

## The spectrum of intermediate SCN8A-related epilepsy

Johannesen, Katrine M; Gardella, Elena; Encinas, Alejandra C; Lehesjoki, Anna-Elina; Linnankivi, Tarja; Petersen, Michael B; Lund, Ida Charlotte Bay; Blichfeldt, Susanne; Miranda, Maria J; Pal, Deb K; Lascelles, Karine; Procopis, Peter; Orsini, Alessandro; Bonuccelli, Alice; Giacomini, Thea; Helbig, Ingo; Fenger, Christina D; Sisodiya, Sanjay M; Hernandez-Hernandez, Laura; Krithika, Sundararaman; Rumpel, Melissa; Masnada, Silvia; Valente, Marialuisa; Cereda, Cristina; Giordano, Lucio; Accorsi, Patrizia; Bürki, Sarah E; Mancardi, Margherita; Korff, Christian; Guerrini, Renzo; von Spiczak, Sarah; Hoffman-Zacharska, Dorota; Mazurczak, Tomasz; Coppola, Antonietta; Buono, Salvatore; Vecchi, Marilena; Hammer, Michael F; Varesio, Costanza; Veggianti, Pierangelo; Lal, Dennis; Brünger, Tobias; Zara, Federico; Striano, Pasquale; Rubboli, Guido; Møller, Rikke S

*Published in:*  
Epilepsia

*DOI (link to publication from Publisher):*  
[10.1111/epi.14705](https://doi.org/10.1111/epi.14705)

*Publication date:*  
2019

*Document Version*  
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

*Citation for published version (APA):*  
Johannesen, K. M., Gardella, E., Encinas, A. C., Lehesjoki, A.-E., Linnankivi, T., Petersen, M. B., Lund, I. C. B., Blichfeldt, S., Miranda, M. J., Pal, D. K., Lascelles, K., Procopis, P., Orsini, A., Bonuccelli, A., Giacomini, T., Helbig, I., Fenger, C. D., Sisodiya, S. M., Hernandez-Hernandez, L., ... Møller, R. S. (2019). The spectrum of intermediate SCN8A-related epilepsy. *Epilepsia*, 60(5), 830-844. <https://doi.org/10.1111/epi.14705>

**General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

**Take down policy**

If you believe that this document breaches copyright please contact us at [vbn@aub.aau.dk](mailto:vbn@aub.aau.dk) providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from [vbn.aau.dk](http://vbn.aau.dk) on: December 05, 2025

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

DR. ELENA GARDELLA (Orcid ID : 0000-0002-7138-6022)

DR. THEA GIACOMINI (Orcid ID : 0000-0002-7802-8789)

DR. PASQUALE STRIANO (Orcid ID : 0000-0002-6065-1476)

PROF. GUIDO RUBBOLI (Orcid ID : 0000-0002-5309-2514)

Article type : Full length original research paper

### The spectrum of intermediate *SCN8A*-related epilepsy

Katrine M Johannesen (MD)<sup>1,2\*</sup>, Elena Gardella (MD, PhD)<sup>1,2\*</sup>, Alejandra C Encinas (MSc)<sup>3</sup>, Anna-Elina Lehesjoki (MD, Prof.)<sup>4,5</sup>, Tarja Linnankivi (MD, PhD)<sup>6</sup>, Michael B Petersen (MD)<sup>7,8</sup>, Ida Charlotte Bay Lund (MD)<sup>7</sup>, Susanne Blichfeldt (MD)<sup>9</sup>, Maria J Miranda (MD, PhD)<sup>9</sup>, Deb K Pal (MD, PhD)<sup>10,11,12,13</sup>, Karine Lascelles (MD)<sup>10</sup>, Peter Procopis (MD)<sup>14,15</sup>, Alessandro Orsini (MD)<sup>16</sup>, Alice Bonuccelli (MD)<sup>16</sup>, Thea Giacomini (MD)<sup>17</sup>, Ingo Helbig (MD)<sup>18,19</sup>, Christina D Fenger<sup>1</sup>, Sanjay M Sisodiya (MD, Prof.)<sup>20,21</sup>, Laura Hernandez-Hernandez (MD)<sup>20,21</sup>, S Krithika (PhD)<sup>20,21</sup>, Melissa Rumple (MD)<sup>22</sup>, Silvia Masnada (MD)<sup>23</sup>, Marialuisa Valente<sup>24</sup>, Cristina Cereda<sup>24</sup>, Lucio Giordano (MD)<sup>25</sup>, Patrizia Accorsi (MD)<sup>25</sup>, Sarah E Bürki (MD)<sup>26</sup>, Margherita Mancardi (MD)<sup>27</sup>, Christian Korff (MD)<sup>28</sup>, Renzo Guerrini (MD, Prof.)<sup>29</sup>, Sarah von Spiczak (MD)<sup>30,31</sup>, Dorota Hoffman-Zacharska (MD, PhD)<sup>32</sup>, Tomasz Mazurczak (MD, PhD)<sup>33</sup>, Antonietta Coppola (MD, PhD)<sup>34</sup>, Salvatore Buono (MD)<sup>35</sup>, Marilena Vecchi<sup>36</sup>, Michael F Hammer (MSc, PhD)<sup>37</sup>, Costanza Varesio (MD)<sup>38,39</sup>, Pierangelo Veggiotti (MD, Prof.)<sup>40,41</sup>, Dennis Lal<sup>42,43,44,45,46</sup>, Tobias Brünner<sup>46</sup>, Federico Zara (PhD)<sup>47</sup>, Pasquale Striano (MD, PhD)<sup>48</sup>, Guido Rubboli (MD, Prof.)<sup>1,49</sup>, Rikke S Møller (MSc, PhD)<sup>1,2</sup>

\* These authors contributed equally

<sup>1</sup> Department of Epilepsy Genetics and Personalized Treatment, The Danish Epilepsy Centre  
Filadelfia, Dianalund, Denmark

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/EPI.14705](#)

This article is protected by copyright. All rights reserved

- <sup>2</sup> Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark
- <sup>3</sup> Graduate Interdisciplinary Program of Genetics, University of Arizona, Arizona, United States
- <sup>4</sup> Folkhälsan Research Center, Helsinki, Finland
- <sup>5</sup> Research Programs Unit, Molecular Neurology and Medicum, University of Helsinki, Helsinki, Finland
- <sup>6</sup> Department of Child Neurology, Children's Hospital, University of Helsinki and Helsinki University Hospital, Finland
- <sup>7</sup> Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark
- <sup>8</sup> Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
- <sup>9</sup> Department of Pediatrics, Herlev Hospital, Herlev, Denmark
- <sup>10</sup> Department of Basic & Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom
- <sup>11</sup> King's College Hospital, London, United Kingdom
- <sup>12</sup> Evelina London Children's Hospital, London, United Kingdom
- <sup>13</sup> MRC Centre for Neurodevelopmental Disorders, King's College, London, United Kingdom
- <sup>14</sup> The Children's Hospital, Westmead, Sydney, Australia
- <sup>15</sup> Discipline of Child and Adolescent Health, Sydney Medical School University of Sydney, Australia
- <sup>16</sup> Pediatric Neurology, Pediatric Clinic, University of Pisa, Pisa, Italy
- <sup>17</sup> Child Neuropsychiatry Unit, Istituto Giannina Gaslini, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics and Maternal and Children's Sciences, University of Genoa, Genoa, Italy
- <sup>18</sup> Department of Neuropediatrics, University Medical Center Schleswig Holstein, Kiel, Germany
- <sup>19</sup> Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States
- <sup>20</sup> Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London WC1N3BG, United Kingdom
- <sup>21</sup> Chalfont Centre for Epilepsy, Bucks, United Kingdom
- <sup>22</sup> Pediatric Neurology, Banner Children's Specialists, Glendale, Arizona, United States
- <sup>23</sup> Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy
- <sup>24</sup> Genomic and post-Genomic Center, IRCCS Mondino Foundation, Pavia, Italy

