection of the differential developmental expression of *SCN2A* vs. *SCN8A*, as *SCN2A* is expressed neonatally and earlier than *SCN8A*[43, 44]. An additional difference is that *SCN2A* variant carriers have better defined electroclinical epilepsy syndromes. Both groups share a phenotype of BFNIE/BFIE, with a later onset in *SCN8A*-related epilepsy. Some individuals carrying *SCN8A* variants develop paroxysmal kinesigenic dyskinesia[3], whereas *SCN2A* variant carriers may develop a form of later onset episodic ataxia[45]. There were also differences regarding the syndromes of LOF variant carriers in both genes. Generalized epilepsy was not generally observed in *SCN2A*, although absence epilepsy has been reported in mice with complete LOF of *Scn2a*, when knocked out in excitatory neurons[46]. Instead, West syndrome was a common phenotype in human *SCN2A*, but also phenotypes with generalized seizures and EEG abnormalities were found in the LOF group (epilepsy with myoclonic-atonic seizures or Lennox-Gastaut syndrome). For *SCN8A*, the phenotypes were rather unspecific generalized epilepsy, mainly with absence seizures. However, we found that a LOF subgroup had a phenotype resembling *SCN1A* LOF, with febrile seizures preceding additional seizure types, resembling a generalized/genetic epilepsy with febrile seizure plus (GEFS+) phenotype. *SCN8A* is expressed in both excitatory and inhibitory neurons[47], which may well explain the phenotypic similarity between some of the *SCN1A*- and *SCN8A*-related phenotypes, since *SCN1A* is considered to be the main sodium channel in inhibitory GABAergic neurons[48, 49]. Additionally, it has been found that knockout of *Scn8a* in mice decreased cortical excitability resulting in convulsive seizure protection[40]. Furthermore, mice with global reduction of *Scn8a* had fewer seizures compared to mice with *Scn8a* deletion only in inhibitory neurons[41]. In contrast, mice carrying a GOF *Scn8a*

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variant (p. (Arg1872Trp)) exhibited convulsive seizures and premature death; however, activation of this variant only in inhibitory neurons did not induce seizures or overt neurological dysfunctions[20]. Altogether, *SCN8A* GOF variants may mainly cause hyperexcitation in excitatory neurons leading to TC and focal seizures, whereas *SCN8A* LOF variants mainly induce hypoexcitation in inhibitory neurons causing generalized and particularly absence seizures. Further studies may elucidate the distinct roles of *SCN8A* and the other sodium channel genes in different neuronal circuits.

Genetic landscape

The vast majority of the detected variants were missense, and we found several recurrent variants. Those detected in more than ten affected individuals include p.(Arg850Gln/Gly) seen in 12 affected individuals, p.(Gly1475Arg) seen in 19 affected individuals, p.(Arg1617Trp/Gln/Leu) seen in 18 affected individuals, p.(Arg1872Trp/Gln/Leu) seen in 41 affected individuals and p.(Asn1877Ser) seen in 23 affected individuals. All of these variants cause a clear GOF. Why some of these variants, especially p.(Gly1475Arg) and p.(Arg1617Trp/Gln/Leu), have a high phenotypic variability and others not, remains to be elucidated. Other genetic or environmental factors might play an important role. Additionally, we found families with intra-familial variability, such as the family of proband #377, whose mother and two older siblings have BFIE and are seizure-free with normal intellect, whereas the proband suffers from pharmacoresistant DEE.

Estimated frequency

We found the frequency of *SCN8A* related disorders in the Danish population to be 1/56,247, which is higher than for *SCN2A* (1/78,608)[42] but lower than for *SCN1A* (1/22,000)[50]. Since these three genes are of similar size and homology, we would assume a similar variant frequency. Large-scale genetic testing in isolated ASD cohorts have failed to detect large numbers of *SCN8A* affected individuals[51], suggesting that a large number of undiagnosed *SCN8A*-ASD affected individuals does not exist and that this is not the cause behind the discrepancy in numbers. It could be that some individuals with truncating/LOF variants are too mildly affected (learning disabilities, mild ID etc.) to be candidates for genetic testing, but are also not healthy enough to be part of control populations (such as blood donors), as reflected in the almost complete absence of truncating variants in gnomAD (pLI score:1). Intra-uterine death in *SCN8A*-related disease could be a third cause not investigated so far.

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Treatment implications

In general, and as expected, affected individuals with GOF effects showed an overall positive response to treatment with SCBs, as has been hypothesized previously[22]. However, many of the affected individuals with DEE still have pharmacoresistant epilepsy (81%), underlining the severity of *SCN8A*-related epilepsy. A recent study evaluated the effect of a novel SCB: GS967, which primarily targets the elevated persistent currents without affecting peak currents, as well as having increased potency compared to PHT[52]. Long-term treatment with GS967 was shown to protect *Scn8a*$^{N1768/+}$ mice against premature death, and also alleviated seizure burden[52]. GS967/PRAX-330 (https://adisinsight.springer.com/drugs/800050600) and another new drug - XEN901 (https://clinicaltrials.gov/ct2/show/NCT03467100), specifically targeting *SCN8A* with a goal of rectifying the effects of GOF variants, are currently in Phase I clinical trials. Furthermore, anti-sense oligonucleotide therapy was recently shown to prolong survival in *Scn8a*$^{R1872W/+}$ (GOF) mice[53]. These novel compounds provide promising advances for precision medicine in *SCN8A* affected individuals. As functional testing is not yet readily available for all affected individuals, it will be important to have clinical markers that may predict the underlying functional effect, when specific blockers of the $Na_V1.6$ channel become available on the market. Our study provides an important contribution in this direction, as genotype-phenotype correlations revealed a clearly differential pattern for both GOF and LOF variants with very few exceptions.

