

RESEARCH ARTICLE

# Evaluation of Presumably Disease Causing *SCN1A* Variants in a Cohort of Common Epilepsy Syndromes

Dennis Lal<sup>1,2,3,4</sup>\*, Eva M. Reinthaler<sup>5</sup>, Borislav Dejanovic<sup>6</sup>, Patrick May<sup>7</sup>, Holger Thiele<sup>1</sup>, Anna-Elina Lehesjoki<sup>8,9,10</sup>, Günter Schwarz<sup>6</sup>, Erik Riesch<sup>11</sup>, M. Arfan Ikram<sup>12</sup>, Cornelia M. van Duijn<sup>12</sup>, Andre G. Uitterlinden<sup>13</sup>, Albert Hofman<sup>14</sup>, Hannelore Steinböck<sup>15</sup>, Ursula Gruber-Sedlmayr<sup>16</sup>, Birgit Neophytou<sup>17</sup>, Federico Zara<sup>18</sup>, Andreas Hahn<sup>19</sup>, Genetic Commission of the Italian League against Epilepsy<sup>1</sup>, EuroEPINOMICS CoGIE Consortium<sup>1</sup>, Padhraig Gormley<sup>2,3,4</sup>, Felicitas Becker<sup>20</sup>, Yvonne G. Weber<sup>20</sup>, Maria Roberta Cilio<sup>21</sup>, Wolfram S. Kunz<sup>22</sup>, Roland Krause<sup>7</sup>, Fritz Zimprich<sup>5</sup>, Johannes R. Lemke<sup>23</sup>, Peter Nürnberg<sup>1,24</sup>, Thomas Sander<sup>1</sup>, Holger Lerche<sup>20</sup>, Bernd A. Neubauer<sup>19</sup>



CrossMark  
click for updates

**OPEN ACCESS**

**Citation:** Lal D, Reinthaler EM, Dejanovic B, May P, Thiele H, Lehesjoki A-E, et al. (2016) Evaluation of Presumably Disease Causing *SCN1A* Variants in a Cohort of Common Epilepsy Syndromes. PLoS ONE 11(3): e0150426. doi:10.1371/journal.pone.0150426

**Editor:** Markus Schuelke, Charité Universitätsmedizin Berlin, NeuroCure Clinical Research Center, GERMANY

**Received:** June 16, 2015

**Accepted:** February 12, 2016

**Published:** March 18, 2016

**Copyright:** This is an open access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the [Creative Commons CC0](https://creativecommons.org/licenses/by/4.0/) public domain dedication.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** HL BN PN TS F. Zimprich received grants by the EuroEPINOMICS programme (DFG grant numbers: HL: LE1030/11-1, BN: BN416/5-1, PN: NU50/8-1, TS: SA434/5-1, FWF grant number: F. Zimprich: 1643-B09) within the EUROCORES framework of the European Science Foundation (ESF). The generation and management of genomics data for the Rotterdam Study are supported by the Netherlands Organization of Scientific Research NOW Investments (nr. 175.010.2005.011, 911-03-

1 Cologne Center for Genomics, University of Cologne, Cologne, Germany, 2 Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States of America, 3 Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States of America, 4 Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States of America, 5 Department of Neurology, Medical University of Vienna, Vienna, Austria, 6 Institute of Biochemistry, Department of Chemistry, University of Cologne, Cologne, Germany, 7 Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch-sur-Alzette, Luxembourg, 8 Folkhälsan Institute of Genetics, Helsinki, Finland, 9 Neuroscience Center, University of Helsinki, Helsinki, Finland, 10 Research Programs Unit, Molecular Neurology, University of Helsinki, Helsinki, Finland, 11 CeGaT GmbH—Centre for Genomics and Transcriptomics, Tübingen, Germany, 12 Departments of Epidemiology, Neurology, Radiology, Erasmus Medical Center, Rotterdam, Netherlands, 13 Department of Epidemiology, Erasmus Medical Center, Rotterdam, Netherlands, 14 Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands, 15 Private Practice for Pediatrics, Vienna, Austria, 16 Department of Pediatrics, Medical University of Graz, Graz, Austria, 17 St. Anna Children's Hospital, Department of Neuropediatrics, Vienna, Austria, 18 Laboratory of Neurogenetics and Neuroscience, Institute G. Gaslini, Genova, Italy, 19 Department of Neuropediatrics, University Medical Center Giessen and Marburg, Giessen, Germany, 20 Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany, 21 Departments of Neurology and Pediatrics, University of California San Francisco, San Francisco, California, United States of America, 22 Department of Epileptology, University of Bonn, Bonn, Germany, 23 Institute of Human Genetics, University of Leipzig, Leipzig, Germany, 24 Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany

\* These authors contributed equally to this work.

† Membership of the Genetic Commission of the Italian League against Epilepsy and the EuroEPINOMICS CoGIE Consortium are listed in the Acknowledgments.

\* [Dennis.Lal@uni-koeln.de](mailto:Dennis.Lal@uni-koeln.de)

## Abstract

### Objective

The *SCN1A* gene, coding for the voltage-gated Na<sup>+</sup> channel alpha subunit Na<sub>v</sub>1.1, is the clinically most relevant epilepsy gene. With the advent of high-throughput next-generation sequencing, clinical laboratories are generating an ever-increasing catalogue of *SCN1A* variants. Variants are more likely to be classified as pathogenic if they have already been

012) and the Netherlands Genomics Initiative (NGI)/NOW project nr. 050-060-810 (Netherlands Consortium for Healthy Ageing; NCHA). The authors thank the members of the Genomics Lab and the ERGO support team for their help in sampling the data and in creating the database, the study participants, the staff of the Rotterdam Study and the participating general practitioners and pharmacists. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

identified previously in a patient with epilepsy. Here, we critically re-evaluate the pathogenicity of this class of variants in a cohort of patients with common epilepsy syndromes and subsequently ask whether a significant fraction of benign variants have been misclassified as pathogenic.

## Methods

We screened a discovery cohort of 448 patients with a broad range of common genetic epilepsies and 734 controls for previously reported *SCN1A* mutations that were assumed to be disease causing. We re-evaluated the evidence for pathogenicity of the identified variants using *in silico* predictions, segregation, original reports, available functional data and assessment of allele frequencies in healthy individuals as well as in a follow up cohort of 777 patients.

## Results and Interpretation

We identified 8 known missense mutations, previously reported as pathogenic, in a total of 17 unrelated epilepsy patients (17/448; 3.80%). Our re-evaluation indicates that 7 out of these 8 variants (p.R27T; p.R28C; p.R542Q; p.R604H; p.T1250M; p.E1308D; p.R1928G; NP\_001159435.1) are not pathogenic. Only the p.T1174S mutation may be considered as a genetic risk factor for epilepsy of small effect size based on the enrichment in patients ( $P = 6.60 \times 10^{-4}$ ; OR = 0.32, fishers exact test), previous functional studies but incomplete penetrance. Thus, incorporation of previous studies in genetic counseling of *SCN1A* sequencing results is challenging and may produce incorrect conclusions.