- <sup>25</sup> Child Neurology and Psychiatry Unit, Spedali Civili, Brescia, Italy
- <sup>26</sup> Department of Pediatrics, Division of Child Neurology, University Children's Hospital Bern, University of Bern, Bern, Switzerland
- <sup>27</sup> Unit of Child Neuropsychiatry, Epilepsy Centre, Department of Clinical and Surgical Neurosciences and Rehabilitation Giannina Gaslini Institute, Genoa, Italy
- <sup>28</sup> Child Neurology Unit, University Children's Hospital, Geneva, Switzerland
- <sup>29</sup> Neuroscience Department, Children's Hospital Anna Meyer-University of Florence, Florence, Italy
- <sup>30</sup> Department of Neuropediatrics, Christian-Albrechts-University, Kiel, Germany
- <sup>31</sup> Northern German Epilepsy Center for Children & Adolescents, Schwentinental/OT Raisdorf, Germany
- <sup>32</sup> Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland
- <sup>33</sup> Department of Neurology of Children and Adolescents, Institute of Mother and Child, Warsaw, Poland
- <sup>34</sup> Department of Neuroscience, Reproductive and Odontostomatological Sciences, Federico II University, Naples, Italy
- <sup>35</sup> Neurology Division, AORN, Santobono Pausilipon, Naples, Italy
- <sup>36</sup> Pediatric Clinic – Azienda Ospedaliero, University of Padova, Padova, Italy
- <sup>37</sup> University of Arizona Genetic Core, University of Arizona, Tucson, Arizona, United States
- <sup>38</sup> Brain and Behaviour Department, University of Pavia, Italy
- <sup>39</sup> Child and Adolescence Neurology Department, IRCCS C. Mondino National Neurological Institute, Pavia, Italy
- <sup>40</sup> Department of Child Neurology, Childrens Hospital V. Buzzi, Milan, Italy
- <sup>41</sup> Department of Biomedical and Clinical Sciences, L. Sacco – University of Milan, Milan, Italy
- <sup>42</sup> Epilepsy Center, Neurological Institute, Cleveland Clinic, Cleveland, Ohio, United States
- <sup>43</sup> Genomic Medicine Institute, Lerner Research Institute Cleveland Clinic, Cleveland, Ohio, United States
- <sup>44</sup> Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States

<sup>45</sup> Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts, United States

<sup>46</sup> Cologne Center for Genomics (CCG), University of Cologne, Cologne, Germany

<sup>47</sup> Laboratory of Neurogenetics and Neuroscience, Department Head-Neck and Neuroscience, Istituto Giannina Gaslini, Genova, Italy

<sup>48</sup> Pediatric Neurology, Pediatric Clinic, University of Studies of Pisa, Pisa, Italy

<sup>49</sup> University of Copenhagen, Copenhagen, Denmark

**Corresponding author:**

Rikke Steensbjerre Møller and Elena Gardella

Kolonivej 1, 4193 Dianalund, Denmark

e-mail: [rimeo@filadelfia.dk](mailto:rimeo@filadelfia.dk), [elga@filadelfia.dk](mailto:elga@filadelfia.dk)

**Key words:** *SCN8A*, epilepsy, genetics, intellectual disability

Page count: 18

Word count: 2945

Reference count: 35

Figure count: 2

Table count: 1

**Abstract:**

Objective: Pathogenic variants in *SCN8A* have been associated with a wide spectrum of epilepsy phenotypes, ranging from benign familial infantile seizures (BFIS) to epileptic encephalopathies with variable severity. Furthermore, a few patients with intellectual disability (ID) or movement disorders without epilepsy have been reported. The vast majority of the published *SCN8A* patients suffers from severe developmental and epileptic encephalopathy (DEE). In this study, we aimed to provide further insight on the spectrum of milder *SCN8A*-related epilepsies.

Methods: A cohort of 1095 patients was screened using a next-generation sequencing panel.

Further patients were ascertained from a network of epilepsy genetics clinics. Patients with severe DEE and BFIS were excluded from the study.

**Results:** We found 36 probands who presented with a *SCN8A*-related epilepsy and normal intellect (33%) or mild (61%) to moderate ID (6%). All patients presented with epilepsy between age 1.5 months and seven years (mean 13.6 months), and 58% of these became seizure free, 2/3 on monotherapy. Neurological disturbances included ataxia (28%) and hypotonia (19%) as the most prominent features. Interictal EEG was normal in 41%. Several recurrent variants were observed including Ile763Val, Val891Met, Gly1475Arg, Gly1483Lys, Phe1588Leu, Arg1617Gln, Ala1650Val/Thr, Arg1872Gln and Asn1877Ser.

**Significance:** With this study, we explore the electro-clinical features of an intermediate *SCN8A*-related epilepsy with mild cognitive impairment and for the majority a treatable epilepsy.

**Key words:** *SCN8A*, voltage-gated sodium channels, epilepsy, epilepsy genetics, intellectual disability

#### **Introduction:**

*SCN8A* encodes the voltage-gated sodium channel  $Na_v1.6$ , which is primarily expressed in excitatory neurons with high concentrations at the axon initial segment and the node of Ranvier. Pathogenic variants in *SCN8A* are associated with a spectrum of epilepsy phenotypes, ranging from rare families with benign familial infantile seizures (BFIS) to severe early onset developmental and epileptic encephalopathies (DEE) (EIEE13, OMIM #614558). Since the first case report published by Veeramah and colleagues in 2012<sup>1</sup>, several reports have confirmed the role of *SCN8A*, primarily in patients with DEE<sup>2-9</sup>. The majority of patients with *SCN8A* DEE, described so far, have a severe phenotype characterized by early seizure onset, difficult to treat seizures, severe intellectual disability (ID), motor disorders and a relatively high mortality<sup>2-10</sup>.

Functional studies of selected variants causing DEE, have revealed gain-of-function as the main pathogenic mechanism<sup>1; 6; 11; 12</sup>. This gain-of-function comes from hyperactivity of the ion channel, due to elevated persistent sodium currents, hyperpolarizing shifts in the voltage dependence of activation or impaired channel current inactivation<sup>12; 13</sup>. This mechanism is the opposite of the one that has been demonstrated in Dravet syndrome, which is characterized by loss-of-function variants in *SCN1A*<sup>14</sup>. *SCN1A* encodes the voltage gated sodium channel  $Nav1.1$ , which is primarily expressed in inhibitory interneurons. Variants in *SCN2A*, encoding a third voltage-gated sodium

channel in the human CNS, can lead to both gain- and loss-of-function, complicating things even more<sup>15</sup>. Awareness of these important differences in pathophysiology is necessary, as it might have therapeutic implications<sup>16</sup>.

We have recently detected a recurrent *SCN8A* variant, Glu1483Lys, in a very mild familial epilepsy phenotype<sup>17</sup>. We identified three unrelated families with a total of 16 family members who presented with BFIS and normal cognition. Five family members developed paroxysmal kinesigenic dyskinesia (PKD)<sup>17</sup>. Recently, Han et al. confirmed BFIS as part of the phenotypic spectrum in *SCN8A*-related epilepsies<sup>18</sup>. Beside these two extreme phenotypes, there is an increasing number of patients with a milder form of DEE. In this study, we describe the spectrum of the electro-clinical features of this intermediate *SCN8A*-epilepsy phenotype, aiming to provide a clearer picture for clinicians, genetic counselors and affected families.

#### **Methods and material:**

We systematically screened all exons and exon-intron boundaries of *SCN8A* in a cohort of 1095 unselected patients with various forms of epilepsy using different next-generation sequencing (NGS) panels<sup>19</sup>. The panels included from 45 to 500+ genes related to epilepsy, intellectual disability or autism. Variants were assumed to be pathogenic if they arose *de novo*, or were inherited from an affected parent or affected/unaffected mosaic parent, and if they were non-synonymous, splice-site altering or frameshift causing, and not present in controls in the gnomAD browser (see web resources). Sanger sequencing confirmed variants and segregation.