Acknowledgements

We thank the affected individuals and their families for participating in this study.
The authors wish to acknowledge the resources of MSSNG (www.mss.ng), Autism Speaks and The Centre for Applied Genomics at The Hospital for Sick Children, Toronto, Canada. We thank the Epi25 Collaborative for providing the SCN8A variants in three individuals (supporting grants see below). We also thank the participating families for their time and contributions to this database, as well as the generosity of the donors who supported this program.

Competing interests

The authors report no competing interests.

Supplementary material

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Funding

This work was supported by the German research foundation and the Fonds Nationale de la Recherche in Luxembourg (Research Unit FOR-2715, DFG grants Le1030/15-1 and /16-1 to HL, He8155/1-1 to UBSH, He5415/7-1 to IH, FNR grant INTER/DFG/17/11583046 to PM), the German Federal Ministry for Education and Research (BMBF, Treat-ION, 01GM1907A to HL, DL and PM), the foundation no epilep (to HL to partially support LS), the Medical Faculty of the University of Tuebingen (the Fortüne program 2430-00 to SL). KS, PL, MV were funded by grant: MH CR AZV NU20-04-00279. Epi25 was supported by the National Human Genome Research Institute (NHGRI) grants UM1 HG008895 and 5U01HG009088-02, and the Stanley Center for Psychiatric Research at the Broad Institute. SI was employed by and received a salary from Ambry Genetics. SS hold the GlaxoSmithKline Endowed Chair in Genome Sciences at the Hospital for Sick Children and University of Toronto. P.S. has received speaker fees and participated at advisory boards for Biomarin, Zogenyx, GW Pharmaceuticals, and has received research funding by ENECTA BV, GW Pharmaceuticals, Kolfarma Srl., Eisai. EM was funded by grant: NIH NINDS K08 NS097633 and Foerderer Award for Excellence from The Children's Hospital of Philadelphia Research Institute. PV received a speaker fee from Nutricia GmbH, Dr. Sch¨ar AG / SPA, Eisai. Nestle', Pediatrica. FZ and PS developed this work within the framework of the DINOGMI Department of Excellence of MIUR 2018-2022 (legge 232 del 2016). IH was also supported by The Hartwell Foundation through an Individual Biomedical Research Award. This work was also supported by the National Institute for Neurological Disorders and Stroke (K02 NS112600), including support through the Center Without Walls on ion channel function in epilepsy ("Channelopathy-associated Research Center", U54 NS108874), the Eunice Kennedy Shriver National Institute of Child Health and Human Development through the Intellectual and Developmental Disabilities Research Center (IDDRC) at Children's Hospital of Philadelphia and the University of Pennsylvania (U54 HD086984), and by intramural funds of the Children's Hospital of Philadelphia through the Epilepsy NeuroGenetics Initiative (ENGIN). Research reported in this publication was also supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR001878. This project was also supported in part by the Institute for Translational Medicine and Therapeutics' (ITMAT)

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Transdisciplinary Program in Translational Medicine and Therapeutics at the Perelman School of Medicine of the University of Pennsylvania. HEO was funded by K23 NS107646-03, PI Olson.

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Figure legends:

Figure 1. Functional characterizations of *SCN8A* variants in the neuroblastoma cell line ND7/23.

WT or mutant $Na_V1.6$ channels were transfected into ND7/23 cells and recorded in the presence of TTX to block endogenous Na^+ channels. (**A**) Representative Na^+ current traces for transfected $Na_V1.6$ wild-type (WT, black) or mutant channels (colour code in the right lower corner). (**B**) Peak Na^+ currents normalized by cell capacitance were plotted versus voltage. Both the p.(Phe846Ser) and p.(Leu840Pro) variants caused a hyperpolarising shift of the current-voltage relationship, whereas the p.(Val1758Ala) and p.(Thr1787Pro) variants caused a depolarising shift compared to WT channels. P.(Ile1654Asn), p.(Val1758Ala) and p.(Thr1787Pro) variants significantly decreased the current density in comparison to WT. WT: n = 30; mutants: n = 14-19. (**C**) Voltage-dependent steady-state activation curves. Lines represent Boltzmann functions fit to the data points. (**D**) Voltage-dependent steady-state inactivation curves. Lines represent Boltzmann functions fit to the data points. (**E**) Time course of recovery from fast inactivation at -100 mV. The p.(Val1758Ala), p.(Thr1787Pro) and p.(Asn374Lys) variants accelerated the recovery from fast inactivation compared to WT. (**F**) Voltage-dependence of the major time constant of fast inactivation τ_h. Shown are means ± SEM (**B**-**F**). Numbers of recorded cells and statistical analysis for all experiments are provided in Supplementary Table 2.

Figure 2. Effects of *SCN8A* variants in primary cultured hippocampal mouse neurons in the absence of TTX.