## Introduction

The *SCN1A* gene (MIM#182389), coding for the voltage-gated Na<sup>+</sup> channel alpha subunit NaV1.1, is the most clinically relevant epilepsy gene. *SCN1A* variants are associated, at the more benign end of the disease spectrum, with the dominantly inherited genetic epilepsy with febrile seizures plus [1, 2] (GEFS+), and, at the severe end, with Dravet syndrome (DS), an epileptic encephalopathy arising from *de novo* *SCN1A* mutations in the vast majority of DS patients [3]. More rarely, *SCN1A* mutations are also found in other types of infantile epileptic encephalopathies [4]. Common variants in *SCN1A* have been associated with mesial temporal lobe epilepsy and hippocampal sclerosis with febrile seizures in a genome-wide association study [5], and in a recent meta-analysis including 8696 patients with genetic generalized-, focal-, or unclassified epilepsies [6]. In around 88% of epilepsy patients carrying rare *SCN1A* mutations, these arise *de novo*, whereas only 12% of the affected individuals inherit the mutation from a, usually unaffected, parent [4]. Patients with an identical mutation may express a broad spectrum of phenotypes even within a single large family ranging from unaffected, over GEFS+ to Dravet syndrome [7, 8]. Most functional studies of disease-associated variants showed loss-of-function effects of *SCN1A* mutations [9–12]. Mutations with a complete loss of function, in general, lead to more severe epilepsies [3, 13, 14].

Massive parallel sequencing studies have accelerated mutation discovery. With declining costs, more patients will be sequenced, and many newly identified mutations are expected. Determination of the functional consequences and pathogenicity is challenging and molecular studies for each variant are currently not feasible. Databases like the "The Human Gene

Mutation Database" (HGMD; <http://www.hgmd.org/>) constitute a comprehensive collection of mutations in genes underlying or associated with human inherited diseases. The database is routinely accessed and utilized by next generation sequencing (NGS) project researchers, human molecular geneticists, neurologists and genetic counselors.

Interpretation of genetic results is challenging, especially in multifactorial diseases like common epilepsies. The HGMD comprises more than 1000 disease-associated mutations annotated for the *SCN1A* gene (accessed Dec/2014). Here, we investigated a already whole-exome sequenced cohort of 448 patients with a broad range of common genetic epilepsies, for *SCN1A* mutations listed as disease associated in the HGMD. We re-evaluated the identified variants' evidence for pathogenicity by *in silico* prediction, segregation, literature review for clinical, genetic and functional relevance and assessment of allele frequencies in healthy individuals. Finally, we investigated the phenotypic spectrum of the most reliable variant in a diagnostic epilepsy cohort.

## Patients and Methods

### Patients and sequencing

Patients of European and Turkish ancestry were recruited at several centers from Germany, Austria, Italy, Finland, Canada and Turkey. We included Turkish patients since *SCN1A* variant frequencies do not show correlation with the ethnicity when compared between Asian and European patients [4]. Samples from The Rotterdam Study [15] not specifically screened for European ethnicity, which were handled in the same way as our cases, i.e. using the same enrichment and sequencing methods, served as controls for the whole exome dataset. The controls are drawn from the population and are not evaluated for seizure disorders. Informed consent for whole exome sequencing was obtained from all participants or legal representatives respectively. The two institutions in charge of the reported analysis are the University hospitals in Tübingen (for "genetic generalized epilepsy") and Giessen (for "genetic focal epilepsy"), Germany. Both review boards in Tübingen and Giessen approved this study.

High-throughput, targeted sequencing was performed as previously described [16] with the Nimblegen-SeqCapEZ-V244M enrichment kit on the Illumina HiSeq2000 system. For each sample, we calculated the gene-coverage of *SCN1A*. Only samples with a minimum of 90% of all bases in the coding region of the gene (i.e. all exons plus the first and last five intronic bases, NM\_001165963.1) being covered by at least 15 reads were used. After quality control, 448 unrelated epilepsy patients (235 with genetic generalized epilepsy; 182 with rolandic epilepsy; 22 with atypical rolandic epilepsy; 4 with benign familial neonatal seizures; 4 with benign familial infantile epilepsy; 1 with benign adult familial myoclonic epilepsy; 226 males, 222 females) and 734 control individuals (435 females, 299 males) were included in the analysis. All *SCN1A* HGMD variants identified in the patients were validated with Sanger sequencing using standard protocols. Primers are available upon request. Rare *SCN1A* sequence variants, which are not annotated by HGMD, were not investigated in this study.

### HGMD annotation and assessment of the literature

Variant annotation was performed using the HGMD Professional 2013.4–15th December 2013 Version [17]. We assessed original reports cited by the HGMD for each identified HGMD patient mutation. We re-analyzed the mutations, and if the original report used the sequence of a shorter transcript of the *SCN1A* gene for numbering we numbered the variant accordingly to the RefSeqGene (NM\_001165963.1; which represent the longest transcript and reference protein NP\_001159435.1) used in our data set.

## Assessment of mutation frequency in controls

Besides our control dataset, variant frequencies were taken from the ExAC collection (<http://exac.broadinstitute.org/>). Note that this database also includes patients from the "Swedish Schizophrenia & Bipolar Studies". Furthermore, it is not clear if patients with mild seizure disorders have been excluded.

## In silico prediction

Functional prediction scores were obtained from the dbNSFP database version 2.8 (<http://sites.google.com/site/jpopgen/dbNSFP>, accessed 01/2015). In total we used six prediction scores (SIFT, Polyphen-2-HVAR, Polyphen-2-HDIV, Mutation Assessor, FATHMM, LRT) and two conservation scores (GERP++, PhyloP). We did not use the dbNSFP generated ensemble scores, as they integrate the prediction of the tool Mutation Taster, which itself incorporates HMGD entries. We classified the variant as "damaging" when the majority of the tools predicted a functional effect for the variant (i.e. a minimum of 5 out of 8 tools). Topology and domain structure were taken from UniProt entry P35498.

## Screening of the p.T1174S mutation in a diagnostic cohort of epilepsy

To further investigate the frequency and phenotypic spectrum of the p.T1174S mutation, we evaluated NGS panel sequencing data of epilepsy-associated genes [18] of 777 individuals provided by the CeGaT diagnostic lab (<http://www.cegat.de>, genes listed in S1 Table). Patients covered a broad spectrum of epilepsy phenotypes, often associated with intellectual disability.

## Criteria for variant re-classification

We consider a variant as pathogenic if all of the following three criteria are met: i) The variant is statistically enriched in the patient cohort and/or absent in controls; ii) the segregation pattern analysis and the re-evaluation of original reports do not weaken the epilepsy association, iii) the variant is predicted to be pathogenic by the majority of *in silico* classifiers and/or molecular functional analyses supports its pathogenicity.