Furthermore, detected variants were tested (PolyPhen-2, SIFT and MutationTaster) for predicted pathogenicity. ACMG and MPC scores are noted in table 1. All variants, except for one, were either pathogenic or likely pathogenic according to the ACMG criteria<sup>20</sup>.

Four different metrics were used for in silico variant pathogenicity prediction:

- 1) The 'Missense badness, PolyPhen-2, and Constraint score' (MPC), which demonstrates a 5.8 times increased variant enrichment in cases compared to individuals from the general population for MPC scores >2<sup>21</sup>
- 2) The 'paralog conservation score' (parazscore), which quantifies the amino-acid positions conservation across human proteins of the same gene family. A significant enrichment of disease-associated missense variants was observed at paralog-conserved sites<sup>22</sup>;

- 3) The Grantham score, which accesses the effect of the amino acid substitution based on the properties of the amino acid exchange. It ranks amino acid substitutions from similar amino acids substitutions (0) to substitution which differs in their chemical properties ones (215)<sup>23</sup>.
- 4) The allele frequency analysis based on the alleleFrequencyApp (see web resources), which calculates a maximum credible number of possible pathogenic alleles observed in gnomAD. The allele count is estimated based on the disease prevalence, the allelic and genetic heterogeneity and the variant penetrance. For *SCN8A* we specified the disease prevalence as one in 300 the allelic heterogeneity as 0.01, the genetic heterogeneity as 0.1 and the penetrance as 50% resulting in a maximum number of a single allele in gnomAD. We compared the corresponding value with the allele frequency of variants present in gnomAD.

*SCN8A* positive patients underwent a detailed clinical evaluation and patients with severe DEE and BFIS were excluded from the study. The criteria for severe DEE were defined as severe, pharmaco-resistant epilepsy and developmental impairment, as previously reported<sup>24</sup>. BFIS is a self-limiting epilepsy syndrome characterized by afebrile seizures typically in clusters, with onset between four and eight months. Neurological examination, psychomotor development and the interictal EEG are normal and the children become seizure free within the first years of life and sustain seizure freedom without the aid of antiepileptic drugs (AEDs)<sup>25</sup>.

Additional probands were collected from an international network of epilepsy genetics clinics. Data on clinical phenotype, genetics, neuroimaging and EEG were requested for all patients (and if possible relatives), who were included in the study. Seizures were classified according to the International League Against Epilepsy (ILAE)<sup>26</sup>. All probands, and in case of minors, legal parents, provided written informed consent. The study was approved by the local ethical committees. Sodium channel blockers (SCBs) were defined as AEDs that target sodium channels and included carbamazepine (CBZ), oxcarbazepine (OXC), lamotrigine (LTG) and phenytoin (PHT).

Data sharing: anonymized data will be shared by request from any qualified investigator.

## Results:

By the use of targeted NGS screening of *SCN8A* in a cohort of 1095 patients, we identified 12 (1.2%) probands with a predicted pathogenic variant in *SCN8A*. Six of the patients fulfilled the

criteria for a severe DEE and thus excluded from this study. Of the 1095 patients screened, approximately 326 fulfilled a DEE diagnosis (this might be an underestimate, as referrals to our center are often lacking clinical information). In addition to the remaining six patients, we ascertained 30 additional probands/families with predicted pathogenic *SCN8A* variants through collaborating diagnostic and research laboratories. The Asn1877Ser variant was seen in three controls in gnomAD, but as the phenotype resembles a benign familial epilepsy, with several family members affected, and was seen in three probands in this study, it was included as being pathogenic. All variants, except #10 p.Tyr1241Cys, were classified as pathogenic or likely pathogenic according to the ACMG criteria<sup>20</sup>. Not all missense variants in *SCN8A* are pathogenic and at 384 amino acid positions variants have been reported in the general population<sup>27</sup>. Distinguishing disease-causing variants from benign is still a challenge in clinical genetics. Rarity of an allele is widely recognized as a necessary (though not sufficient) criterion for variant pathogenicity<sup>20</sup> (ACMG guidelines, but the key question “how common is too common?” remains poorly answered for many diseases.) Estimating the genetic and allelic heterogeneity offers an opportunity to identify variant cut-off frequency filters. Genetic heterogeneity is the maximum proportion of disease that is attributable to variation in a single gene, and allelic heterogeneity is the maximum proportion of variation within a gene that is attributable to a single allele. The patient variant 10 (c.3722A>G, p.Tyr1241Cys, NM\_014191.3) was found once in the gnomAD<sup>27</sup> and discovEHR<sup>28</sup> database. The presence of a variant in databases represents not a pathogenicity exclusion criterion based on our allele frequency analysis for mild forms of epilepsy. The amino acid residue position is relatively conserved across voltage-gated sodium channels (positive parazscore of 0.23<sup>22</sup>). In addition, the MPC score (MPC = 2.48) supports variant pathogenicity and the Grantham score of 194 indicates a likely pathogenic variant. One of the probands (#30) has previously been mentioned briefly<sup>29</sup>, but was included in this study since additional data had been collected. In total, 36 probands/families were included. The detected *SCN8A* variants were mainly missense variants (33/36) scattered throughout the gene. In addition, two truncating variants (one frameshift (#4) and one stop (#30)), and one variant causing a nucleotide change eight basepairs upstream of the start codon (#1) were detected. Fourteen variants were located in the transmembrane domains and two in the poreforming domain, of the cytoplasmic variants, we

found one in the D1/D2 domain. The variant Asn544fs\* was a frameshift variant with a clinical picture of absence epilepsy, inherited from an affected parent, and classified pathogenic according to the ACMG classification guidelines.

The variants either occurred *de novo* or segregated within the family in a dominant fashion (#2, #4, #7, #14, #15, #33 and #34, see figure 1 for selected pedigrees). In the family of proband #33 the variant was inherited from an affected mosaic parent. Mosaicism was also suspected in #15, but has not yet been confirmed. All variants were located at highly conserved residues and predicted possibly damaging according to computational prediction software (see methods and web resources). Mining the available literature and databases, eight variants were found to be recurrent either within this study or overall; Ile763Val<sup>30</sup>, Val891Met<sup>31</sup>, Gly1475Arg<sup>10; 32</sup>, Gly1483Lys<sup>17</sup>, Phe1588Leu, Arg1617Gln<sup>5; 7; 8; 33; 34</sup>, Ala1650Val<sup>5; 7; 35</sup> (different amino acid substitution, see discussion section), Arg1872Gln<sup>7; 12; 36</sup> and Asn1877Ser<sup>30; 35; 37</sup>, with five of them (amino acid positions 763, 1475, 1617, 1650, 1872) seen in severe DEE phenotypes as well. Clinical data on all 36 probands and families are presented in table 1. Age at inclusion varied from nine months to 35 years, with a mean of 7.9 years. Seizure onset was between six weeks and seven years, with a mean of 13.6 months (SD  $\pm$ 17 m). Seizure semiology was very diverse, and included generalized tonic-clonic seizures (GTCs) (21), focal (14), tonic (8), myoclonic (6) and atonic seizures (8), as well as epileptic spasms (2) and atypical / typical absences (12). Seizure severity did not progress over time and seizure triggers were not found. Three probands experienced convulsive status epilepticus (#3, #14, #33).

The interictal EEG was available in 33/36 patients and showed focal epileptiform abnormalities in 15 patients (45%), predominant in the posterior quadrants in (ten patients) or in the central/centro-parietal / fronto-central regions (five patients), with or without bilateral spreading. Four patients (12%) had only generalized abnormalities (#6, #14, #22, #26). In 14 patients (39%) the interictal EEG was normal (#9, #11, #12, #13, #16, #17, #20, #23, #29, #34, #35) or normalized at follow up (#15). Ictal EEG was available in four patients and showed a focal discharge in two (#6, #11) and generalized spike or irregular spike and waves discharges in the other two (#19, #28). Cognitively, these patients fare well. Before seizure onset 26/36 (72%) had normal cognitive development, two/36 (6%) had a mild ID, one/36 had moderate ID (3%) and four/36 (11%) had developmental delay, not classified due to the young age of the patient. At follow-up, a

deterioration from normal intellect/developmental delay to mild ID or from mild ID/developmental delay to moderate ID was seen in seven (19%) patients, whereas 81% did not experience deterioration at seizure onset. After seizure onset (and at the time of follow-up) 22/36 (61%) had mild ID and two/36 (6%) had moderate ID.