Neurons were transfected with WT or mutant $Na_V1.6$ channels and recorded in the absence of TTX. (**A**) Representative voltage traces of evoked action potentials (APs) in the absence of TTX from neurons transfected with WT (black) or mutant neurons (colour code indicated in **B**). (**B**) Numbers of evoked action potentials plotted versus injected current in the absence of TTX. Shown are means ± SEM. (**C**) Area under the curve for the input-output relationships. The p.(Thr1787Pro) variant shows a significantly decreased area under the curve compared to WT channels. (**D** and **E**) Rheobase (**D**) and threshold (**E**) of APs were decreased for neurons transfected with the p.(Phe846Ser) variant, but increased for neurons transfected with the p.(Thr1787Pro) variant compared to WT channels. Box-and-whisker plots (**C**-**E**) show means (plus sign), the 25th, 50th, 75th percentiles, minima and maxima; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; one-way ANOVA

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with Dunnett´s posthoc test or ANOVA on ranks with Dunn´s posthoc test were performed. Numbers of recorded cells and statistical analysis are provided in Supplementary Table 3.

Figure 3. Neuronal properties carried only by transfected WT or mutant $Na_V1.6$ channels. Hippocampal neurons transfected with wild-type or mutant $Na_V1.6$ channels were recorded in the presence of TTX to block endogenous Na^+ channels. (**A**) Representative voltage traces of evoked action potentials (APs) from neurons transfected with WT (black) or mutant channels (colour code in the lower left corner). (**B**) Numbers of evoked action potentials plotted versus injected current in the presence of TTX. Shown are means ± SEM. (**C**) Area under the curve for the input-output relationships. (**D**) Peak Na^+ current amplitudes of neurons transfected with WT or mutant $Na_V1.6$ channels in the presence of TTX. (**E** and **F**) Rheobase (**E**) and Threshold (**F**) were significantly decreased in neurons transfected with p.(Phe846Ser) or p.(Leu840Pro) variants compared to WT channels. The p.(Asn1877Ser) variant also significantly decreased the rheobase. The rheobase or threshold could not be obtained in neurons transfected with p.(Asn374Lys), p.(Val1758Ala), p.(Thr1787Pro) and p.(Ile 1654Asn) mutant channels due to very few evoked APs. Box-and-whisker plots (**C**-**F**) show means (plus sign), the 25th, 50th, 75th percentiles, minima and maxima; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; one-way ANOVA with Dunnett´s posthoc test or ANOVA on ranks with Dunn´s posthoc test were performed. Numbers of recorded cells and statistical analysis are provided in Supplementary Table 4.

Figure 4. Distribution of affected individuals carrying *SCN8A* variants according to phenotype and age of seizure onset.

(**A**-**C**) Phenotypic subgroups of the total cohort (**A**), and individuals carrying LOF (**B**) or GOF (**C**) variants. (**A**) In the total cohort, 225 affected individuals (57.4%) had BFIE, IE or DEE; 20 individuals (5.1%) had GE; 20 individuals (5.1%) had a NDDwoE. (**B**) Twenty-five affected individuals had GE or a NDDwoE, accounting for 73.5% of LOF variant carriers, (**C**) whereas 71.6% of GOF variant carriers had BFIE, IE or DEE. (**D**-**G**) Histogram of the age at seizure onset in affected individuals with BFIE(**D**), IE (**E**), DEE (**F**), GE (**G**)., (**H**) Stacked histogram of age at seizure onset in affected individuals with FE carrying GOF (FE+GOF), LOF (FE+LOF) or *SCN8A* variants which are not functionally characterized (FE). (**I**) Stacked histogram of age at seizure onset in affected individuals with GE carrying LOF (GE+LOF), or *SCN8A* variants which are not functionally characterized (GE). (**J**) Stacked histogram of age at seizure onset in affected

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individuals carrying GOF variants with FE (FE+GOF), or unclassifiable epilepsy (UE+GOF). (**K**) Stacked histogram of age at seizure onset in affected individuals carrying LOF variants with GE (GE+LOF), FE (FE+LOF) or unclassifiable epilepsy (UE+GOF). Affected individuals with BNIE, IE or DEE exhibited a significant earlier age at seizure onset than individuals with GE (Kruskal-Wallis test, $p < 0.001$), which is also observed for GOF vs. LOF variant carriers (Mann-Whitney test, $p < 0.001$). Histogram bin size = 1 month. (**L**-**M**) Seizure type distributions of affected individuals with FE vs. GE (**L**) and GOF vs. LOF variant carriers (**M**). BFIE=Benign Familial Infantile Epilepsy; IE=Intermediate Epilepsy; DEE= Developmental and Epileptic Encephalopathy; FE= Focal Epilepsy; GE= Generalized Epilepsy; NDD= Neurodevelopmental Disorder; GOF= Gain-of-function; LOF=Loss-of-function.

Figure 5. Correlation of a computed electrophysiological score with the clinical severity of *SCN8A* GOF variants.

Electrophysiological scores of *SCN8A* GOF variants were obtained according to the effect of variants on AP firing simulated by a single-compartment conductance based model. (**A**-**F**) The contrast of the simulated area under the input-output curve (AUC, equation 1) as a function of the changes of single Na^+ current gating parameters, such as: (**A**) the $V_{1/2}$ of the activation curve; (**B**) the slope factor k of the activation curve; (**C**) the $V_{1/2}$ of the fast inactivation curve; (**D**) the slope factor k of the fast inactivation curve; (**E**) the persistent Na^+ current; (**F**) the Na^+ conductivity (or current density). Changes of the $V_{1/2}$ and the slope of the activation curve had a much stronger effect on the AUC than other parameters. (**G**) Correlation (dashed gray line) of the simulation-based score (equation 9) with the severity of each *SCN8A* GOF variant averaged over affected individuals (blue dots with respective one-amino acid code). (**H**) Distributions of simulation-based scores of each patient (blue dots) for each of the four categories of clinical severities. * $p < 0.05$; *** $p < 0.001$ (ANOVA on ranks with Dunn´s posthoc test).