## Results

### Case vs. control HGMD analysis

We analyzed sequence data of 448 epilepsy patients and 734 controls for *SCN1A* variants previously reported as disease causing collected by the HGMD. Our mutation screening identified eight distinct HGMD missense mutations (Table 1: p.R27T, p.R28C, p.R604H, p.R542Q, p.T1174S, p.T1250M, p.E1308D, p.R1928G) affecting in total 17 unrelated epilepsy patients (17/448 = 3.80%) compared to six HGMD variants affecting 10 control individuals (10/734 = 1.36%). None of these patients were carrier of a rare loss of function variant in a known epilepsy gene (S2 Table). The *SCN1A* HGMD variant frequency was therefore slightly enriched in the patient cohort ( $P = 8.30 \times 10^{-3}$ ). A high allele frequency in public databases would argue against pathogenicity of the identified *SCN1A* HGMD variants identified in our patients. In addition to our own sequenced controls we investigated the *SCN1A* HGMD variant frequencies further using the ExAC, a publicly accessible variant database of 60706 unrelated individuals. All eight variants were found in individuals collected in the ExAC database (Table 1). After combining our in-house control and ExAC data in a meta-analysis, only the p.T1174S variant remained significantly enriched after correction for multiple testing (Table 1) in epilepsy patients supporting p.T1174S as the only identified *SCN1A* genetic risk factor for epilepsy in our cohort.

**Table 1. HGMD *SCN1A* mutation frequencies in patients and controls.**

Mutation	dbSNP ID	Patient mutation carrier in (N = 448)	Controls (N = 734)	Patient vs. Controls	ExAC (N = 60706)	Patient vs. controls + ExAC
p.R1928G	<a href="#">rs121917956</a>	1	0	-	171/60605	1
p.E1308D	<a href="#">rs121917910</a>	1	2	-	91/60276	0.498
p.T1250M	<a href="#">rs140731963</a>	1	0	-	42/58362	0.277
p.T1174S	<a href="#">rs121918799</a>	6	0	<b><math>3.02 \times 10^{-3}</math></b>	214/60675	<b><math>5.61 \times 10^{-3}</math></b>
p.R604H	<a href="#">rs121918769</a>	5	1	$7.91 \times 10^{-3}$	185/60690	0.013
p.R542Q	<a href="#">rs121918817</a>	1	5	-	186/60687	1
p.R28C	No ID	1	0	-	4/60701	0.036
p.R27T	<a href="#">rs121917906</a>	1	0	-	14/60702	0.103

Accession according to reference transcript NM\_001165963.1; and references protein NP\_001159435.1; P values were calculated either by Fisher's exact test; bold P values are still significant after Bonferroni correction =  $\alpha/n$ . For testing eight mutations with a desired  $\alpha = 0.05$ , the Bonferroni correction tests each individual hypothesis at  $\alpha = 0.05/8 = 0.00625$

doi:10.1371/journal.pone.0150426.t001

### Segregation analysis

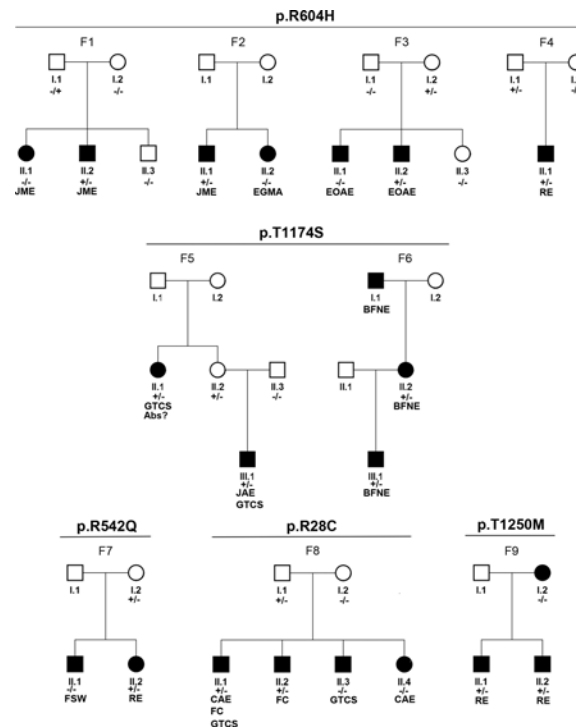
We further analyzed the family segregation pattern whereby a shared segregation of the *SCN1A* HGMD variant and epilepsy was evaluated as support for the variant pathogenicity. For five variants detected in patients (p.T1250M, p.T1174S, p.R604H, p.R542Q, p.R28C), segregation analysis was possible in a total of nine families (Fig 1). All variants were inherited, five times maternally and three times paternally, six times from a healthy parent and only twice from an affected parent. Only the p.T1174S variant segregated with epilepsy in our investigated families (Fig 1).

### Review of the original reports

Next, we went back to the 16 original reports (1–4 cited reports for each mutation, Table 2) of all eight identified patient mutations referenced in the HGMD to summarize inheritance, segregation and available functional data. The majority of the reports do not support the disease association of the variant from a today's perspective. None of the mutations have been described as *de novo*. In three reports (p.R604H, p.E1308D, p.R1928G) an additional *SCN1A* mutation has been reported in the HGMD referenced patient, including a stop codon mutation (p.R604H in combination with p.R1525X; p.R1928G in combination with p.L1207P, and p.E1308D in combination with p.A239V; Table 2). In another study the HGMD referred patient had two additional *SCN1A* mutations, one missense and one splice site acceptor variant (Table 2, p.T1250M in combination with p.R27T and c.3706-2A > G). In four families the mutation was inherited (each one family: p.R27T, p.R542Q, p.T1174S, p.E1308D; Table 2) from a healthy parent and segregation was not determined in 12 families. In contrast, only in three families the mutation segregated with the disease, one affected by the p.R604H and two by the p.T1174S variants. Furthermore, only for the p.T1174S variant functional studies have been conducted. In particular, experiments in tsA-201 cells and computational modeling of mutant properties both supported its epileptogenic effect [19].

### In silico prediction analysis

Six of the eight identified variants (p.R28C, p.R542Q, p.R604H, p.T1250M, p.E1308D and p.R1928G) are predicted to have a damaging effect when both structural prediction and local conservation scores were considered (Table 3). In contrast to previous molecular studies [19]



**Fig 1. Segregation analysis of the identified variants.** Analysis of likely segregation of the respective mutations in family members could be performed in eleven families. Accession is according to reference transcript NM\_001165963.1; and references protein NP\_001159435.1. Abbreviations: FC = febrile convulsion; JME = juvenile myoclonic epilepsy, CAE: childhood absence epilepsy, JAE: juvenile absence epilepsy, EGMA: epilepsy with generalized tonic-clonic seizures predominantly on awakening, EOAE = Early onset absence epilepsy, RE = Rolandic epilepsy, Abs = absence, BFNE = Benign familial neonatal seizure, FSW = Focal Sharp Waves, GTCS = Generalized tonic-clonic seizure

doi:10.1371/journal.pone.0150426.g001

and our statistical enrichment and segregation studies, the p.T1174S variant was not predicted to be damaging by the majority of prediction tools (Table 3).

