Research on the distribution ratio of variants and the proportion of variant types in different conditions of SCN1A Seizure Disorders using ClinVar

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Article

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Abstract

The aim of this study was to clarify the distribution of *SCN1A* variants in each condition of *SCN1A* seizure disorders and the proportion of the *SCN1A* variant types in different conditions of *SCN1A* seizure disorders.This study consisted of querying *SCN1A* variants in ClinVar, reclassificating ClinVar P/LP *SCN1A* missense variants provided by a singgle submitter, validating conditions of *SCN1A* Seisure Disorders by reclassification of P/LP variants and screening variant types of conditions of *SCN1A* Seisure Disorders by validation.The result showed that most of the P/LP *SCN1A* missense variants provided by a single submitter in ClinVar remained at the P/LP level (247/383), and a few variants were converted from P/LP to VUS (136/383). The condition "Early infantile epileptic encephalopathy with suppression bursts" shown in ClinVar was actually all other conditons, such as Dravet syndrome/Severe myoclonic epilepsy of infancy(DS/SMEI)(69/89) and Generalized epilepsy with febrile seizures plus/Borderline severe myoclonic epilepsy of infancy(GEFS+/SMEB) (3/89). Both in missense and trunction, DS/SMEI-related *SCN1A* variants had the highest proportion (291/371, 78.4%),and there were still quite a few some *SCN1A* variants are associated with other conditions such as GEFS+, FMH, DEE, Lennox-Gastaut Syndrome and Intractable childhood epilepsy with generlaized tonic-clonic seizures (80/371, 21.6%).This provided data support for clinicians to grasp the severity of epilepsy caused by these *SCN1A* variants.

Introduction

SCN1A seizure disorders range from mild febrile seizures and generalized epilepsy with febrile seizures plus (GEFS+)[1] to Dravet syndrome[2] and intractable childhood epilepsy with generalized tonic-clonic seizures (ICE-GTC)[3]. Even in developmental and epileptic encephalopathy (DEE), a range of severities is observed, from myoclonic-atonic epilepsy (MAE) to Dravet syndrome (DS), infantile epilepsy with migrating focal epilepsy (EIMFS) and early-onset *SCN1A* DEE[4].

SCN1A encodes the alpha subunit of the neuronal voltage-gated sodium channel (also known as Na v 1.1)[5]. Pathogenic *SCN1A*/NaV1.1 mutations have been studied using several experimental models at different integration levels, revealing important information about the effects of mutations and pathologic mechanisms, particularly the identification of loss-of-function of NaV1.1 caused by epileptogenic mutations and leading to hypoexcitability of GABAergic neurons[6]. Based on gnomAD database query(URL: https://gnomad.broadinstitute.org/), it can also be concluded that loss of function (pLoF) is the main pathogenic mechanism of *SCN1A* seizure disorders (PLI = 1). However, *SCN1A* missense variants also account for a considerable proportion of *SCN1A* seizure disorders (Z = 5.22).

ClinVar is a freely available, public archive of human genomic variants and interpretations of their relationships to diseases and other conditions[7]. It is maintained at the National Center for Biotechnology Information (NCBI) within the National Library of Medicine (NLM) at the National Institutes of Health (NIH)[8]. ClinVar represents interpretations of clinical significance, when that significance was last interpreted by the submitter, the mode of inheritance of a variation relative to a disorder, and qualification of severity of phenotype, among other aspects[9]. According to the guidelines published by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) in 2015[10], rules are formulated to meet one of the following classification terms for sequence variant interpretation of Mendelian genes: pathogenic (P), likely pathogenic (LP), uncertain significance (VUS), likely benign (LB), or benign (B). According to the ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020(URL: https://www.acgs.uk.com/quality/best-practice-guidelines/#VariantGuidelines),VUS is divided into six levels because of the large interval of the posterior probability (10%-90%),including "Hot" (81.2%-90%), "Warm" (67.5%-81.2%), "Tepid" (50%-67.5%), "Cool" (32.5%-50%), "Cold" (18.8%-32.5%), and "Ice cold" (10%-18.8%).

In our study,1139 *SCN1A* gene variants by querying ClinVar are used to research correlation between gene variant types and condition(s) of *SCN1A* seizure disorders. The purpose of this study is to clarify the distribution of *SCN1A* variants in each condition of *SCN1A* seizure disorders and the proportion of the *SCN1A* variant types in different conditions of *SCN1A* seizure disorders.