Additional features included ataxia (10/36), hypotonia (7/36), language delay (5/36), autism/autistic features (4/36), movement disorders (3/36) including paroxysmal dyskinesia (3/36), gait disturbances (2/36), sleep disturbances (1/36), learning difficulties (2/36) and ADHD (1/36). See table 1 for details.

All probands were evaluated for their treatment response. Twenty-one/36 (58%) probands became seizure free. Monotherapy was sufficient in 12/21 (57%) probands, and included lamotrigine (LTG) (2), carbamazepine (CBZ) (1), phenytoin (PHT) (1), valproate (VPA) (1), vigabatrin (VGB) (1), ethosuximide (ETX) (1), phenobarbital (PB) (1) and levetiracetam (LEV) (1). In total 13/36 (36%) probands became seizure free with therapy that included a SCB either in monotherapy or in combination with other antiepileptic drugs (AEDs). One became seizure free with a small dose of cannabidiol. However, 7/36 (20%) probands became seizure free without the use of SCBs. Seizure offset was only available for six patients, and ranged between four and 10 years, mean age at seizure offset was 7.7 years.

Six probands had affected family members, segregating with the variants. For proband #5 a similar phenotype with absences and learning/language difficulties was seen in the sister, as well as the mother. For proband #7, the mother also had unspecified epilepsy and carried the variant; there was also an affected maternal grandfather, who did not have genetic analysis done. Likewise, proband #14 had an affected father, who carried the variant, and an affected paternal grandfather, who was not tested genetically. Proband #15 had a sister with a similar epilepsy phenotype, the variant was suspected to have been inherited from a mosaic parents, but this was not confirmed at the time of preparation of this paper. Proband #33 had an affected sister with a similar phenotype, and genetic investigations showed that the variant was inherited from an affected mosaic (28%) father. Proband #34 had an affected sister and mother, in whom the variant segregated.

## Discussion:

Pathogenic variants in *SCN8A* have so far been described in patients with different epilepsy phenotypes, including rare families with BFIS and in >100 patients with mild to severe DEE. In this study, we describe the electro-clinical phenotype of 36 patients with intermediate epilepsies due to pathogenic variants in *SCN8A*. Patients with *SCN8A*-related severe DEE<sup>21</sup> and BFIS<sup>18</sup> were excluded from the study.

The variant at position c.-8A>G was assumed to be likely pathogenic because it occurred de novo and the clinical features of the patient resembled the phenotype seen in other *SCN8A* patients. The variant is located outside of the Kozak consensus sequence, but may lead to increased RNA stability or translational initiation or result in altered splicing pattern. These theories can only be confirmed by functional testing of the variant, which unfortunately was not possible in this study. Until then, the variant may need to be classified as a variant of unknown significance.

All 36 patients presented with seizures in early childhood (mean 13.6 months). Before seizure onset, 72% probands had normal cognitive development. More than half of the probands became seizure free, 57% of these with monotherapy. Compared to the BFIS families, described by Gardella et al<sup>17</sup>, Anand et al<sup>37</sup> and Han et al<sup>18</sup>, the majority of the probands in this cohort have cognitive impairment, with 6% suffering from moderate ID, and 61% from mild ID. Furthermore, only 58% became seizure free compared to almost 100% of the BFIS patients; seizure freedom is exceptional in the severe DEE phenotype. The patients herein described also appeared to have additional neurological disturbances including primarily ataxia (in 28%) and hypotonia (in 19%). In the severe DEE cohort, the incidence of ataxia is around 11%, compared to the 28% of this cohort. However, it is important to notice that many of the patients suffering from severe *SCN8A* DEE are unable to walk autonomously. Furthermore, the patients in this intermediate cohort do not suffer from the spasticity and paraplegia or the extra-pyramidal/cerebellar symptoms that up to 50% of the severe DEE patients do<sup>24</sup>. A few patients in this cohort (8%) had dyskinesia, which is also seen in both severe DEEs and BFIS families. Growth impairment (microcephaly or reduced growth) observed in severe DEE, was not seen in this cohort. Other prominent phenotypic features included language delay/difficulties in 14% and movement disorders not further specified in 8%. In mouse models, it has been shown that *SCN8A* is widely expressed, both in the motor neurons of the brain stem, as well as in many types of neurons in the cerebellum, where functional deficits in Purkinje cells have been found<sup>38-40</sup>, confirming the importance of *SCN8A* in motor function. This

could explain the involvement of the motor system, and why cerebellar atrophy and ataxia<sup>41</sup>, associated with intellectual impairment, appeared to be major features in subjects harboring *SCN8A* variants. The cerebellum does, however, also play an important role in language and grammar processing, verbal working memory and speech motor planning<sup>42</sup>, and a large proportion of *SCN8A* patients, including those reported in the present cohort, show an impairment of these functions as well.

When comparing the present cohort to the severe *SCN8A* DEE population, in which the patients have earlier seizure onset, with more pronounced cognitive deficits as well as refractory epilepsy, we tried to identify possible predictive factors, that in newly diagnosed patients could help to detect those with a milder course as compared to those with a more severe evolution. First, within our cohort, the majority of probands have normal development prior to seizure onset (and 33% of them continue to develop normally) and maintain a normal EEG (41%). This is often not the case in probands who develop severe DEE, in which the majority usually are cognitively delayed from birth<sup>43</sup>, or will present with cognitive difficulties early on and show changes in their EEGs.

However, it is not an absolute feature, as we have observed several children with an extremely severe follow up, despite early normal development. Second, seizure onset occurred at a mean age of 13.6 months, compared to a mean age of 4 months in the DEE group<sup>43</sup>. Anyway, it is worth to bear in mind that the age deviation in this cohort is quite large ( $SD \pm 17$  months), and thus these numbers should be interpreted carefully when counselling a family. Last, seizure freedom was obtained in 58% of the patients in this cohort and it was achieved rapidly and with monotherapy in 2/3. In contrast, only about 5% of patients in the severe DEE cohort achieve seizure freedom with monotherapy. This is important, albeit also a prerequisite in this study, where the more severe epilepsies were excluded.

Previously, it has been hypothesized, that seizures in patients with *SCN8A* variants should respond to SCBs<sup>16; 17</sup>. A beneficial effect of SCBs was observed in 36% of the patients reported in this study, either as monotherapy or in combination with other AEDs, and supportive of this, previous studies have shown a gain-of-function of the Arg1617Gln and Arg1872Leu<sup>12</sup> variants. Yet, 19% became seizure free without the use of SCBs, suggesting that seizure freedom should not be attributed solely to the use of SCBs, but also considered a phenotypic trait.

Interestingly, a partial benefit of LTG was also observed in the proband with a stop variant (#30). We can speculate that this unexpected finding may depend on genetic modifiers and differences in genetic background, or the fact that as the stop-codon is located at the c-terminal part of the protein, the transcript does not undergo nonsense-mediated decay.