### Replication analysis p.T1174S

Overall, after the initial re-evaluation of the identified *SCN1A* HGMD variants, we suggest that only the p.T1174S variant is potentially a epilepsy associated variant. The p.T1174S variant is enriched in patients, segregates with the phenotype in families and published functional data support pathogenicity of the variant (Table 2). A major factor in our variant classification is comparing the allele frequency differences between patients and controls. To further assess the frequency of p.T1174S alleles in epilepsy patients and replicate our association, we investigated an additional epilepsy cohort provided by the CeGaT diagnostic lab using targeted sequencing. We identified seven out of 777 epilepsy patients (0.90%) carrying the p.T1174S *SCN1A* mutation. Four of the patients were diagnosed with intellectual disability and epilepsy, one patient had temporal lobe epilepsy, and two patients had epileptic seizures without further specification of the phenotype. In five of the seven patients, the mutation had been inherited from a healthy parent. In the remaining two cases, parents were not available for segregation testing. Of note, in one of the seven patients, an additional pathogenic variant was detected by detection of a *de novo* mutation in *GRIN2B* [34] (c.1619G>A, p.R540H).

Combining both epilepsy cohorts together, we have identified 13 out of 1219 patients carrying the p.T1174S mutation. A comparison of all available patient and control data confirms

**Table 2. Summary of previous studies referenced at the HGMD specific for each identified variant.**

Mutation	Previous Phenotype	Previous inheritance	Comment	Ref	Phenotypes in this study
p. R1928G	GEFS+	Not determined	-	[1]	1x CAE
	Control	Not determined	-	[1]	
	SMEI	Not determined	Patient carried an additional p.L1207P mutation in <i>SCN1A</i>	[20]	
p. E1308D	FC	Not determined	-	[21]	1x JAE
	SMEI	Inherited from healthy father	Patient carried an additional m p.A239V mutation in <i>SCN1A</i>	[22]	
p. T1250M	DS	Not determined	Patient has an additional <i>SCN1A</i> missense mutation (p.Arg27Thr) and one variant predicted as splice Site acceptor mutation (c.3706-2A > G)	[23]	1x RE
	GEFS+		-	[21]	
p. T1174S	DS + Migraine	Maternal inherited	Patient has DS, mother has migraine with aura	[24]	1x GTCS, 2x RE, 1x
	SMEI	Not determined	-	[25]	BFNE, 1x CAE, 1x JAE
	Seizures and hemiplegic migraine	Large family: all mutation carriers had epilepsy or migraine. 3x migraine with aura, 1 migraine without aura and benign occipital epilepsy, 2x migraine with aura and benign occipital epilepsy = > inherited	Functional studies, interpretation = modulation of the properties of T1174S can lead to a switch between overall gain and loss of function, consistent with a switch between promigraine end epileptogenic effect and, thus, with coexistence of epileptic and FHM phenotypes in the same family.	[19]	
	MAE	Inherited from healthy mother	-	[26]	
	FHM	Not determined	-	[27]	
p.R604H	Intractable Epilepsy	Not determined		[28]	2x JME;
	FS, FS+	Inherited all affected	With a single family, All three mutation carrier had FS or FS+	[29]	1x CAE, 1xRE, 1x EOAE
	DS	Not determined	Patient carries also a <i>SCN1A</i> Stop codon mutation (p.R1525X)	[30]	
p.R542Q	JME	Not determined	-	[31]	1x RE
	Familial autism	Inherited from healthy father	A sib with autism is affected and carries the mutation as well	[32]	
	ICE	Not determined	-	[28]	
p.R28C	GEFS+	Not determined	-	[33]	1x JAE
p.R27T	GEFS+	Inherited from healthy father	-	[22]	1x JME

Abbreviations: GEFS+ = genetic epilepsy with febrile seizures plus; SMEI = severe myoclonic epilepsy of infancy; FC = febrile convulsion; DS = Dravet Syndrome; MAE = Myoclonic astatic epilepsy (Doose syndrome); FHM = Familial hemiplegic migraine; FS = febrile seizures; JME = juvenile myoclonic epilepsy, ICE = Intractable childhood epilepsy; GGE syndromes: CAE: childhood absence epilepsy, JAE: juvenile absence epilepsy, JME: juvenile myoclonic epilepsy, EGMA: epilepsy with generalized tonic-clonic seizures predominantly on awakening, EGTCS: epilepsy with generalized tonic-clonic seizure

doi:10.1371/journal.pone.0150426.t002

enrichment in epilepsy patients ( $P = 5.68 \times 10^{-4}$ ; OR = 3.08, 95%-CI: 1.61–5.40, fisher exact test, 13/1219 patients vs. 21461409 controls).

## Discussion

In the context of molecular genetic testing, it is often challenging to establish the pathogenicity of an intragenic variant. Lack of parental DNA or control individuals, absence of functional data and association with different phenotypes add to this complexity.

**Table 3. Functional prediction and conservation scores of the 8 HMGD Variations.**

Mutation	SIFT	Polyphen2_HDIV	Polyphen2_HVAR	LRT_pred	MutationAssessor	FATHMM	GERP++ rankscore	PhyloP46way_primate rankscore	predicted outcome (n of tools)
p.R27T	T	B	B	N	L	D	0.589	0.943	functionally neutral (3/8)
p.R28C	D	P	B	D	M	D	0.912	0.943	damaging (7/8)
p.R542Q	D	B	B	D	L	D	0.997	0.943	damaging (5/8)
p.R604H	T	D	D	D	M	D	0.772	0.741	damaging (7/8)
p.T1174S	T	B	B	N	N	D	0.789	0.430	functionally neutral (2/8)
p. T1250M	T	D	B	D	M	D	0.534	0.711	damaging (6/8)
p. E1308D	T	D	P	D	N	D	0.802	0.533	damaging (6/8)
p. R1928G	T	B	B	D	M	D	0.638	0.697	damaging (5/8)

T = tolerated, D = damaging, B = benign, P = possibly damaging, N = neutral, L = predicted non-functional low, M = predicted functional medium, N = predicted non-functional neutral. For GERP++ and PhyloP rankscores a cutoff of 0.5 was used in order to rank the variant site as conserved. The variant was classified as “damaging” when the majority of tools predicted a functional effect (damaging, possibly damaging, predicted functional-damaging) and classified as „functionally neutral”when the majority of the tools predicted no functional effect (neutral, tolerated, benign, non-functional).

doi:10.1371/journal.pone.0150426.t003



In this study, we investigated the liability of *SCN1A* variants that were previously classified as pathogenic, in a cohort of common epilepsy syndromes. We detected an enrichment of HGMD annotated variants in our epilepsy patient cohort compared to controls ( $P = 8.30 \times 10^{-3}$ ). All identified HGMD *SCN1A* variants in our patients have also been detected in control individuals arguing against completely penetrant causal mutations. None of the variants were localized in the transmembrane regions of the protein (S1 Fig), where mutations are associated with more severe channel dysfunctions [13]. We considered seven, out of the eight (p.R27T, p.R28C, p.R542Q, p.R604H, p.T1250M, p.E1308D, p.R1928G) analyzed HGMD variants, as obvious benign based on the original reports, the high frequency in control individuals and missing or negative segregation and functional results (Tables 1 and 2; Fig 1).