Figure 6. Location of *SCN8A* variants associated with neurodevelopmental disorders.

Schematic 2D representation of the $Na_V1.6$ channel displaying the location of pathogenic variants. A comparison of the location of missense variants with proven GOF or a BFIE/IE/DEE phenotype (without functional analysis) on one hand, and variants with proven LOF or GE/NDDwoE phenotypes (without functional analysis) on the other revealed a significant difference in the distribution of variants (see main text and supplement). Recurring variants are indicated with larger

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symbol size relative to the number of patients and in frame indels or deletions are indicated showing the whole affected regions (p.(Ile888_Val892delinsMet), p.(Glu1774_Ala1777del) and p.(Pro1428_Lys1473del)).
Abbreviations: BFIE=Benign Familial Infantile Epilepsy; IE=Intermediate Epilepsy; DEE= Developmental and Epileptic Encephalopathy; GE= Generalized Epilepsy; NDDwoE=NeuroDevelopmental Disorder without Epilepsy; GOF= Gain-of-function; LOF=Loss-of-function.

Figure 7. Treatment responses to anti-seizure medications in affected individuals carrying *SCN8A* variants.

(**A**, **B**) Treatment effects of anti-seizure medications (ASM) on seizures in affected individuals with BFIE, IE or DEE versus those with GE (**A**) and those carrying *SCN8A* GOF vs. LOF variants (**B**). KD = ketogenic diet; CLZ = clonazepam; VGB = vigabatrin; PB = phenobarbital; CLB = clobazam; ESM = ethosuximide; VPA = valproate; TPM = topiramate; LEV = levetiracetam; PHT = phenytoin; CBZ = carbamazepine; LTG = lamotrigine; OXC= oxcarbazepine; ZNS = zonisamide; LCM = lacosamide. PHT, CBZ, LTG, OXC, ZNS and LCM are sodium channel blockers (SCBs). (**C, D**) Individuals with BFIE, IE or DEE and those carrying *SCN8A* GOF variants responded significantly better to SCBs than non-SCBs, whereas treatment of SCBs or non-SCBs did not cause a different effect in individuals with GE and those carrying *SCN8A* LOF variants (please consider the very small numbers in these latter categories). P values derived from Fisher's exact test are provided in the Figure. Responders were defined as those becoming seizure free or experienced a seizure reduction staying on the drug; non-responders were defined as those experiencing no effect or seizure worsening.

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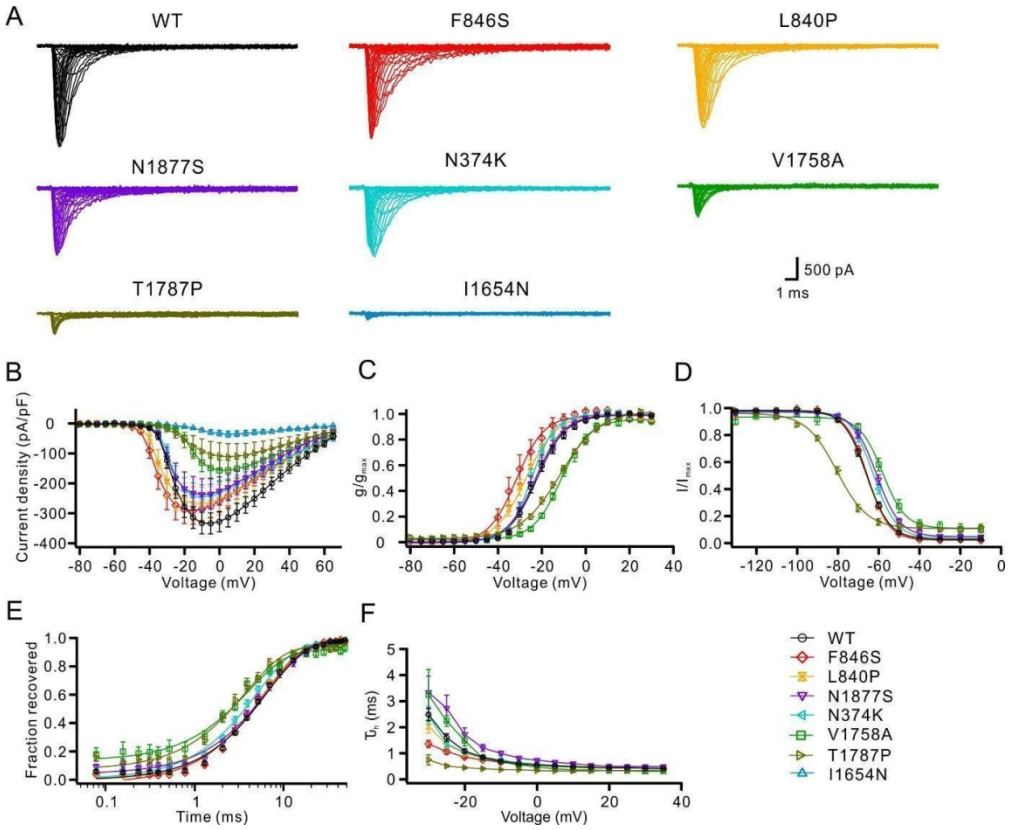