Methods

1. Querying SCN1A variants in ClinVar

Using ClinVar (URL: https://www.ncbi.nlm.nih.gov/clinvar/), we searched for "*SCN1A*". We selected "Likely pathogenic" and "Pathogenic" in the "Clinical significance" module on the left.Then we selected “Frameshift””Missense””Nonsens e” and ”Splice site” .Finally we screened 1139 *SCN1A* gene variants of them(Figure.1).

2. Reclassification of ClinVar P/LP SCN1A missense variants provided by a singgle submitter

We selected 383 ClinVar P/LP *SCN1A* missense variants provided by a singgle submitter in order to reclassify the Pathogenicity and then we abandoned VUS variants by the reclassification. At last,we screened 1002 P/LP *SCN1A* gene variants out by the reclassification for the further reseach (Figure.1).Pathogenicity reclassification and data interpretation rules for mutations are based on guidelines of the American College of Medical Genetics and Genomics (ACMG) [10]and ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020.We determined the use of PM2/BA1/BS1/BS2 evidence by querying the 1000 Genomes, ExAC and gnomAD databases. We performed gene function prediction using software, such as REVEL, VEST, FATHMM, SIFT, Polyphen2, and CADD, and compared clinical symptoms.The interpretation databases used included DGV, DECIPHER, ClinGen, OMIM, UCSC, ClinVar, HGMD and PubMed.

3. Validation of conditions of SCN1A **Seisure Disorders by reclassification of P/LP variants**

We classified the 1002 P/LP *SCN1A* gene variants by reclassification according to the condition(s) shown in ClinVar and divided them into 6 conditions: early infantile epileptic encephalopathy with suppression bursts(EIEESB), severe myoclonic epilepsy in infancy(SMEI)/Dravet syndrome(DS), febrile seizures plus in a family (GEFS+)/borderline severe myoclonic epilepsy in infancy (SMEB), Phenotype heterogeneity(Mix) and other condition(s). We validated these 6 conditions by querying case report in Pubmed.0 EIEESB, 291 SMEI/DS, 19 GEFS+/SMEB, 35 Mix and 26 other conditions were finally determined(Figure.1).

4. Statistical analysis of variant types of conditions of SCN1A **Seisure Disorders by validation**

We divided the variant types of conditions of *SCN1A* Seizure Disorder in ClinVar by validation into "Missense" and "Truncation", and divided "Truncation" into "Nonsense", "Deletion/duplication" and "Splice site".We classifed and counted the number of these variant types.

5. Analysis of rare conditions of SCN1A **Seizure Disorder in ClinVar by validation**

From 26 other condition(s), we screened 11 *SCN1A* gene variants out with rare conditons for pathogenicity analysis and research.

6. Statistical analysis

Statistical figure and statistical table analyses were performed in SPSS 22.0.

Results

1. Reclassification of ClinVar P/LP SCN1A **missense variants provided by a singgle submitter**

We reclassified 383 ClinVar P/LP *SCN1A* missense variants provided by a single submitter and found that 129 variants (129/383) had no change in classification; 115 variants (115/383) changed from Pathogenic to Likely pathogenic (P_LP); 3 variants (3/383) changed from Likely pathogenic to Pathogenic (LP_P); 21 variants (21/383) changed from Pathogenic to Hot VUS (P_Hot VUS); 70 variants (70/383) changed from Likely pathogenic Hot VUS (LP_Hot VUS); 5 variants (5/383) changed from Pathogenic to Warm VUS (P_Warm VUS); 24 variants (24/383) changed from Likely pathogenic to Warm VUS (LP_Warm VUS); 1 variant (1/383) from Pathogenic to Tepid VUS (P_Tepid VUS); 14 variants (14/383) from Likely pathogenic to Tepid VUS (LP_Tepid VUS); 1 variant (1/383) from Likely pathogenic to Cold VUS (LP_Cold VUS)(Figure .2) .Finally, by reclassification, we classified 16 Pathogenic variants (4.2%), 231 Likely pathogenic variants (60.3%), 91 Hot VUS variants (23.8%), and 29 Warm VUS variants (7.6%), 15 Tepid VUS variants (3.9%) and 1 Cold VUS variant (0.2%)(Table.1 and Figure.2A).