In contrast to the other mutations classification of the p.T1174S mutation is less trivial. On the one hand, support as a potential genetic risk factor for epilepsy is based on several reasons. Firstly, the p.T1174S variant is overrepresented in epilepsy patients ( $P = 5.68 \times 10^{-4}$ ). Secondly, it segregates with the disease in our, and, previously analyzed families (Table 2, Fig 1). It was previously described in patients diagnosed with severe myoclonic epilepsy of infancy (inheritance not determined), Dravet syndrome (inherited from a mother with migraine), myoclonic astatic epilepsy (inherited from a healthy mother), and repetitively with familial hemiplegic migraine (FHM) [24, 27]. In our cohort, the p.T1174S mutation was found in patients with rolandic epilepsy, childhood absence epilepsy, juvenile absence epilepsy, benign familial neonatal seizures epilepsy and patients with a single generalized tonic-clonic seizure. In the diagnostic cohort we found patients with unclassified epilepsy with intellectual disability as well as temporal lobe epilepsy. Additionally, another family with the p.T1174S mutation has been identified (personal communication with Arvid Suls, University of Antwerp). In this family the mother carries the variant, as do the six elder siblings of seven. Two of these six carriers are affected by epilepsy with myoclonic seizures. Finally, previous *in vitro* electrophysiological recordings of the mutant p.T1174S Nav1.1 channel support the variant pathogenicity [19]. The molecular analysis revealed two divergent effects: a positive shift of the activation curve and deceleration of recovery from fast inactivation—consistent with a loss of function, and an increase of persistent current (I (NaP))—consistent with a gain of function [19]. On the other hand, the p.T1174S variant was only predicted to be damaging by one *in silico* program (FATHMM, Table 3) and was inherited by unaffected parent in the replication cohort five out of seven times.

Besides the unclear role of the p.T1174S variant, the majority of our investigated *SCN1A* HGMD variants cannot be classified as clearly pathogenic. Based on our results, we assume that a significant fraction of patients diagnosed with pathogenic *SCN1A* mutations may actually not carry an *SCN1A* variant of relevance. The role of *SCN1A* missense mutations in the pathogenesis of common epilepsies may thus be overstated (in general and e. g. studies in Table 2). Previous conclusions were frequently based on comparisons of allele frequencies between patients and small control datasets often without molecular follow up of the variant (Table 2). By accessing large gene mutation databases of non-epileptic individuals (e.g. <http://exac.broadinstitute.org>) we show that previous studies have been underpowered. In order to draw a definitive conclusion about pathogenicity for variants in common epilepsy syndromes, which are also present in healthy individuals, functional studies are mandatory (e.g. functional characterization of patient neurons derived from induced pluripotent cells).

The conclusions drawn from our study can be translated to other fields of research. Several prediction tools [35–39] evaluating the pathogenic potential of DNA sequence alterations are trained with variant lists extracted from the HGMD. Given that the disease association of the annotated *SCN1A* variants have to be questioned, it is likely that a similar picture can be seen for other complex diseases and genes. Simply extracting HGMD variants as training sets might bias prediction algorithms and machine learning approaches.

Overall, we highlight the ambiguities of variant classification in common epilepsy syndromes and emphasize that the majority of *SCN1A* variants could not be re-classified as pathogenic. Furthermore, our results warrant careful assessment of variants previously reported in small cohort studies.

## Supporting Information

**S1 Fig. *SCN1A* domain organization and mutations.** The schematic diagram showing the domain organization of the alpha subunit of the voltage-gated sodium ion channel coded by the *SCN1A* gene and the positions of the missense mutations (shown as orange circles). The complex consists of four homologous domains (I-IV), each containing six transmembrane segments (S1-S6). IQ indicates the localization of the IQ calmodulin-binding motif.

(PDF)

**S1 Table. List of genes included in the gene panel.**

(XLSX)

**S2 Table. Rare loss of function variants in 17 epilepsy patients with known HGMD *SCN1A* variants.**

(XLSX)

## Acknowledgments

We thank all participants and their families for participating in this study. The authors would like to thank the Exome Aggregation Consortium and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at (<http://exac.broadinstitute.org/>). H.L., B.N., P.N., T.S. and F.Z. received grants by the EuroEPINOMICS programme (DFG grant numbers: HL: LE1030/11-1, BN: BN416/5-1, PN: NU50/8-1, TS: SA434/5-1, FWF grant number: FZ: 1643-B09) within the EUROCORES framework of the European Science Foundation (ESF). The generation and management of genomics data for the Rotterdam Study are supported by the Netherlands Organization of Scientific Research NOW Investments (nr. 175.010.2005.011, 911-03-012) and the Netherlands Genomics Initiative (NGI)/NOW project nr. 050-060-810 (Netherlands Consortium for Healthy Ageing; NCHA). We thank the members of the Genomics Lab and the ERGO support team for their help in sampling the data and in creating the database, the study participants, the staff of the Rotterdam Study and the participating general practitioners and pharmacists. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Contributors

**EuroEPINOMICS CoGIE Consortium members are:** Consortium leader: Holger Lerche ([holger.lerche@uni-tuebingen.de](mailto:holger.lerche@uni-tuebingen.de)); Aarno Palotie, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK; Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland; Program in Medical and Population Genetics and Genetic Analysis Platform, The Broad Institute of MIT and Harvard, Cambridge, USA; Anna-Elina Lehesjoki, Folkhälsan Institute of Genetics, Helsinki, Finland; Neuroscience

Center, University of Helsinki, Helsinki, Finland; Research Programs Unit, Molecular Neurology, University of Helsinki, Helsinki, Finland; **Ann-Kathrin Ruppert**, Cologne Center for Genomics, University of Cologne, Cologne, Germany; **Arvid Suls**, Neurogenetics group, Department of Molecular Genetics, VIB, Antwerp, Belgium; Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium; **Auli Siren**, Outpatient Clinic for Persons with Intellectual Disability, Tampere University Hospital, Tampere, Finland; **Birgit Neophytou**, St. Anna Children's Hospital, Department of Neuropediatrics, Vienna, Austria; **Bobby Koeleman**, Department of Genetics, Center for Molecular Medicine, University Medical Center Utrecht, The Netherlands; **Dennis Lal**, Cologne Center for Genomics, University of Cologne, Cologne, Germany; **Edda Haberlandt**, Department of Pediatrics, Medical University of Innsbruck, Innsbruck, Austria; **Eva Maria Reinthaler**, Department of Neurology, Medical University of Vienna, Vienna, Austria; **Federico Zara**, Laboratory of Neurogenetics, Pediatric Neurology and Muscular Diseases Unit, Department of Neurosciences, Gaslini Institute, Genova, Italy; **Felicitas Becker**, Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany; **Fritz Zimprich**, Department of Neurology, Medical University of Vienna, Vienna, Austria; **Gabriel M Ronen**, Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada; **Hande Caglayan**, Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey; **Helle Hjalgrim**, Danish Epilepsy Centre, Dianalund, Denmark; Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark; **Hiltrud Muhle**, University Medical Center Schleswig-Holstein, Christian-Albrechts University, Kiel, Germany; **Hannelore Steinböck**, Private Practice of Pediatrics, 1150 Vienna, Austria; **Herbert Schulz**, Cologne Center for Genomics, University of Cologne, Cologne, Germany; **Holger Lerche**, Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Germany; **Holger Thiele**, Cologne Center for Genomics, University of Cologne, Cologne, Germany; **Ingo Helbig**, University Medical Center Schleswig-Holstein, Christian-Albrechts University, Kiel, Germany; **Janine Altmüller**, Cologne Center for Genomics, University of Cologne, Cologne, Germany; **Julia Geldner**, Department of Pediatrics, Hospital SMZ Süd Kaiser—Franz—Josef Spital, Vienna, Austria; **Julian Schubert**, Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany; **Kamel Jabbari**, Cologne Center for Genomics, University of Cologne, Cologne, Germany; **Kate Everett**, Cardiovascular and Cell Sciences Research Institute, St George's University of London, London, UK; **Martha Feucht**, Department of Pediatrics, Medical University of Vienna, Vienna, Austria; **Martina Balestri**, Division of Neurology, Bambino Gesù Children's Hospital and Research Institute, Rome, Italy; **Michael Nothnagel**, Cologne Center for Genomics, University of Cologne, Cologne, Germany; **Pasquale Striano**, Pediatric Neurology and Muscular Diseases Unit, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, G. Gaslini Institute, Genoa, Italy; **Patrick May**, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg; **Peter Nürnberg**, Cologne Center for Genomics, University of Cologne, Cologne, Germany, Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany; Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany; **Rikke S Møller**, Danish Epilepsy Centre, Dianalund, Denmark, Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark; **Rima Nabbout**, Centre de Référence Epilepsies Rares, Inserm U1129, Neuropediatrics Department, Necker-Enfants Malades Hospital, APHP, Paris Descartes University, CEA, Orsay, France; **Roland Krause**, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg; **Rudi Balling**, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-