Figure 1

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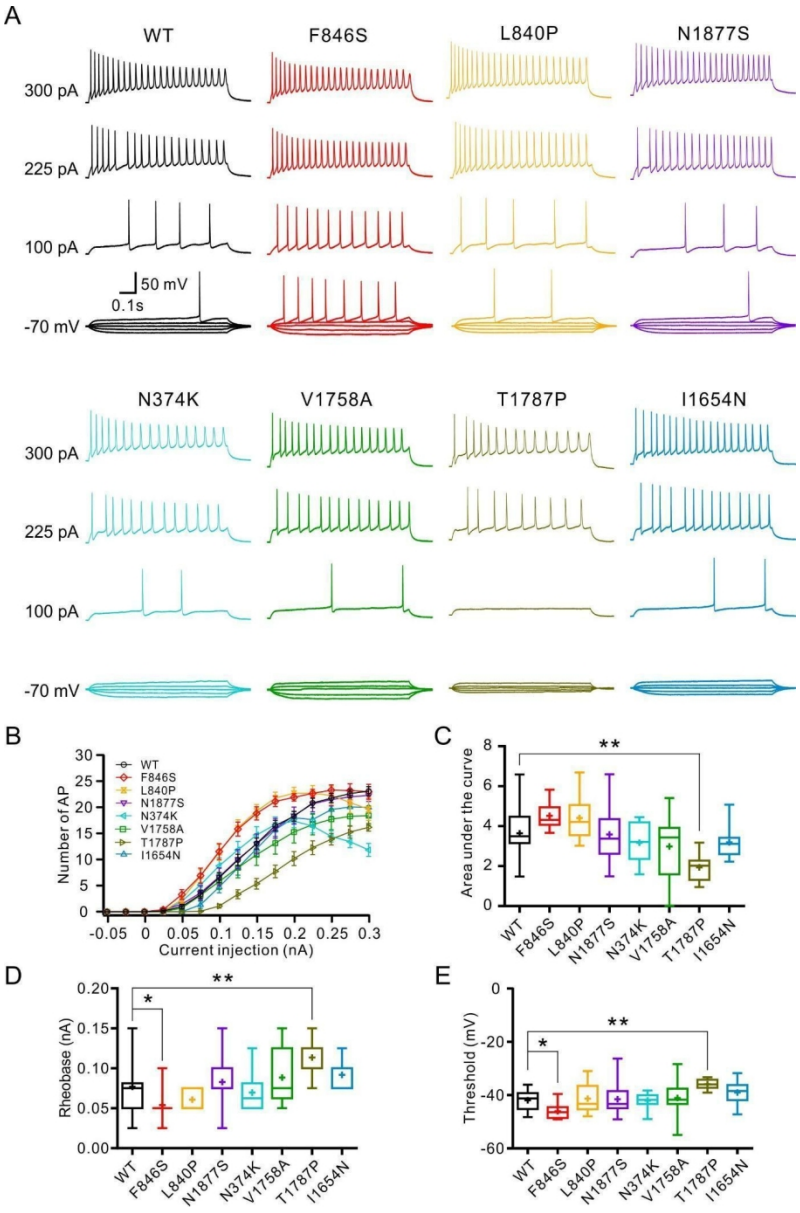

Figure 2

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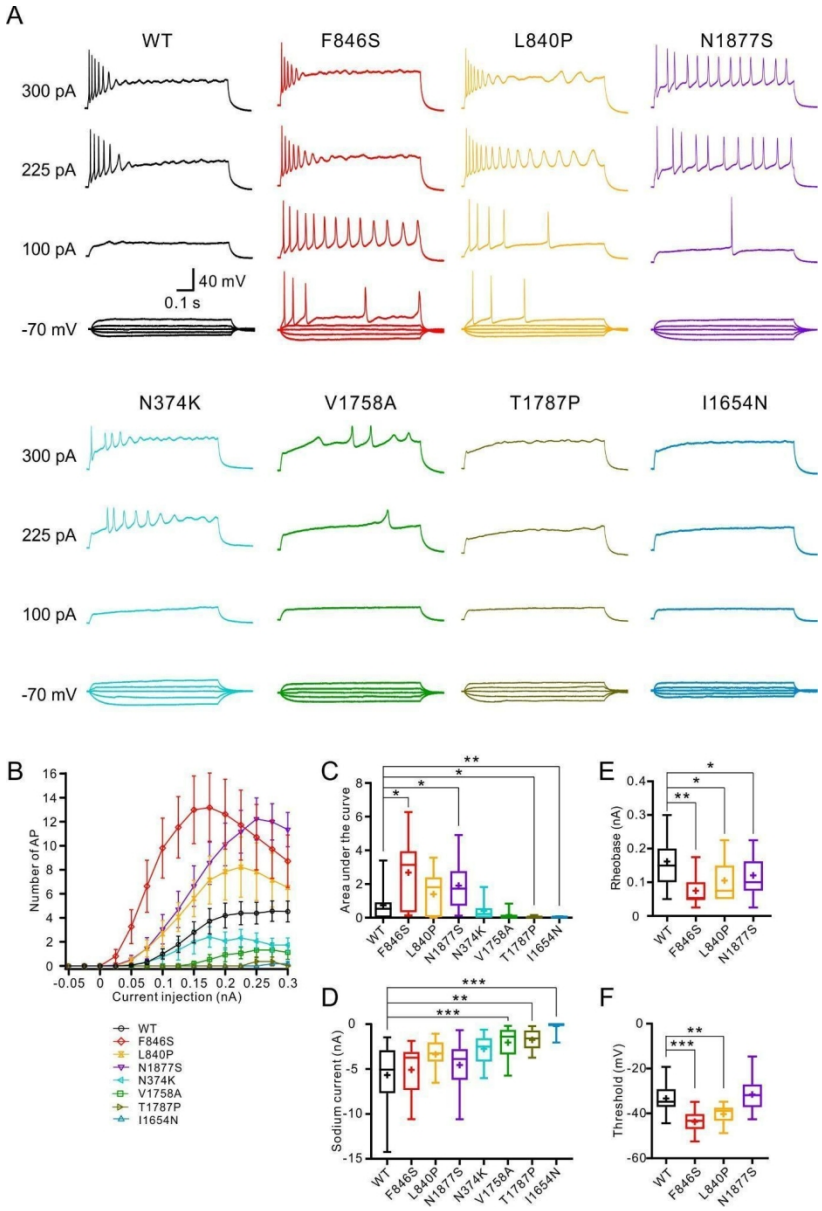