Furthermore, we found three variants, i.e. one eight basepairs upstream of the start codon (#1), one frameshift variant (#4) and one stop variant (#30), all suspected to be loss-of-function, and all of them associated with an epilepsy phenotype. Previously, it has been hypothesized, that loss-of-function variants would not cause epilepsy<sup>44</sup>, but rather present with ID with or without motor function abnormalities, such as ataxia<sup>2; 34; 41</sup>. However, Blanchard et al. also describe a patient with epilepsy and ID, carrying a LOF variant<sup>11</sup>, and two existing *Scn8a* knock-out mice models, supports the notion of epilepsy also being a feature of LOF of NaV1.6<sup>45; 46</sup>. Other ion channel genes, including *SCN2A* have been shown to have a similar clinical picture, where both GOF and LOF variants may cause epilepsy. The underlying functional causes are yet to be fully elucidated. Variants were found throughout the gene (see figure 3), including the transmembrane segments, cytoplasmic loops and the inactivation gate, thus location in the gene is likely not predictable of functional or clinical effects of the variant, however, the figure does display the fact that the majority of the variants are found in domain three and four, the inactivation gate and the c-terminal, which may help guide variant interpretation.

Indeed, we identified several recurrent variants and observed a wide range of phenotypic variability for variant carriers. Ile763Val, Gly1475Arg, Arg1617Gln, Ala1650Val and Arg1872Gln were all seen in this study as well as in patients with DEE. Ile763Val has previously been described in a patient with intractable epilepsy and moderate ID<sup>30</sup>, whereas we found it in two patients with focal epilepsy and mild ID (#5 and #6).

The Gly1475Arg variant is even more diverse and it has previously been identified in several patients, including a child with severe DEE, who died from probable SUDEP<sup>10; 32</sup>. In this cohort, we found it in two patients with mild ID, and epilepsy controlled by SCBs. Both patients did suffer from hypotonia and ataxia.

Arg1617Gln has been seen in several DEE patients previously, including a girl with severe DEE who died after terminal progression of her disease<sup>2, 17</sup>, whereas we found it in two patients with mild ID and treatable epilepsy (#24, #25). Still, they did display additional neurological disturbances

(language delay, dyskinesia) and their seizure onset was earlier (4 months), compared to the rest of this cohort. We also found the Arg1617Gln variant in one patient (#21), who displayed moderate ID as well as autism. Ala1650Val variant has not been described before (#26), but has been seen several times with a threonine (Ala1650Thr) substitution in patients with severe DEE<sup>5; 7; 35</sup>. Arg1872Gln has also been seen several times in patients with severe DEE<sup>7; 36</sup>, however we found it in a sib pair, with normal intellect and focal epilepsy (#34). The Gly1483Lys variant has previously been described in BFIS<sup>17</sup>; the patient in this study (#18) carrying this variant has indeed a very mild phenotype; with only speech delay, sporadic seizures and discrete focal EEG abnormalities. Why some variants show phenotypic heterogeneity and other variants do not, remains elusive. Of course, differences in the amino acid substitution might explain some of the difference, but this is true for just a few of the variants. In other cases, genetic modifiers and differences in the genetic background could underlie these observations. Further studies are needed to investigate this further.

In conclusion, this study we provide further insight on the phenotypic spectrum of *SCN8A* epilepsy by focusing an intermediate phenotype characterized by treatable epilepsy with a later age of onset, mildly impaired cognitive development, as well as variable but in general mild neurological disturbances. A positive response to epilepsy treatment, especially with SCBs, was observed. Even if a wide range of phenotypes related to *SCN8A* variants can be expected (see illustration in figure 2), these findings highlight the presence of an increasing number of *SCN8A* patients with a phenotype of moderate severity.

The partial overlapping of genetic and early clinical features in *SCN8A*-related epilepsies makes it difficult to provide proper counselling in these children so far. Further investigations are warranted to clarify this issue, as well as explore possible prognostic factors.

#### **Acknowledgements:**

We thank the patients and families for participating in our study.

#### **Conflicts of interest:**

None of the other authors have any conflict of interest to disclose.

**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.

**Web resources:**

- The ExAC browser: <http://exac.broadinstitute.org>
- The GnomAD database: <http://gnomad.broadinstitute.org/>
- SIFT: <http://sift.jcvi.org>
- Poly-Phen2: <http://genetics.bwh.harvard.edu/pph2/>
- MutationTaster: <http://www.mutationtaster.org>
- The allele frequency app: <https://www.cardiodb.org/allelefrequencyapp>

**Key Bullets:**

- The phenotypic spectrum of *SCN8A* is wide, from BFIS to DEE
- The intermediate phenotype is characterized by a treatable epilepsy and mild cognitive delay
- Prognostic markers remain elusive

**References:**

1. Veeramah KR, O'Brien JE, Meisler MH, 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;90:502-510.
2. O'Brien JE, Meisler MH. Sodium channel *SCN8A* (Nav1.6): properties and de novo mutations in epileptic encephalopathy and intellectual disability. *Front Genet* 2013;4:213.
3. Vaher U, Noukas M, Nikopentis T, et al. De novo *SCN8A* mutation identified by whole-exome sequencing in a boy with neonatal epileptic encephalopathy, multiple congenital anomalies, and movement disorders. *J Child Neurol* 2014;29:Np202-206.

4. Estacion M, O'Brien JE, Conravey A, et al. A novel de novo mutation of SCN8A (Nav1.6) with enhanced channel activation in a child with epileptic encephalopathy. *Neurobiol Dis* 2014;69:117-123.
5. Ohba C, Kato M, Takahashi S, et al. Early onset epileptic encephalopathy caused by de novo SCN8A mutations. *Epilepsia* 2014;55:994-1000.
6. de Kovel CG, Meisler MH, Brilstra EH, et al. Characterization of a de novo SCN8A mutation in a patient with epileptic encephalopathy. *Epilepsy Res* 2014;108:1511-1518.
7. Larsen J, Carvill GL, Gardella E, et al. The phenotypic spectrum of SCN8A encephalopathy. *Neurology* 2015;84:480-489.
8. Kong W, Zhang Y, Gao Y, et al. SCN8A mutations in Chinese children with early onset epilepsy and intellectual disability. *Epilepsia* 2015;56:431-438.
9. Singh R, Jayapal S, Goyal S, et al. Early-onset movement disorder and epileptic encephalopathy due to de novo dominant SCN8A mutation. *Seizure* 2015;26:69-71.
10. Johannesen KM, Gardella E, Scheffer I, et al. Early mortality in SCN8A-related epilepsies. *Epilepsy Res* 2018;143:79-81.
11. Blanchard MG, Willemsen MH, Walker JB, et al. De novo gain-of-function and loss-of-function mutations of SCN8A in patients with intellectual disabilities and epilepsy. *J Med Genet* 2015;52:330-337.
12. Wagnon JL, Barker BS, Hounshell JA, et al. Pathogenic mechanism of recurrent mutations of SCN8A in epileptic encephalopathy. *Ann Clin Transl Neurol* 2016;3:114-123.
13. Lopez-Santiago LF, Yuan Y, Wagnon JL, et al. Neuronal hyperexcitability in a mouse model of SCN8A epileptic encephalopathy. *Proc Natl Acad Sci U S A* 2017;114:2383-2388.
14. Oliva M, Berkovic SF, Petrou S. Sodium channels and the neurobiology of epilepsy. *Epilepsia* 2012;53:1849-1859.
15. Wolff M, Johannesen KM, Hedrich UBS, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain* 2017;140:1316-1336.
16. Moller RS, Johannesen KM. Precision Medicine: SCN8A Encephalopathy Treated with Sodium Channel Blockers. *Neurotherapeutics* 2016;13:190-191.
17. Gardella E, Becker F, Moller RS, et al. Benign infantile seizures and paroxysmal dyskinesia caused by an SCN8A mutation. *Ann Neurol* 2016;79:428-436.