Table.1 Reclassification outcomes of ClinVar P/LP *SCN1A* missense variants provided by a singgle submitter

ClinVar classification	Reclassification outcomes, n(%)						All
	P	LP	Hot VUS	Warm VUS	Tepid VUS	Cold VUS	
P	13 (8.3)	115 (74.2)	21 (13.5)	5 (3.2)	1 (0.6)	0	155 (100)
LP	3 (1.3)	116 (50.9)	70 (30.7)	24 (10.5)	14 (6.2)	1 (0.4)	228 (100)
Total	16 (4.2)	231 (60.3)	91 (23.8)	29 (7.6)	15 (3.9)	1 (0.2)	383 (100)
P:Pathogenic;							

LP:Likely pathogenic

2. Validation of conditions of SCN1A Seizure Disorders in ClinVar

We conducted a case report query validation of 1002 variants related to conditions of *SCN1A* Seizure Disorders that completed ClinVar P/LP variant reclassification and found that the number of actual case reports from 413 "Early infantile epileptic encephalopathy with suppression bursts (EIEESB)" shown in ClinVar was 0 (Supplementary Table 2;Table.2); in addition, among the 1002 cases of conditions of *SCN1A* Seizure Disorders in ClinVar, 371 cases were validated by case reports, including 291 (78.4%) cases of Dravet syndrome/Severe myoclonic epilepsy of infancy (DS/ SMEI) (Supplementary Table 2;Table.2); 19 (5.2%) cases of Generalized epilepsy with febrile seizures plus/Borderline severe myoclonic epilepsy of infancy (GEFS+/SMEB)(Supplementary Table 3;Table.2); 35 (9.4%) cases of Phenotype heterogeneity (MIX) (Supplementary Table 4;Table.2)and 26 (7%) cases of other condition(s)(Supplementary Table 5;Table.2).

Table.2 Validation outcomes of variants related to conditions of *SCN1A* Seizure Disorders in ClinVar

ClinVar condition(s)	Validation outcomes, n(%)					All
	EIEESB	DS/SMEI	GEFS+/SMEB	MIX	Other conditon(s)	
EIEESB	0	69 (77.5)	3 (3.4)	10 (11.2)	7 (7.8)	89 (100)
DS/SMEI	0	207 (90.8)	2 (0.9)	10 (4.4)	9 (3.9)	228 (100)
GEFS+/SMEB	0	2 (11.1)	12 (66.7)	3 (16.7)	1 (5.5)	18 (100)
MIX	0	13 (41.9)	2 (6.5)	12 (38.7)	4 (12.9)	31 (100)
Other condition(s)	0	0	0	0	5 (100)	5 (100)
Toal	0	291 (78.4)	19 (5.2)	35 (9.4)	26 (7.0)	371 (100)

EIEESB:Early infantile epileptic encephalopathy with suppression bursts;

DS:Dravet syndrome;

SMEI:Severe myoclonic epilepsy of infancy;

GEFS+:Generalized epilepsy with febrile seizures plus;

SMEB:Borderline severe myoclonic epilepsy of infancy;

MIX:Phenotype heterogeneity

3. Statistical analysis of variant types of conditions of SCN1A Seisure Disorders by validation

Statistical analysis of the above 371 variant types of conditions of *SCN1A* Seisure Disorders by validation shows that the ratio of missense and truncation (nonsense, deletion/duplication and splice site) was comparable (140:151) in the condition of DS/SMEI. However, in GEFS+/SMEB, MIX and other condition(s), the ratio of missense was significantly higher than that of truncation (nonsense, deletion/duplication and splice site), which were 16:3, 22:13 and 23:3, respectively(Table.3).At the same time, by comparing the distribution of conditions of *SCN1A* Seisure Disorders in each variant type, we found that the cases of missense variants in the condition of DS/SMEI accounted for 69.7% of the total conditions, while truncation (Nonsense, Deletion/ The cases of duplication and Splice site) variants all exceeded 80% of the total conditons(Table.3 and Figure.2B).

Table.3 Variant types of conditions of *SCN1A* Seizure Disorder in ClinVar by validation

Conditions by validation	Variant types,n(%)[%]				
	Missense	Nonsense	Deletion/duplication	Splice site	All
DS/SMEI	140 (48.1) [69.7]	66 (22.7) [89.1]	54 (18.6) [93.1]	31 (10.6) [81.6]	291 (100)
GEFS+/SMEB	16 (84.1) [8.0]	1 (5.3) [1.4]	1 (5.3) [1.7]	1 (5.3) [2.6]	19 (100)
MIX	22 (62.9) [10.9]	5 (14.3) [6.8]	3 (8.5) [5.2]	5 (14.3) [13.2]	35 (100)
Other condition(s)	23 (88.5) [11.4]	2 (7.7) [2.7]	0	1 (3.8) [2.6]	26 (100)
Total	201 [100]	74 [100]	58 [100]	38 [100]	371
DS:Dravet syndrome;					

SMEI:Severe myoclonic epilepsy of infancy;

GEFS+:Generalized epilepsy with febrile seizures plus;

SMEB:Borderline severe myoclonic epilepsy of infancy;

MIX:Phenotype heterogeneity

4. Analysis of variants related to rare conditions of SCN1A **Seizure Disorder in ClinVar by validation**

Among the above 26 cases of other conditions by validation, we screened 11 variants of rare conditions of *SCN1A* Seizure Disorder, in which conditions included 1 case of Lennox-Gastaut Syndrome, 2 cases of malignant migrating partial seizures of infancy, 2 cases of retractable childhood epilepsy with generlaized tonic-clonic seizures (ICE-GTC), 3 cases of familial hemiplegic migraine and 3 cases of Developmental and epileptic encephalopathy 6B(Table.4).