Figure 3

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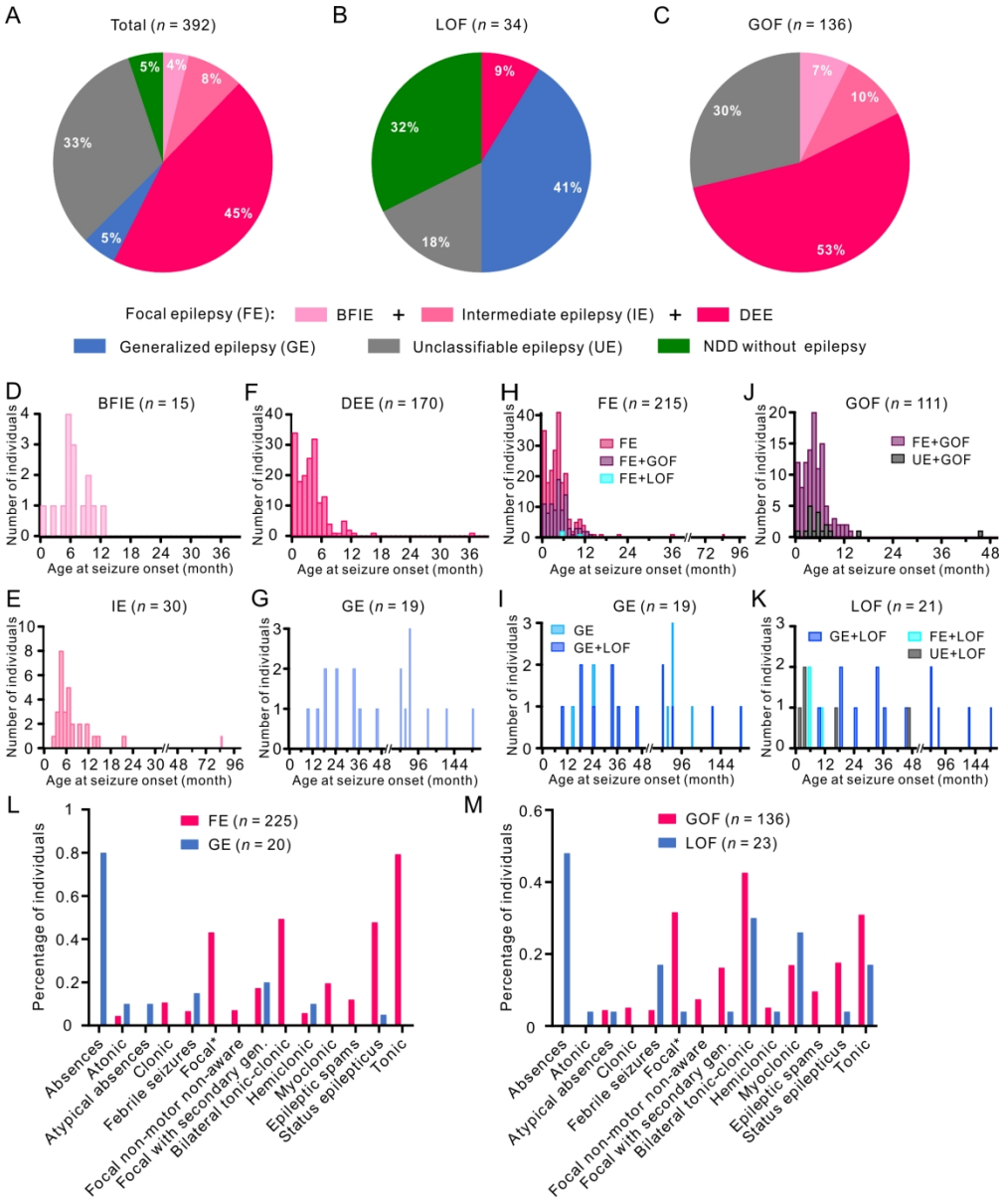