18. Han JY, Jang JH, Lee IG, et al. A Novel Inherited Mutation of SCN8A in a Korean Family with Benign Familial Infantile Epilepsy Using Diagnostic Exome Sequencing. *Ann Clin Lab Sci* 2017;47:747-753.
19. Moller RS, Dahl HA, Helbig I. The contribution of next generation sequencing to epilepsy genetics. *Expert Rev Mol Diagn* 2015;15:1531-1538.
20. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-424.
21. Samocha KE, Kosmicki JA, Karczewski KJ, et al. Regional missense constraint improves variant deleteriousness prediction. *bioRxiv* 2017.
22. Lal D, May P, Samocha K, et al. Gene family information facilitates variant interpretation and identification of disease-associated genes. *bioRxiv* 2017.
23. Grantham R. Amino acid difference formula to help explain protein evolution. *Science* 1974;185:862-864.
24. Gardella E, Marini C, Trivisano M, et al. The phenotype of SCN8A developmental and epileptic encephalopathy. *Neurology* 2018;91:e1112-e1124.
25. Vigevano F, Fusco L, Di Capua M, et al. Benign infantile familial convulsions. *Eur J Pediatr* 1992;151:608-612.
26. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017;58:512-521.
27. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285-291.
28. Dewey FE, Murray MF, Overton JD, et al. Distribution and clinical impact of functional variants in 50,726 whole-exome sequences from the DiscovEHR study. *Science* 2016;354.
29. Moller RS, Larsen LH, Johannesen KM, et al. Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies. *Mol Syndromol* 2016;7:210-219.
30. Butler KM, da Silva C, Shafir Y, et al. De novo and inherited SCN8A epilepsy mutations detected by gene panel analysis. *Epilepsy Res* 2017;129:17-25.
31. Wang J, Gao H, Bao X, et al. SCN8A mutations in Chinese patients with early onset epileptic encephalopathy and benign infantile seizures. *BMC Med Genet* 2017;18:104.

32. Xiao Y, Xiong J, Mao D, et al. Early-onset epileptic encephalopathy with de novo SCN8A mutation. *Epilepsy Res* 2017;139:9-13.
33. Dymont DA, Tetreault M, Beaulieu CL, et al. Whole-exome sequencing broadens the phenotypic spectrum of rare pediatric epilepsy: a retrospective study. *Clin Genet* 2015;88:34-40.
34. Rauch A, Wieczorek D, Graf E, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 2012;380:1674-1682.
35. Parrini E, Marini C, Mei D, et al. Diagnostic Targeted Resequencing in 349 Patients with Drug-Resistant Pediatric Epilepsies Identifies Causative Mutations in 30 Different Genes. *Hum Mutat* 2017;38:216-225.
36. Arafat A, Jing P, Ma Y, et al. Unexplained Early Infantile Epileptic Encephalopathy in Han Chinese Children: Next-Generation Sequencing and Phenotype Enriching. *Sci Rep* 2017;7:46227.
37. Anand G, Collett-White F, Orsini A, et al. Autosomal dominant SCN8A mutation with an unusually mild phenotype. *Eur J Paediatr Neurol* 2016;20:761-765.
38. Chen K, Godfrey DA, Ilyas O, et al. Cerebellum-related characteristics of Scn8a-mutant mice. *Cerebellum* 2009;8:192-201.
39. Levin SI, Khaliq ZM, Aman TK, et al. Impaired motor function in mice with cell-specific knockout of sodium channel Scn8a (NaV1.6) in cerebellar purkinje neurons and granule cells. *J Neurophysiol* 2006;96:785-793.
40. Woodruff-Pak DS, Green JT, Levin SI, et al. Inactivation of sodium channel Scn8A (Na-sub(v)1.6) in Purkinje neurons impairs learning in Morris water maze and delay but not trace eyeblink classical conditioning. *Behav Neurosci* 2006;120:229-240.
41. Trudeau MM, Dalton JC, Day JW, et al. Heterozygosity for a protein truncation mutation of sodium channel SCN8A in a patient with cerebellar atrophy, ataxia, and mental retardation. *J Med Genet* 2006;43:527-530.
42. Marien P, Ackermann H, Adamaszek M, et al. Consensus paper: Language and the cerebellum: an ongoing enigma. *Cerebellum* 2014;13:386-410.

43. Hammer MF, Wagnon JL, Mefford HC, et al. SCN8A-Related Epilepsy with Encephalopathy. In Adam MP, Ardinger HH, Pagon RA, et al. (Eds) GeneReviews(R), University of Washington, Seattle  
University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.: Seattle (WA); 1993.
44. Wagnon JL, Barker BS, Ottolini M, et al. Loss-of-function variants of SCN8A in intellectual disability without seizures. *Neurol Genet* 2017;3:e170.
45. Papale LA, Beyer B, Jones JM, et al. Heterozygous mutations of the voltage-gated sodium channel SCN8A are associated with spike-wave discharges and absence epilepsy in mice. *Hum Mol Genet* 2009;18:1633-1641.
46. Makinson CD, Tanaka BS, Sorokin JM, et al. Regulation of Thalamic and Cortical Network Synchrony by Scn8a. *Neuron* 2017;93:1165-1179.e1166.

Table 1. Clinical characteristics of the probands

Proband #	Age at inclusion	Variant, Inheritance, location	Pathogenicity (ACMG guidelines, MPC)	Family history	Age at seizure onset	Seizure types	Interictal EEG / Ictal EEG	Cognition before sz onset / cognition after sz onset	Treatment response	Successful monotherapy	Other features
1	2 y 8 m	c.-8A>G <i>de novo</i> 5'UTR	Pathogenic	None	11 m	GTC, AA	EDs on the P-temporal regions bilaterally, with rare diffuse spreading	N/ MID	Sz reduction: VPA 50%		Hypotonia, uncoordinated movements, gait disturbance
2	9 y	c.411C>G p.Ile137Met <i>maternal</i> transmembrane domain D1S1	Likely pathogenic (PM1, PM2, PP1, PP2, PP3) MPC=1.66	Mother, at 10-11 y: episodes of loss of balance, lower limb hyposthenia and falls, gait disturbance and hand tremor	2 y 2 m	Staring/ hypotonia to GTC, A, F (visual)	Temporal-O spikes / SW (left side predominance)	MID/MID	Sz reduction: LTG, VPA, ZNS No effect: LEV, RFN, TPM		Hypotonia, Hypothyroidism Enuresis, sleep disorders
3	9 y 4 m	c.1122C>G p.Asn374Lys <i>de novo</i> poreforming	Likely pathogenic (PS2, PP2, PP3)	maternal cousin with sz	7 y	Nocturnal frontal, T, GTC, SE	Multifocal SW, paroxysmal beta activity that lasts	N / MID	Sz free: PHT, VPA No effect: OXC, LEV,		Gait disturbance

		domain	MPC=1.83				between 1-2 minutes		ZNS, CLB		
4		c.1630_1631del p.Asn544fs*39 <i>maternal</i> cytoplasmic domain D1/D2	Pathogenic (PVS1, PM2, PP1)	See fig 1.	2 y 9 m	Ab	Slow wave and SW discharges	N / MID	Sz free: VPA + ESM+ AMD Sz reduction: VPA + ESM		
5	13 y	c.2287A>G p.Ile763Val <i>de novo</i> transmembrane domain D2S1	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=1.51	Cousin with febrile sz	7 wk	GTC	NA	NA / MID	Sz free: CLB, ZNS, PHT, LTG, CBZ Adverse effects: LEV (behavior), CLZ and LZP (sleepiness)		Hypotonia, ataxia, chronic constipation, premature adrenarche, periodic leg movement
6	20 y	c.2287A>G p.Ile763Val <i>de novo</i> transmembrane domain D2S1	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=1.51	None	3 m	S, F	EDs, both hemispheres Ictal: right F-temporal onset, then rhythmic slow activity propagating	NA / MID	No effect: VPA, CBZ, LTG, CLB, acetazolamide		Ataxia

							into the right parasagittal regions				
7	11 y	c.2671G>A p.Val891Met <i>maternal</i> transmembrane domain D2S5	Pathogenic (PS1, PM1, PM2, PP1, PP2)  MPC=3.14	See fig. 1	3 y	GTC, AA	CP and midline spikes	N / MID	Sz free: CBZ	CBZ	ADHD
8	10 y	c.2806G>A p.Glu936Lys <i>non-maternal</i> poreforming domain	Likely pathogenic (PM1, PM2, PP1, PP2)  MPC=3.33	Father with ID and ADHD	5 y	F, AA, FS	SW left F, C, right P	NA / MD	Sz free: LEV Adverse effects: TPM	LEV	Autistic features, behavioral problems
9	9 y 9 m	c.3601G>A p.Glu1201Lys <i>de novo</i> transmembrane domain D3S1	Likely pathogenic (PS2, PM1, PM2, PP2)  MPC=2.37	None	8 m	M	Initially normal (1y - 3y4 m), then BG slowing (7-10y) Postictal: abundant beta, diffusely slow BG	DD / MID	Sz reduction: VPA, LEV, CLB No effect: STP Sz aggravation. OXC		Hypotonia, ataxia, paroxysmal dystonia