Table.4 Variants related to rare conditions of *SCN1A* Seizure Disorder in ClinVar by validation

No.	GRCh37 location	HGVS	Significance	Condition(s)	Reference(s)
1	Chr2:166848563	NM_001165963.4(*SCN1A*): c.5222G > C (p.Cys1741Ser)	Pathogenic	Lennox-Gastaut Syndrome	11,12
2	Chr2:166848779	NM_001165963.4(*SCN1A*): c.5006C > A (p.Ala1669Glu)	Likely pathogenic	Malignant migrating partial seizures of infancy	13
3	Chr2:166894297	NM_001165963.4(*SCN1A*): c.2935G > A (p.Gly979Arg)	Pathogenic	Intractable childhood epilepsy with generlaized tonic-clonic seizures	14
4	Chr2:166895938	NM_001165963.4(*SCN1A*): c.2584C > G (p.Arg862Gly)	Pathogenic	Migrating partial seizures of infancy	15
5	Chr2:166850677	NM_001165963.4(*SCN1A*): c.4831G > T (p.Val1611Phe)	Pathogenic	Intractable childhood epilepsy with generalized tonic-clonic seizures	14
6	Chr2:166852609	NM_001165963.4(*SCN1A*): c.4495T > C (p.Phe1499Leu)	Likely pathogenic	Familial hemiplegic migraine	16
7	Chr2:166854557	NM_001165963.4(*SCN1A*): c.4467G > C (p.Gln1489His)	Likely pathogenic	Familial hemiplegic migraine	16
8	Chr2:166854559	NM_001165963.4(*SCN1A*): c.4465C > A (p.Gln1489Lys)	Likely pathogenic	Familial hemiplegic migraine	17
9	Chr2:166903393	NM_001165963.4(*SCN1A*): c.1264G > T (p.Val422Leu)	Pathogenic	Developmental and epileptic encephalopathy 6B	18
10	Chr2:166859233	NM_001165963.4(*SCN1A*): c.4033C > T (p.Pro1345Ser)	Likely pathogenic	Developmental and epileptic encephalopathy 6B	19,20
11	Chr2:166909379	NM_001165963.4(*SCN1A*): c.677C > T (p.Thr226Met)	Pathogenic	Developmental and epileptic encephalopathy 6B	19,21,22,23

Discussion

SCN1A Seizure Disorders are generally considered to have a broad phenotype ranging from simple febrile seizures and generalized epilepsy with febrile seizures plus to Dravet syndrome and retractable childhood epilepsy with generalized tonic-clonic seizures (ICE-GTC)[24]. However, the distribution ratio of these phenotypes in *SCN1A* Seizure Disorders is unclear due to the limited number of cases in various institutions. So far, ClinVar has included 1360 variations related to *SCN1A* Seizure Disorders. When CNV is removed and only single-nucleotide variations are retained, there are 1,139 variations being screened. Among these ClinVar variants, 1* variants provided by a single submitter have lower associated evidence than 2* or 3* variants[25]. At the same time, not all of the conditions of *SCN1A* Seizure Disorders shown by ClinVar have case reports. The evidences for some conditions are insufficient to support the correlation of the target variants with the conditions. Therefore, in our study, ClinVar P/LP *SCN1A* missense variants provided by a single submitter were reclassified, and then these reclassified conditions of *SCN1A* Seizure Disorders were validated by querying case reports in Pubmed.