Figure 4

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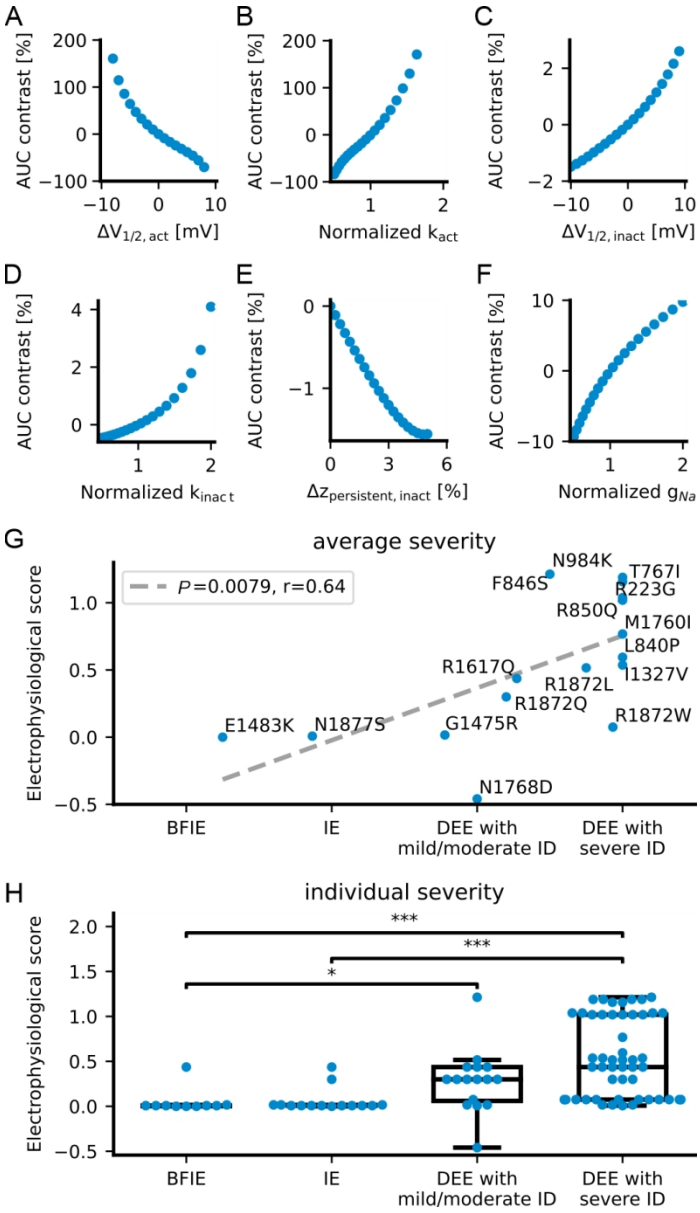

Figure 5

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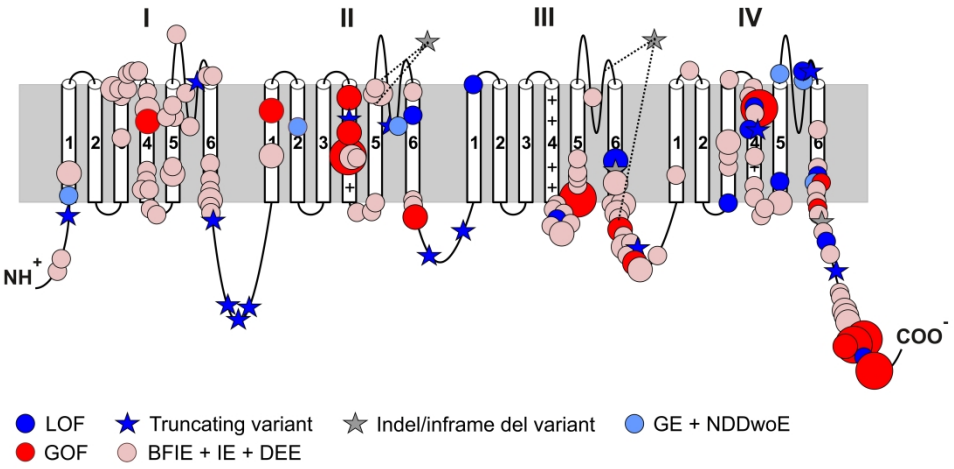