10	2 y	c.3722A>G p.Tyr1241Cys <i>pending</i> transmembrane domain D3S2	VUS (PM1, PP2)  MPC=2.48	None	8 m	F, A	Focal posterior bilateral EDs	NA / MID	Sz free: VGB	VGB	Paroxysmal dyskinesia
11	2 y 1 m	c.3953A>G p.Asn1318Ser <i>de novo</i> cytoplasmic linker D3S4/D3S5	Likely pathogenic (PS2, PM2, PP2)  MPC=2.04	None	3 m	F, GTC	Normal Ictal: EDs on the P regions bilaterally	N / N	Sz reduction: CBZ, PER No effect: PB, OXC, CLB, ZNS Adverse effects: TPM (hyperthermia)		Ataxia
12	2 y 3 m	c.3956C>A p.Ala1319Asp <i>de novo</i> cytoplasmic linker D3S4/D3S5	Likely pathogenic (PS2, PM2, PP2)  MPC=2.69	None	2 m	C, T, GTC	Normal Ictal EEG: parasagittal EDs, F rhythmic delta	N / MID	Sz reduction: PHT No effect: VPA, PB		Ataxia, Tremor, language delay

13	7 y 11 m	c.3967G>A p.Ala1323Thr, de novo cytoplasmic domain D3S4/D3S5	Likely pathogenic (PS2, PM2, PP2)  MPC=2.22	None	5 m	FS, GTC	Normal	N / N	Sz free: VPA	VPA	
14		c.4391T>C p.Ile1464Thr <i>paternal</i> inactivation gate	Likely pathogenic (PM1, PM2, PP1, PP2)  MPC=2.65	See fig. 1	7 m	A, GTC, SE	Diffuse abnormal	NA / MID	Sz reduction: TPM, VPA		
15	10 y 6 m	c.4423G>A p.Gly1475Arg, <i>paternal</i> , inactivation gate	Likely pathogenic (PS1, PM2, PP1, PP2)  MPC=1.54	See fig. 1	9 m	F to GTC, AA	Slowing over the temporal region (3y), then normal (9y)	NA / MID	Sz free: LTG  Sz reduction: OXC, CBZ  No effect: LEV	LTG	Hypotonia, ataxia, autistic features
16	9 m	c.4423G>A p.Gly1475Arg <i>de novo</i> , inactivation	Pathogenic (PS1, PS2, PP2)  MPC=1.54	None	4 m	F, T	Ictal: Slowing and EDs on both hemispheres, mainly	NA / MID	Sz free: PHT  No effect: LEV	PHT	Hypotonia, ataxia

		gate					temporal regions				
17	9 y 9 m	c.4423G>A p.Gly1475Arg <i>de novo</i> <i>Blood</i> <i>mosaicism</i> 10% inactivation gate	Pathogenic (PS1, PS2, PP2)  MPC=1.54	None	11 m	GTC, F	Normal	N / N	Sz free: CBZ  Sz reduction: VPA	CBZ	
18	8 y	c.4447 G>A, p.Glu1483Lys <i>de novo</i> inactivation gate	Pathogenic (PS1, PS2, PS3, PM2, PP2)  MPC=2.13	None	11 m	F, GTC	Normal  background, small Spikes / theta pointu in the central regions	N / N	SZ free: VPA, CBZ, currently no AED		Clumsy, shy, speech delay
19	2 y	c.4585A>G p.Met1529Val <i>de novo</i> transmembr ane D4S1	Likely pathogenic (PS2, PM1, PM2, PP2)  MPC=1.2	None	4 m	GTC	Right posterior  slow activity during sleep  Ictal: generalized spike discharges	N / N	No effect: LEV, PB, VPA		

20	11 y	c.4764C>G p.Phe1588L eu <i>de novo</i> transmembr ane D4S3	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4	None	3.5 m	GTC, AA, T, F	Normal	DD / MID	Sz free: CBZ	CBZ	Autism, language delay
21	13 y	c.4764C>A p.Phe1588L eu <i>de novo</i> , transmembr ane D4S3	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4	None	5 m	F, GTC	NA	N / MID	Sz reduction: PB, CBZ		Obesity
22	2 y 8 m	c.4840A>G p.Thr1614Al a <i>de novo</i> extracellular domain D4S3/D4S4	Likely pathogenic (PS2, PM2, PP2) MPC=2.17	None	4.5 m	AA, GTC, M, A	Generalized SW	N / MID	Sz reduction: VPA, OXC		
23	7 y 5 m	c.4850G>C p.Arg1617Gl n <i>de novo</i> transmembr ane D4S4	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4	None	5 m	GTC	Normal	DD / MD	Sz free: LTG	LTG	Autism

24	9 y	c.4850G>A p.Arg1617Gln <i>de novo</i> transmembrane D4S4	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4	Paternal first cousin with microcephaly	5 m	GTC, M, A	NA	DD / MID			Language delay, paroxysmal dyskinesia
25	24 y	c.4850G>A p.Arg1617Gln <i>de novo</i> transmembrane D4S4	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4	None	NA	GTC, F	NA	MID / MID	Sz free: CBZ, VPA		Extra-pyramidal signs
26	7 y	c.4949 C>T p.Ala1650Val <i>de novo</i> cytoplasmic D4S4/D4S5	Pathogenic (PS1, PS2, PM2, PP2) MPC=2.26	None	11 m	M, A, T, S, AA	Generalized EDs	NA / MID	Sz free: CLB, RUF, KDSz adverse effects: LEV (increased frequency), ZNS (new sz type), VPA (neurodevelopmental regression)		Hypotonia, supraventricular tachycardia
27	9 y	c.4961T>A	Likely	None	2 y 8 m	Ab	Generalized 3	NA / MID	No effect:		Ataxia

		p.Ile1654As n <i>unknown</i> transmembr ane domain D4S5	pathogenic (PM1, PM2, PP2) MPC=2.92				Hz SW. Spikes occipital right and left		VPA		
28	4 y	c.5273T>C p.Val1758Al a <i>de novo</i> transmembr ane D4S6	Likely pathogenic (PS2, PM1, PM2, PP2) MPC=2.46	None	2 y	Ab, FS	Mild BG slowing, irregular generalized EDs, OIRDA Ictal: irregularly generalized, 3Hz SW followed by rhythmic diffuse delta activity (clinically: arrest of ongoing activity)	N / N	Sz free: ETX No effect: LEV	ETX	
29	9 y	c.5311G>A p.Val1771Ile <i>de novo</i>	Likely pathogenic (PS2, PM2, PP2)	None	6 m	GTC, T	Normal	N / N	Sz reduction: CBZ		

		C-terminal	MPC=2.02								
30	6 y	c.5458C>T p.Arg1820* <i>de novo</i> C-terminal	Pathogenic (PVS1, PS1, PM2, PP2)	None	3-4 m	M in hands and fingers	Trains of SW, right CP. Irregular delta activity on the left side	NA / MID	Sz reduction: LTG		Ataxia, language delay
31	15 y	c.5497G>C p.Asp1833H <i>is</i> <i>unknown</i> C-terminal	Likely pathogenic (PM1, PM2, PP2) MPC=2.44	None	6 m	T	EDs over the posterior lobe	N / N	Sz free: PB No effect: LEV	PB	Reflux
32	3 y	c.5597G>A, p.Arg1866G <i>n</i> , <i>de novo</i> C-terminal	Likely pathogenic (PS2, PM2, PP2) MPC=2.39	None	9 m	F, A, AA	EDs, mid CP	N / Mild learning disability	Sz free: CBD No effect: CBZ, CLB, LEV, TPM, VGB, VPA, ST Adverse effects: CBZ (drowsiness and severe cognitive disturbance)	CBD	