1. **Reclassification of ClinVar P/LP** SCN1A **missense variants provided by a singgle submitter**

Reclassification outcomes of 383 ClinVar P/LP *SCN1A* missense variants provided by a single submitter shows that most of the P/LP variants remained at the P/LP level (247/383)(Table.1), and a few variants were converted from P/LP to VUS (136/383)(Table.1). Among them, there are 10 variants changed from P/LP to VUS due to the reduced level of PS2 evidence (Supplementary Table 1), including

p.Met1348Ile, p.Pro657Leu, p.Ser626Gly, p.Val422Ala, p.Met350Val, p.Ala342Ser, p.Ala333Thr, p.Val250Ile, p.Asn115Lys and p.Ala24Thr. According to specifications for the ACMG/AMP recommendations developed by the ClinGen Sequence Variant Interpretation Working Group (SVI) and various disease-specific variant curation expert panels (VCEP) (https://clinicalgenome.org/working-groups/sequence-variant-interpretation/), when the phenotype is consistent with the gene but not highly specific and if there is a de novo status with validated parental relationships, the PS2 evidence strength level is 1 point (PS2_Moderate); if there is a de novo status with unvalidated parental relationships, the PS2 evidence strength level is 0.5 points (PS2_Supporting). Seizure is not a highly specific phenotype[26].Therefore,PS2_Moderate is applied according to the SVI recommendation for de novo criteria.

In addition, p. Gly682Val (Supplementary Table 1) changed from the PS3 level to PS3_Supporting, resulting in a change of P/LP to VUS. According to recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework[27], if the number of benign/pathogenic variant controls used in a functional study is less than 10, PS3 should be changed to PS3_Supporting. Regarding p.Gly682Val, as only one pathogenic variant was used and a benign variant was not used as controls, PS3_Supporting fits the pathogenicity assessment for this variant according to the SVI recommendation.

Besides, the assignment of p. Asp1742Asn (Supplementary Table 1) is changed from P/LP to VUS due to reduced PM2 levels. Based on SVI Recommendation for Absence/Rarity (PM2) (https://www.clinicalgenome.org/site/assets/files/5182/pm2_-_svi_recommendation_-_approved_sept2020.pdf), the weight of criterion PM2 has been changed from a moderate level to a supporting level (PM2_Supporting), which resulted in a change in the classification of p.Asp1742Asn.

2. Validation of conditions of SCN1A **Seizure Disorders in ClinVar and statistical analysis of variant types of conditions of** SCN1A **Seisure Disorders by validation**

Validation outcomes of variants related to the conditions of *SCN1A* Seizure Disorders in ClinVar suggests that the condition "Early infantile epileptic encephalopathy with suppression bursts" shown in ClinVar is actually all other conditons, such as Dravet syndrome/Severe myoclonic epilepsy of infancy(69/89) and Generalized epilepsy with febrile seizures plus/Borderline severe myoclonic epilepsy of infancy (3/89)(Supplementary Table 2). Among all conditions of *SCN1A* Seizure Disorders, DS/SMEI accounts for the highest proportion (291/371) (Table.2), which is much higher than other conditions. GEFS+/SMEB accounts for a lower proportion (19/371)(Table.2). In addition, There are 35 *SCN1A* gene variants having Phenotype heterogeneity of varying severity and there are 26 *SCN1A* gene variants associated with rare conditions such as familial hemiplegic migraine(Table.2).

According to the Gene-Disease Validity presented by CinGen(URL: https://clinicalgenome.org/), the *SCN1A* gene is classified as "Definitive" in generalized epilepsy with febrile seizures plus, "Definitive" in Dravet syndrome, "Strong" in developmental and epileptic encephalopathy and "Moderate" in familial hemiplegic migraine. The OMIM(URL: https://www.omim.org/) database suggests that the *SCN1A* gene is associated with Developmental and epileptic encephalopathy 6B, non-Dravet, Dravet syndrome, February seizures, familial, 3A, Generalized epilepsy with febrile seizures plus, type 2 and Migraine, familial hemiplegic, 3. No relevant database is found showing the association of *SCN1A* gene with Early infantile epileptic encephalopathy with suppression bursts. Therefore, the condition "Early infantile epileptic encephalopathy with suppression bursts" submitted in ClinVar is suspected to be excluded from *SCN1A* Seizure Disorders.

Distribution of variant types of the conditions of *SCN1A* Seizure Disorder in ClinVar by validation shows that among all truncation variants, DS/SMEI-related *SCN1A* variants account for the vast majority (151/170, 88.8%)(Figure.2B). This is consistent with the fact that the pathogenic mechanism of DS/SMEI-related *SCN1A* variants is loss of function[28,29].Severe Myoclonic Epilepsy in infancy (SMEI, or Dravet syndrome) is a severe condition on the genetic epilepsy with febrile seizures plus (GEFS+) spectrum of seizure disorders[30,31]. The clinical presentation of DS/SMEI is characterized by frequent febrile seizures followed by non-febrile seizures, mainly clonic and unilateral, of prolonged duration and frequent status epilepticus[32]. There is sufficient case-level and experimental data evidence to support this gene-disease pair[29,33–36]. In addition, expression, protein interaction, and biochemical function studies as well as mouse models also provide experimental evidence that *SCN1A* variants cause DS[37,38].