Figure 6

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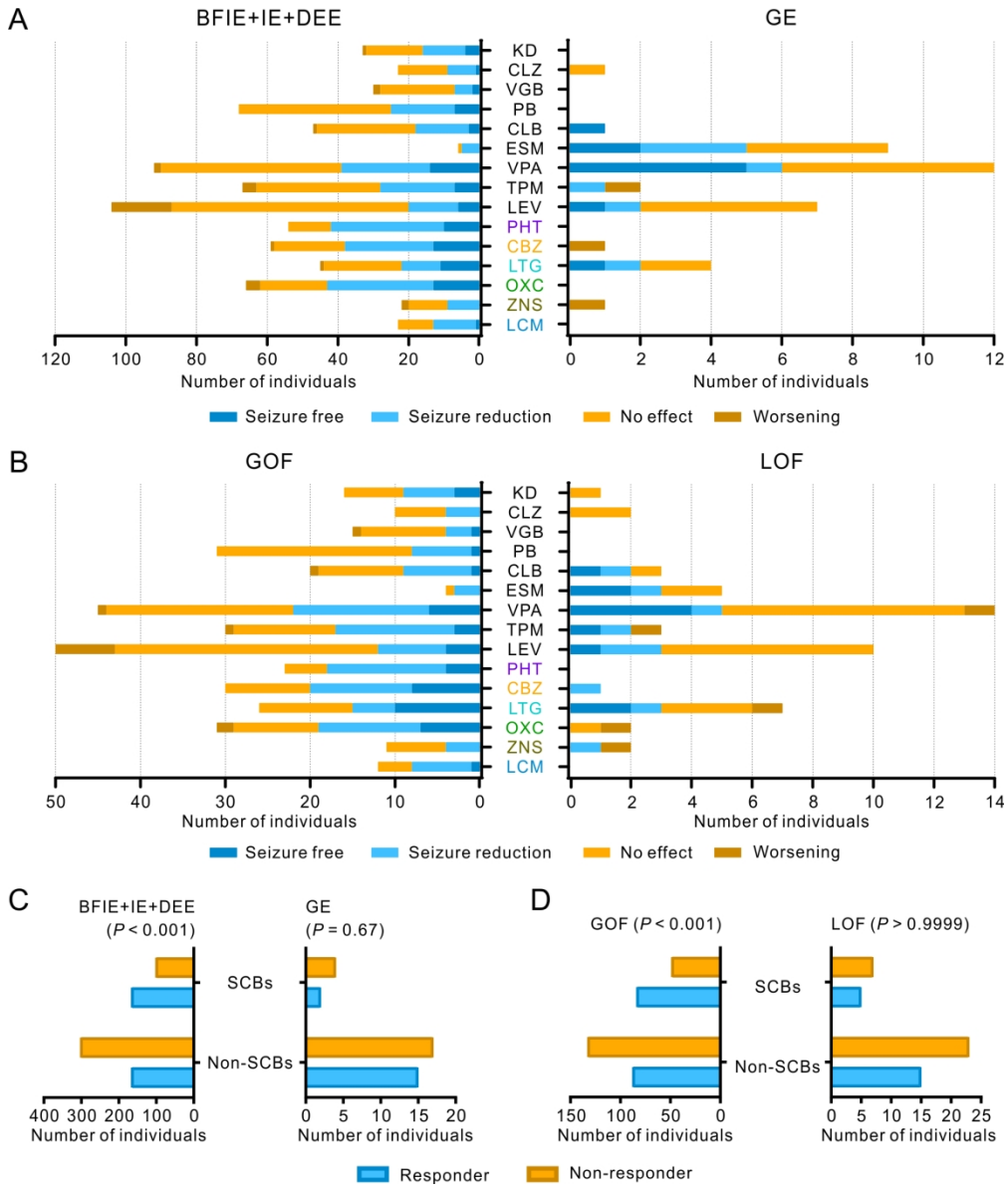

Figure 7

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Table 1 Clinical characteristics of LOF and GOF variants + phenotypic subgroups

	BFIE	Intermediate epilepsy	DEE	Generalized epilepsy	Neurodevelopmental disorder without epilepsy	LOF	GOF
Number of patients	15	33	177	20	20	34	136
Percentage with epilepsy	100%	100%	100%	100%	0%	68%	100%
Median age at seizure onset	6 months	5 months	3 months	42 months	-	24 months	4 months
Most common seizure types	Focal, focal to bilateral TC and bilateral TC	Focal, bilateral TC and tonic	Bilateral TC, focal and tonic	Absences, bilateral TC and febrile seizures	-	Absence seizures, bilateral TC and myoclonic seizures	Bilateral TCs, tonic and focal
Phenotype subgroups	-	-	-	-	-	GE 41% NDDwoE 32% DEE 9% UE 18%	BFIE 7% IE 10% DEE 53% UE 30%
Cognition	Normal 100%	Normal 33% Mild ID 52% Moderate ID 15%	Moderate ID 22% Severe ID 73% Unknown 5%	Normal 20% Mild ID 30% Moderate ID 35% Severe ID 10% Unknown 5%	Normal 10% Mild ID 45% Moderate ID 20% Severe ID 15% Unknown 10%	Normal 15% Mild ID 36% Moderate ID 15% Severe ID 21% Unknown 15%	Normal 11% Mild ID 9% Moderate ID 15% Severe ID 39% Unknown 26%
Comorbidities	Paroxysmal kinesigenic dyskinesia	Language delay, behavioral issues	Hypotonia, CVI, ataxia	Language delay, behavioral issues	Behavioral disorders (ASD, ADHD), delayed speech, microcephaly	Language delay, autism, behavioral issues, ataxia	Hypotonia, CVI, dyskinesia, ataxia
Mortality	0%	0%	10.2 %	0%	0%	0%	9.0%
Precision medicine	Sodium-channel blockers	Sodium-channel blockers	Sodium-channel blockers				Sodium-channel blockers

Abbreviations: BFIE: Benign familial infantile epilepsy, bilateral TCs: bilateral tonic clonic seizures, CVI: Cortical vision impairment, DEE: Developmental and epileptic encephalopathy, GE: Generalized epilepsy, GOF: Gain of function, ID: Intellectual disability, IE: Intermediate epilepsy, LOF: Loss of function, NDDwoE: Neurodevelopmental disorder without epilepsy, UE: Unclassified epilepsy

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