sur-Alzette, Luxembourg; **Stephanie Baulac**, Inserm U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France; **Thomas Sander**, Cologne Center for Genomics, University of Cologne, Cologne, Germany; **Ursula Gruber-Sedlmayr**, Department of Pediatrics, Medical University of Graz, Graz, Austria; **Wolfram Kunz**, Department of Epileptology and Life & Brain Center, University of Bonn Medical Center, Bonn, Germany; **Yvonne G. Weber**, Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany

**Italian League against Epilepsy members are:** Consortium leader: **Amedeo Bianchi** ([bianchi.epigenet@tin.it](mailto:bianchi.epigenet@tin.it)); **Amedeo Bianchi**, Centro epilessia, UO Neurologia, Ospedale San Donato Arezzo, Italy; **Angela La Neve**, Neurology Clinic, University of Bari, 70124 Bari, Italy **Pasquale Striano**, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa and Giannina Gaslini Institute, 16148 Genova, Italy **Carlo Minetti**, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa and Giannina Gaslini Institute, 16148 Genova, Italy **Capovilla Giuseppe**, Department of Child Neuropsychiatry, C. Poma Hospital, Mantova, Italy

## Author Contributions

Conceived and designed the experiments: DL EMR BD BAN. Performed the experiments: DL EMR BD. Analyzed the data: DL EMR BD ER JRL. Contributed reagents/materials/analysis tools: A-EL MAI CMvD AGU A. Hofman HS UGS BN F. Zara A. Hahn PM HT GS FB YW MRC WK FZ AU ER JRL [Genetic Commission of the Italian League against Epilepsy] [EuroE-PINOMICS CoGIE Consortium]. Wrote the paper: DL EMR BD RK FZ JRL PN TS HL BAN PG. Revising and reviewing the manuscript: DL EMR BD PM HT A-EL GS ER MAI CMvD AGU A. Hofman HS UGS BN F. Zara A. Hahn PG FB YW MRC WK RK F. Zimprich JRL PN TS HL BAN.

## References

1. Wallace RH, Scheffer IE, Barnett S, Richards M, Dibbens L, Desai RR, et al. Neuronal sodium-channel alpha1-subunit mutations in generalized epilepsy with febrile seizures plus. *American journal of human genetics*. 2001; 68(4):859–65. Epub 2001/03/20. doi: [10.1086/319516](https://doi.org/10.1086/319516) PMID: [11254444](https://pubmed.ncbi.nlm.nih.gov/11254444/); PubMed Central PMCID: PMC1275639.
2. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. *Nature genetics*. 2000; 24(4):343–5. Epub 2000/03/31. doi: [10.1038/74159](https://doi.org/10.1038/74159) PMID: [10742094](https://pubmed.ncbi.nlm.nih.gov/10742094/).
3. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *American journal of human genetics*. 2001; 68(6):1327–32. Epub 2001/05/19. doi: [10.1086/320609](https://doi.org/10.1086/320609) PMID: [11359211](https://pubmed.ncbi.nlm.nih.gov/11359211/); PubMed Central PMCID: PMC1226119.
4. Parihar R, Ganesh S. The SCN1A gene variants and epileptic encephalopathies. *Journal of human genetics*. 2013; 58(9):573–80. Epub 2013/07/26. doi: [10.1038/jhg.2013.77](https://doi.org/10.1038/jhg.2013.77) PMID: [23884151](https://pubmed.ncbi.nlm.nih.gov/23884151/).
5. Kasperaviciute D, Catarino CB, Matarin M, Leu C, Novy J, Tostevin A, et al. Epilepsy, hippocampal sclerosis and febrile seizures linked by common genetic variation around SCN1A. *Brain: a journal of neurology*. 2013; 136(Pt 10):3140–50. Epub 2013/09/10. doi: [10.1093/brain/awt233](https://doi.org/10.1093/brain/awt233) PMID: [24014518](https://pubmed.ncbi.nlm.nih.gov/24014518/); PubMed Central PMCID: PMC3784283.
6. International League Against Epilepsy Consortium on Complex Epilepsies. Electronic address e-aeua. Genetic determinants of common epilepsies: a meta-analysis of genome-wide association studies. *The Lancet Neurology*. 2014; 13(9):893–903. Epub 2014/08/05. doi: [10.1016/S1474-4422\(14\)70171-1](https://doi.org/10.1016/S1474-4422(14)70171-1) PMID: [25087078](https://pubmed.ncbi.nlm.nih.gov/25087078/); PubMed Central PMCID: PMC4189926.
7. Goldberg-Stern H, Aharoni S, Afawi Z, Bennett O, Appenzeller S, Pendziwiat M, et al. Broad phenotypic heterogeneity due to a novel SCN1A mutation in a family with genetic epilepsy with febrile seizures plus. *Journal of child neurology*. 2014; 29(2):221–6. Epub 2013/11/22. doi: [10.1177/0883073813509016](https://doi.org/10.1177/0883073813509016) PMID: [24257433](https://pubmed.ncbi.nlm.nih.gov/24257433/).