Generalized epilepsy with febrile seizures plus (GEFS+) is a rare autosomal dominant, familial condition with incomplete penetrance and variable expressivity, which includes seizures ranging from usually mild (eg, isolated febrile seizures) to less severe (including medically treatable generalized epilepsy, refractory generalized epilepsy, or in some cases Dravet syndrome)[39]. The penetrance of the GEFS + phenotype has been estimated to be 70%[40].In affected families, parents of more than 95% of individuals with GEFS + also have the same *SCN1A* pathogenic variant[41]. Distribution of variant types of the conditions of *SCN1A* Seizure Disorder in ClinVar by validation shows that the number of variants in GEFS+/SMEB is low (5.2%)(Table.2), indicating that although *SCN1A* variants have conditions ranging from mild to severe, variants associated with severe conditions are still the mainstream of *SCN1A* variants.

Analysis of variants related to rare conditions of *SCN1A* Seizure Disorder in ClinVar by validation shows that p.Val422Leu(c.1264G > T), p.Pro1345Ser(c.4033C > T) and p.Thr226Met (c.677C > T) are all associated with developmental and epileptic encephalopathy (DEE) (Supplementary Table 5). Distinguished by DS/SMEI, DEE has a younger age at onset, beginning at < 3 months compared with the typical seizure onset age range of 4 to 15 months in Dravet syndrome[19]. Unlike DS/SMEI, DEE has Severe developmental disability and reported movement disorders including choreoathetosis, dystonia, and perioral hyperkinesia. Currently the 3 *SCN1A* variants that have been reported to be associated with DEE[42] are all missense variants, the most notable of which is p.Thr226Met (c.677C > T)(Table.4), a variant that has been identified in several individuals with similar characteristics and identified as de novo[19,43,44]. Because these three *SCN1A* variants is discovered for a short time, the association of *SCN1A* with DEE is not well documented. It is currently known that the mechanism by which *SCN1A* variation leads to the phenotype of DEE is mainly due to gain of function[42].

Analysis of variants related to rare conditions of *SCN1A* Seizure Disorder in ClinVar by validation shows that three missense variants in *SCN1A* have been reported to associated with familial hemiplegic migraine (FHM) (Supplementary Table 5,Table.4), including one de novo variant[45,46]. FHM is a rare autosomal dominant disorder characterized by migraine attacks with aura and associated hemiplegic attacks, in which *SCN1A*-associated FHM has a penetrance approaching 100%[47].The recognized mechanism of aura in hemiplegic migraine is cortical spreading depression. Because the effects of FHM variants in *SCN1A* are often complex and diverse, the exact mechanism by which defects in *SCN1A* may lead to cortical spreading inhibition is unknown. According to ClinGen's gene curation classification, the association between *SCN1A* and FHM is "Moderate", which means that the pathogenic classification of all *SCN1A* variants associated with FHM can only be classified as "Likely pathogenic" at the highest level.

Both in missense and trunction, DS/SMEI-related *SCN1A* variants have the highest proportion (291/371, 78.4%)(Figure.2B), which shows that although DS/SMEI is the most important condition in *SCN1A* Seizure Disorders, there are still quite a few some *SCN1A* variants are associated with other conditions such as GEFS+, FMH, DEE, Lennox-Gastaut Syndrome and Intractable childhood epilepsy with generlaized tonic-clonic seizures (80/371, 21.6%)(Supplementary Table 5).

Conclusions

SCN1A Seizure Disorders have a fairly broad phenotypic spectrum, which often makes it difficult for clinicians to judge the severity of the disease. In our study, the distribution ratio of *SCN1A* variants in these conditions is clarified, and the proportion of variant types in each condition of *SCN1A* Seizure Disorders is determined. This provides data support for clinicians to grasp the severity of epilepsy caused by these *SCN1A* variants, and plays a guiding role in the clinical diagnosis and treatment for *SCN1A* Seizure Disorders.

Declarations

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Code availability

Codes are available from the corresponding author upon request.

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Author contributions

Yuan Tian worked on ACMG guideline reclassification and manuscript writing; Shihong Cui ,Chenchen Ren and Enwu Yuan provided analysis platform and basic work; Hua Zhang worked on clinical phenotyping; Ying Li, Bo Yang,Jia Peng, Erfeng Yuan and Yaqing Guo worked on ClinVar variant screening and statistics ; Ling Liu provided the general idea of the manuscript.