33		c.5615G>A p.Arg1872Gln <i>paternal</i> ( <i>mosaic</i> ) cytoplasmic c-terminal	Likely pathogenic (PS1, PM2, PP1, PP2) MPC=2.39	See fig. 1	6 wk	F, clusters of GTC, SE	Normal	N / N	Sz free: CBZ + LEV		Learning difficulties
34	14 y	c.5630A>G, p.Asn1877Ser, pending cytoplasmic c-terminal	Likely pathogenic (PS1, PM2, PP2) MPC=2.04	None	5 m	F clustering	Normal	N / N	Sz free: LTG	LTG	Language delay
35	4 y 4 m	c.5630A>G Asn1877Ser <i>paternal</i> cytoplasmic c-terminal	Likely pathogenic (PS1, PM2, PP1, PP2) MPC=2.04	See fig. 1	6 m		Normal	N / MID	Sz free: LTG, VPA		
36	4 y 6 m	c.5630A>G p.Asn1877Ser <i>unknown</i> cytoplasmic c-terminal	Likely pathogenic (PS1, PM2, PP2) MPC=2.04	None	7 m	GTC, T, AA	Sporadic EDs, FC bilateral > right	N/ MID	Sz reduction: VPA, PB, TPM		Ataxia, clumsiness

**Abbreviations:** A: Atonic, AA: Atypical Absences, Ab: Absences, ADHD: Attention Deficit Hyperactivity Disorder, AMD: amantadine, BG: Background, CAE: Childhood Absence Epilepsy, CBZ: Carbamazepine, CLB: Clobazam, CLZ: Clonazepam, D: Domain, DD: Developmental Delay, ED: Epileptiform Discharges, FS: Febrile seizures, F: Focal, GTC: Generalized Tonic-Clonic, KD: Ketogenic diet, LAC: Lacosamide, LEV: Levetiracetam, LTG: Lamotrigine, , LZP: Lorazepam, MD: Moderate Intellectual Disability, MID: Mild Intellectual Disability, m: months, M: Myoclonic, NA: Not available, N: Normal, O: Occipital, OXC: Oxcarbazepine, P: Parietal, PER: Perampanel, PHT: Phenytoin, RFM: Rufinamide, S: SE: Status Epilepticus, ST: Stiripentol, S: Spasms, SW: Spike and Wave complexes, sz: Seizures, T: Tonic, TPM: Topiramate, VGB: Vigabatrin, VPA: Valproic Acid, y: years, ZNS: Zonisamide

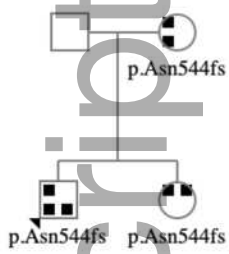
#### **Figure legends:**

Figure 1. Pedigrees of *SCN8A*-related epilepsies showing segregation of the variant with the phenotype

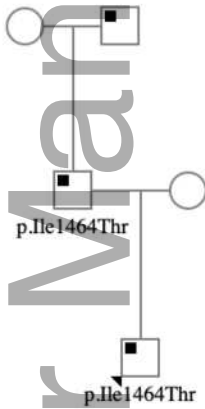
Figure 2. The spectrum of *SCN8A*-related epilepsy

Figure 3. A partial display of published variants, with the recurrent DEE and known LOF variants, as well as those identified in this study

Family of proband #4



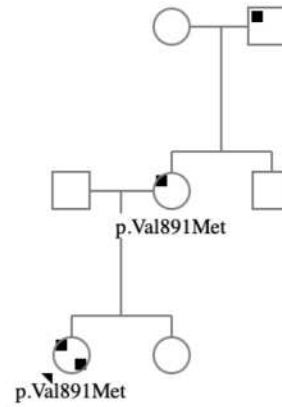
Family of proband #14



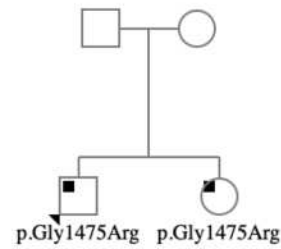
Family of proband #33



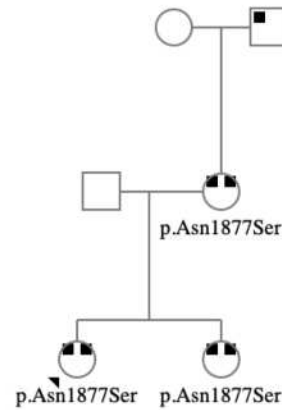
Family of proband #7



Family of proband #15



Family of proband #35

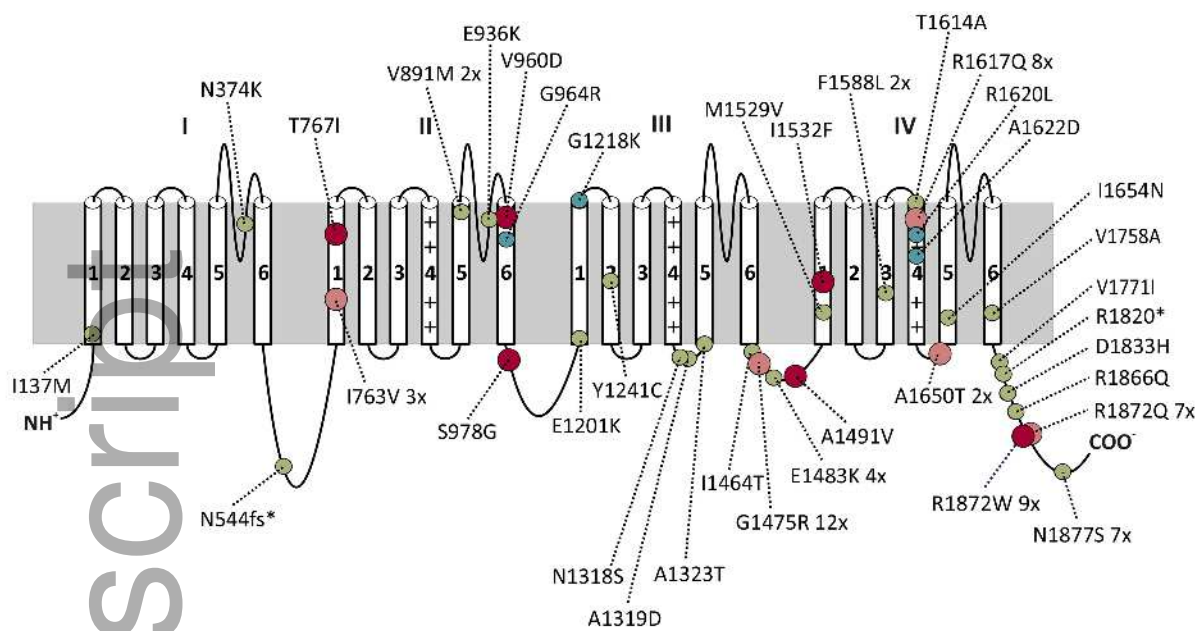


Epilepsy    Mild ID    Behavioral issues    Learning difficulties

This article is protected by copyright. All rights reserved



epi\_14705\_f2.tiff



- Intellectual disability or ataxia without epilepsy (Wagnon et al., 2017; Liu et al., 2019)
- Intermediate epilepsy (this study)
- DEE variants (previously published)
- Recurrent variants - DEE and intermediate epilepsy

epi\_14705\_f3.tif