8. Passamonti C, Petrelli C, Mei D, Foschi N, Guerrini R, Provinciali L, et al. A novel inherited SCN1A mutation associated with different neuropsychological phenotypes: Is there a common core deficit? *Epilepsy & behavior: E&B*. 2015; 43C:89–92. Epub 2015/01/09. doi: [10.1016/j.yebeh.2014.11.009](https://doi.org/10.1016/j.yebeh.2014.11.009) PMID: [25569746](https://pubmed.ncbi.nlm.nih.gov/25569746/).
9. Lossin C, Rhodes TH, Desai RR, Vanoye CG, Wang D, Carniciu S, et al. Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2003; 23(36):11289–95. Epub 2003/12/16. PMID: [14672992](https://pubmed.ncbi.nlm.nih.gov/14672992/).
10. Mantegazza M, Gambardella A, Rusconi R, Schiavon E, Annesi F, Cassulini RR, et al. Identification of an Nav1.1 sodium channel (SCN1A) loss-of-function mutation associated with familial simple febrile seizures. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(50):18177–82. Epub 2005/12/06. doi: [10.1073/pnas.0506818102](https://doi.org/10.1073/pnas.0506818102) PMID: [16326807](https://pubmed.ncbi.nlm.nih.gov/16326807/); PubMed Central PMCID: PMC1312393.
11. Ragsdale DS. How do mutant Nav1.1 sodium channels cause epilepsy? *Brain research reviews*. 2008; 58(1):149–59. Epub 2008/03/18. doi: [10.1016/j.brainresrev.2008.01.003](https://doi.org/10.1016/j.brainresrev.2008.01.003) PMID: [18342948](https://pubmed.ncbi.nlm.nih.gov/18342948/).
12. Tang B, Dutt K, Papale L, Rusconi R, Shankar A, Hunter J, et al. A BAC transgenic mouse model reveals neuron subtype-specific effects of a Generalized Epilepsy with Febrile Seizures Plus (GEFS+) mutation. *Neurobiology of disease*. 2009; 35(1):91–102. Epub 2009/05/05. doi: [10.1016/j.nbd.2009.04.007](https://doi.org/10.1016/j.nbd.2009.04.007) PMID: [19409490](https://pubmed.ncbi.nlm.nih.gov/19409490/); PubMed Central PMCID: PMC2735447.
13. Catterall WA, Kalume F, Oakley JC. Nav1.1 channels and epilepsy. *The Journal of physiology*. 2010; 588(Pt 11):1849–59. Epub 2010/03/03. doi: [10.1113/jphysiol.2010.187484](https://doi.org/10.1113/jphysiol.2010.187484) PMID: [20194124](https://pubmed.ncbi.nlm.nih.gov/20194124/); PubMed Central PMCID: PMC2901973.
14. Marini C, Mantegazza M. Na<sup>+</sup> channelopathies and epilepsy: recent advances and new perspectives. *Expert review of clinical pharmacology*. 2010; 3(3):371–84. Epub 2010/05/01. doi: [10.1586/ecp.10.20](https://doi.org/10.1586/ecp.10.20) PMID: [22111617](https://pubmed.ncbi.nlm.nih.gov/22111617/).
15. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, et al. The Rotterdam Study: 2012 objectives and design update. *European journal of epidemiology*. 2011; 26(8):657–86. Epub 2011/08/31. doi: [10.1007/s10654-011-9610-5](https://doi.org/10.1007/s10654-011-9610-5) PMID: [21877163](https://pubmed.ncbi.nlm.nih.gov/21877163/); PubMed Central PMCID: PMC3168750.
16. Reinthaler EM, Lal D, Jurkowski W, Feucht M, Steinbock H, Gruber-Sedlmayr U, et al. Analysis of ELP4, SRPX2, and interacting genes in typical and atypical rolandic epilepsy. *Epilepsia*. 2014; 55(8):e89–93. Epub 2014/07/06. doi: [10.1111/epi.12712](https://doi.org/10.1111/epi.12712) PMID: [24995671](https://pubmed.ncbi.nlm.nih.gov/24995671/).
17. Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Human genetics*. 2014; 133(1):1–9. Epub 2013/10/01. doi: [10.1007/s00439-013-1358-4](https://doi.org/10.1007/s00439-013-1358-4) PMID: [24077912](https://pubmed.ncbi.nlm.nih.gov/24077912/); PubMed Central PMCID: PMC3898141.
18. Lemke JR, Riesch E, Scheurenbrand T, Schubach M, Wilhelm C, Steiner I, et al. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia*. 2012; 53(8):1387–98. Epub 2012/05/23. doi: [10.1111/j.1528-1167.2012.03516.x](https://doi.org/10.1111/j.1528-1167.2012.03516.x) PMID: [22612257](https://pubmed.ncbi.nlm.nih.gov/22612257/).
19. Cestele S, Labate A, Rusconi R, Tarantino P, Mumoli L, Franceschetti S, et al. Divergent effects of the T1174S SCN1A mutation associated with seizures and hemiplegic migraine. *Epilepsia*. 2013; 54(5):927–35. Epub 2013/02/13. doi: [10.1111/epi.12123](https://doi.org/10.1111/epi.12123) PMID: [23398611](https://pubmed.ncbi.nlm.nih.gov/23398611/).
20. Zucca C, Redaelli F, Epifanio R, Zanotta N, Romeo A, Lodi M, et al. Cryptogenic epileptic syndromes related to SCN1A: twelve novel mutations identified. *Archives of neurology*. 2008; 65(4):489–94. Epub 2008/04/17. doi: [10.1001/archneur.65.4.489](https://doi.org/10.1001/archneur.65.4.489) PMID: [18413471](https://pubmed.ncbi.nlm.nih.gov/18413471/).
21. Orrico A, Galli L, Grosso S, Buoni S, Pianigiani R, Balestri P, et al. Mutational analysis of the SCN1A, SCN1B and GABRG2 genes in 150 Italian patients with idiopathic childhood epilepsies. *Clinical genetics*. 2009; 75(6):579–81. Epub 2009/06/13. PMID: [19522081](https://pubmed.ncbi.nlm.nih.gov/19522081/).
22. Nicita F, Spalice A, Papetti L, Ursitti F, Parisi P, Gennaro E, et al. Genotype-phenotype correlations in a group of 15 SCN1A-mutated Italian patients with GEFS+ spectrum (seizures plus, classical and borderline severe myoclonic epilepsy of infancy). *Journal of child neurology*. 2010; 25(11):1369–76. Epub 2010/08/24. doi: [10.1177/0883073810365737](https://doi.org/10.1177/0883073810365737) PMID: [20729507](https://pubmed.ncbi.nlm.nih.gov/20729507/).
23. Catarino CB, Liu JY, Liagkouras I, Gibbons VS, Labrum RW, Ellis R, et al. Dravet syndrome as epileptic encephalopathy: evidence from long-term course and neuropathology. *Brain: a journal of neurology*. 2011; 134(Pt 10):2982–3010. Epub 2011/07/02. doi: [10.1093/brain/awr129](https://doi.org/10.1093/brain/awr129) PMID: [21719429](https://pubmed.ncbi.nlm.nih.gov/21719429/); PubMed Central PMCID: PMC3187538.
24. Frosk P, Mhanni AA, Rafay MF. SCN1A mutation associated with intractable myoclonic epilepsy and migraine headache. *Journal of child neurology*. 2013; 28(3):389–91. Epub 2012/05/03. doi: [10.1177/0883073812443309](https://doi.org/10.1177/0883073812443309) PMID: [22550089](https://pubmed.ncbi.nlm.nih.gov/22550089/).