Competing interests

The authors declare no competing interests.

References

1. Camfield P, Camfield C. Febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+). Epileptic Disord 2015;17:124–133.
2. Lagae L. Dravet syndrome. Curr Opin Neurol 2021;34:213–218.
3. Scheffer IE, Nabbout R. SCN1A-related phenotypes: Epilepsy and beyond. Epilepsia 2019;60 Suppl 3:S17-S24.
4. de Lange IM, Gunning B, Sonsma A, van Gemert L, van Kempen M, Verbeek NE et al. Outcomes and comorbidities of SCN1A-related seizure disorders. Epilepsy Behav 2019;90:252–259.
5. Colasante G, Lignani G, Brusco S, Di Berardino C, Carpenter J, Giannelli S et al. dCas9-Based Scn1a Gene Activation Restores Inhibitory Interneuron Excitability and Attenuates Seizures in Dravet Syndrome Mice. Mol Ther 2020;28:235–253.
6. Mantegazza M, Broccoli V. SCN1A/NaV 1.1 channelopathies: Mechanisms in expression systems, animal models, and human iPSC models. Epilepsia 2019;60 Suppl 3:S25-S38.
7. Landrum MJ, Chitipiralla S, Brown GR, Chen C, Gu B, Hart J et al. ClinVar: improvements to accessing data. Nucleic Acids Res 2020;48:D835-D844.
8. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018;46:D1062-D1067.
9. Wright CF, Eberhardt RY, Constantinou P, Hurles ME, FitzPatrick DR, Firth HV. Evaluating variants classified as pathogenic in ClinVar in the DDD Study. Genet Med 2021;23:571–575.
10. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J 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.
11. Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE et al. De novo mutations in epileptic encephalopathies. Nature 2013;501:217–221.
12. Zhu X, Padmanabhan R, Copeland B, Bridgers J, Ren Z, Kamalakaran S et al. A case-control collapsing analysis identifies epilepsy genes implicated in trio sequencing studies focused on de novo mutations. PLoS Genet 2017;13:e1007104.
13. Freilich ER, Jones JM, Gaillard WD, Conry JA, Tsuchida TN, Reyes C et al. Novel SCN1A mutation in a proband with malignant migrating partial seizures of infancy. Arch Neurol 2011;68:665–671.
14. Fujiwara T, Sugawara T, Mazaki-Miyazaki E, Takahashi Y, Fukushima K, Watanabe M et al. Mutations of sodium channel alpha subunit type 1 (SCN1A) in intractable childhood epilepsies with frequent generalized tonic-clonic seizures. Brain 2003;126:531–546.
15. Carranza RD, Hamiwka L, McMahon JM, Dibbens LM, Arsov T, Suls A et al. De novo SCN1A mutations in migrating partial seizures of infancy. Neurology 2011;77:380–383.
16. Vahedi K, Depienne C, Le Fort D, Riant F, Chaine P, Trouillard O et al. Elicited repetitive daily blindness: a new phenotype associated with hemiplegic migraine and SCN1A mutations. Neurology 2009;72:1178–1183.
17. Dichgans M, Freilinger T, Eckstein G, Babini E, Lorenz-Depiereux B, Biskup S et al. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet 2005;366:371–377.
18. Ohashi T, Akasaka N, Kobayashi Y, Magara S, Kawashima H, Matsumoto N et al. Infantile epileptic encephalopathy with a hyperkinetic movement disorder and hand stereotypies associated with a novel SCN1A mutation. Epileptic Disord 2014;16:208–212.
19. Sadleir LG, Mountier EI, Gill D, Davis S, Joshi C, DeVile C et al. Not all SCN1A epileptic encephalopathies are Dravet syndrome: Early profound Thr226Met phenotype. Neurology 2017;89:1035–1042.
20. Carvill GL, Heavin SB, Yendle SC, McMahon JM, O'Roak BJ, Cook J et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. Nat Genet 2013;45:825–830.
21. Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM et al. The spectrum of SCN1A-related infantile epileptic encephalopathies. Brain 2007;130:843–852.
22. Mulley JC, Hodgson B, McMahon JM, Iona X, Bellows S, Mullen SA et al. Role of the sodium channel SCN9A in genetic epilepsy with febrile seizures plus and Dravet syndrome. Epilepsia 2013;54:e122-e126.
23. Muona M, Berkovic SF, Dibbens LM, Oliver KL, Maljevic S, Bayly MA et al. A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. Nat Genet 2015;47:39–46.
24. Aljaafari D, Fasano A, Nascimento FA, Lang AE, Andrade DM. Adult motor phenotype differentiates Dravet syndrome from Lennox-Gastaut syndrome and links SCN1A to early onset parkinsonian features. Epilepsia 2017;58:e44-e48.
25. Xiang J, Yang J, Chen L, Chen Q, Yang H, Sun C et al. Reinterpretation of common pathogenic variants in ClinVar revealed a high proportion of downgrades. Sci Rep 2020;10:331.