25. Rilstone JJ, Coelho FM, Minassian BA, Andrade DM. Dravet syndrome: seizure control and gait in adults with different SCN1A mutations. *Epilepsia*. 2012; 53(8):1421–8. Epub 2012/07/12. doi: [10.1111/j.1528-1167.2012.03583.x](https://doi.org/10.1111/j.1528-1167.2012.03583.x) PMID: [22780858](https://pubmed.ncbi.nlm.nih.gov/22780858/).
26. Yordanova I, Todorov T, Dimova P, Hristova D, Tincheva R, Litvinenko I, et al. One novel Dravet syndrome causing mutation and one recurrent MAE causing mutation in SCN1A gene. *Neuroscience letters*. 2011; 494(2):180–3. Epub 2011/03/15. doi: [10.1016/j.neulet.2011.03.008](https://doi.org/10.1016/j.neulet.2011.03.008) PMID: [21396429](https://pubmed.ncbi.nlm.nih.gov/21396429/).
27. Gargus JJ, Tournay A. Novel mutation confirms seizure locus SCN1A is also familial hemiplegic migraine locus FHM3. *Pediatric neurology*. 2007; 37(6):407–10. Epub 2007/11/21. doi: [10.1016/j.pediatrneurol.2007.06.016](https://doi.org/10.1016/j.pediatrneurol.2007.06.016) PMID: [18021921](https://pubmed.ncbi.nlm.nih.gov/18021921/); PubMed Central PMCID: [PMC2151385](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC2151385/).
28. Wang JW, Shi XY, Kurahashi H, Hwang SK, Ishii A, Higurashi N, et al. Prevalence of SCN1A mutations in children with suspected Dravet syndrome and intractable childhood epilepsy. *Epilepsy research*. 2012; 102(3):195–200. Epub 2012/12/01. doi: [10.1016/j.eplepsyres.2012.06.006](https://doi.org/10.1016/j.eplepsyres.2012.06.006) PMID: [23195492](https://pubmed.ncbi.nlm.nih.gov/23195492/).
29. Fendri-Kriaa N, Kammoun F, Rebai A, Kolsi D, Hadj Salem I, Fakhfakh F, et al. Genetic screening of two Tunisian families with generalized epilepsy with febrile seizures plus. *European journal of neurology: the official journal of the European Federation of Neurological Societies*. 2009; 16(6):697–704. Epub 2009/02/25. doi: [10.1111/j.1468-1331.2009.02570.x](https://doi.org/10.1111/j.1468-1331.2009.02570.x) PMID: [19236456](https://pubmed.ncbi.nlm.nih.gov/19236456/).
30. Heron SE, Scheffer IE, Iona X, Zuberi SM, Birch R, McMahon JM, et al. De novo SCN1A mutations in Dravet syndrome and related epileptic encephalopathies are largely of paternal origin. *Journal of medical genetics*. 2010; 47(2):137–41. Epub 2009/07/11. doi: [10.1136/jmg.2008.065912](https://doi.org/10.1136/jmg.2008.065912) PMID: [19589774](https://pubmed.ncbi.nlm.nih.gov/19589774/).
31. Escayg A, Heils A, MacDonald BT, Haug K, Sander T, Meisler MH. A novel SCN1A mutation associated with generalized epilepsy with febrile seizures plus—and prevalence of variants in patients with epilepsy. *American journal of human genetics*. 2001; 68(4):866–73. Epub 2001/03/20. doi: [10.1086/319524](https://doi.org/10.1086/319524) PMID: [11254445](https://pubmed.ncbi.nlm.nih.gov/11254445/); PubMed Central PMCID: [PMC1275640](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC1275640/).
32. Weiss LA, Escayg A, Kearney JA, Trudeau M, MacDonald BT, Mori M, et al. Sodium channels SCN1A, SCN2A and SCN3A in familial autism. *Molecular psychiatry*. 2003; 8(2):186–94. Epub 2003/03/01. doi: [10.1038/sj.mp.4001241](https://doi.org/10.1038/sj.mp.4001241) PMID: [12610651](https://pubmed.ncbi.nlm.nih.gov/12610651/).
33. Lossin C. A catalog of SCN1A variants. *Brain & development*. 2009; 31(2):114–30. Epub 2008/09/23. doi: [10.1016/j.braindev.2008.07.011](https://doi.org/10.1016/j.braindev.2008.07.011) PMID: [18804930](https://pubmed.ncbi.nlm.nih.gov/18804930/).
34. Lemke JR, Hendrickx R, Geider K, Laube B, Schwake M, Harvey RJ, et al. GRIN2B mutations in West syndrome and intellectual disability with focal epilepsy. *Annals of neurology*. 2014; 75(1):147–54. Epub 2013/11/26. doi: [10.1002/ana.24073](https://doi.org/10.1002/ana.24073) PMID: [24272827](https://pubmed.ncbi.nlm.nih.gov/24272827/); PubMed Central PMCID: [PMC4223934](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC4223934/).
35. Javed A, Agrawal S, Ng PC. Phen-Gen: combining phenotype and genotype to analyze rare disorders. *Nature methods*. 2014; 11(9):935–7. Epub 2014/08/05. doi: [10.1038/nmeth.3046](https://doi.org/10.1038/nmeth.3046) PMID: [25086502](https://pubmed.ncbi.nlm.nih.gov/25086502/).
36. Robinson PN, Kohler S, Oellrich A, Sanger Mouse Genetics P, Wang K, Mungall CJ, et al. Improved exome prioritization of disease genes through cross-species phenotype comparison. *Genome research*. 2014; 24(2):340–8. Epub 2013/10/29. doi: [10.1101/gr.160325.113](https://doi.org/10.1101/gr.160325.113) PMID: [24162188](https://pubmed.ncbi.nlm.nih.gov/24162188/); PubMed Central PMCID: [PMC3912424](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC3912424/).
37. Bendl J, Stourac J, Salanda O, Pavelka A, Wieben ED, Zendulka J, et al. PredictSNP: robust and accurate consensus classifier for prediction of disease-related mutations. *PLoS computational biology*. 2014; 10(1):e1003440. Epub 2014/01/24. doi: [10.1371/journal.pcbi.1003440](https://doi.org/10.1371/journal.pcbi.1003440) PMID: [24453961](https://pubmed.ncbi.nlm.nih.gov/24453961/); PubMed Central PMCID: [PMC3894168](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC3894168/).
38. Mort M, Sterne-Weiler T, Li B, Ball EV, Cooper DN, Radivojac P, et al. MutPred Splice: machine learning-based prediction of exonic variants that disrupt splicing. *Genome biology*. 2014; 15(1):R19. Epub 2014/01/24. doi: [10.1186/gb-2014-15-1-r19](https://doi.org/10.1186/gb-2014-15-1-r19) PMID: [24451234](https://pubmed.ncbi.nlm.nih.gov/24451234/); PubMed Central PMCID: [PMC4054890](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC4054890/).
39. Frousios K, Iliopoulos CS, Schlitt T, Simpson MA. Predicting the functional consequences of non-synonymous DNA sequence variants—evaluation of bioinformatics tools and development of a consensus strategy. *Genomics*. 2013; 102(4):223–8. Epub 2013/07/09. doi: [10.1016/j.ygeno.2013.06.005](https://doi.org/10.1016/j.ygeno.2013.06.005) PMID: [23831115](https://pubmed.ncbi.nlm.nih.gov/23831115/).