26. Zhang J, Yao Y, He H, Shen J. Clinical Interpretation of Sequence Variants. Curr Protoc Hum Genet 2020;106:e98.
27. Brnich SE, Abou TA, Couch FJ, Cutting GR, Greenblatt MS, Heinen CD et al. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. Genome Med 2019;12:3.
28. Tsuji M, Mazaki E, Ogiwara I, Wada T, Iai M, Okumura A et al. Acute encephalopathy in a patient with Dravet syndrome. Neuropediatrics 2011;42:78–81.
29. Gaily E, Anttonen AK, Valanne L, Liukkonen E, Traskelin AL, Polvi A et al. Dravet syndrome: new potential genetic modifiers, imaging abnormalities, and ictal findings. Epilepsia 2013;54:1577–1585.
30. Dravet C, Oguni H. Dravet syndrome (severe myoclonic epilepsy in infancy). Handb Clin Neurol 2013;111:627–633.
31. Wolff M, Casse-Perrot C, Dravet C. Severe myoclonic epilepsy of infants (Dravet syndrome): natural history and neuropsychological findings. Epilepsia 2006;47 Suppl 2:45–48.
32. Wirrell EC, Laux L, Donner E, Jette N, Knupp K, Meskis MA et al. Optimizing the Diagnosis and Management of Dravet Syndrome: Recommendations From a North American Consensus Panel. Pediatr Neurol 2017;68:18–34.
33. Wu YW, Sullivan J, McDaniel SS, Meisler MH, Walsh EM, Li SX et al. Incidence of Dravet Syndrome in a US Population. Pediatrics 2015;136:e1310-e1315.
34. Liu J, Tong L, Song S, Niu Y, Li J, Wu X et al. Novel and de novo mutations in pediatric refractory epilepsy. Mol Brain 2018;11:48.
35. Kalume F, Yu FH, Westenbroek RE, Scheuer T, Catterall WA. Reduced sodium current in Purkinje neurons from Nav1.1 mutant mice: implications for ataxia in severe myoclonic epilepsy in infancy. J Neurosci 2007;27:11065–11074.
36. Liu Y, Lopez-Santiago LF, Yuan Y, Jones JM, Zhang H, O'Malley HA et al. Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism. Ann Neurol 2013;74:128–139.
37. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci 2006;9:1142–1149.
38. Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I et al. Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. J Neurosci 2007;27:5903–5914.
39. Camfield P, Camfield C. Febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+). Epileptic Disord 2015;17:124–133.
40. Bonanni P, Malcarne M, Moro F, Veggiotti P, Buti D, Ferrari AR et al. Generalized epilepsy with febrile seizures plus (GEFS+): clinical spectrum in seven Italian families unrelated to SCN1A, SCN1B, and GABRG2 gene mutations. Epilepsia 2004;45:149–158.
41. 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. Proc Natl Acad Sci U S A 2005;102:18177–18182.
42. Berecki G, Bryson A, Terhag J, Maljevic S, Gazina EV, Hill SL et al. SCN1A gain of function in early infantile encephalopathy. Ann Neurol 2019;85:514–525.
43. Ishikawa N, Tateishi Y, Tani H, Kobayashi Y, Itai T, Miyatake S et al. Successful treatment of intractable life-threatening seizures with perampanel in the first case of early myoclonic encephalopathy with a novel de novo SCN1A mutation. Seizure 2019;71:20–23.
44. Beck VC, Hull JM, Isom LL. Beyond Dravet Syndrome: Characterization of a Novel, More Severe SCN1A-Linked Epileptic Encephalopathy. Epilepsy Curr 2019;19:266–268.
45. Chastan N, Lebas A, Legoff F, Parain D, Guyant-Marechal L. Clinical and electroencephalographic abnormalities during the full duration of a sporadic hemiplegic migraine attack. Neurophysiol Clin 2016;46:307–311.
46. Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I et al. Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. J Neurosci 2007;27:5903–5914.
47. Shao N, Zhang H, Wang X, Zhang W, Yu M, Meng H. Familial Hemiplegic Migraine Type 3 (FHM3) With an SCN1A Mutation in a Chinese Family: A Case Report. Front Neurol 2018;9:976.

Figures

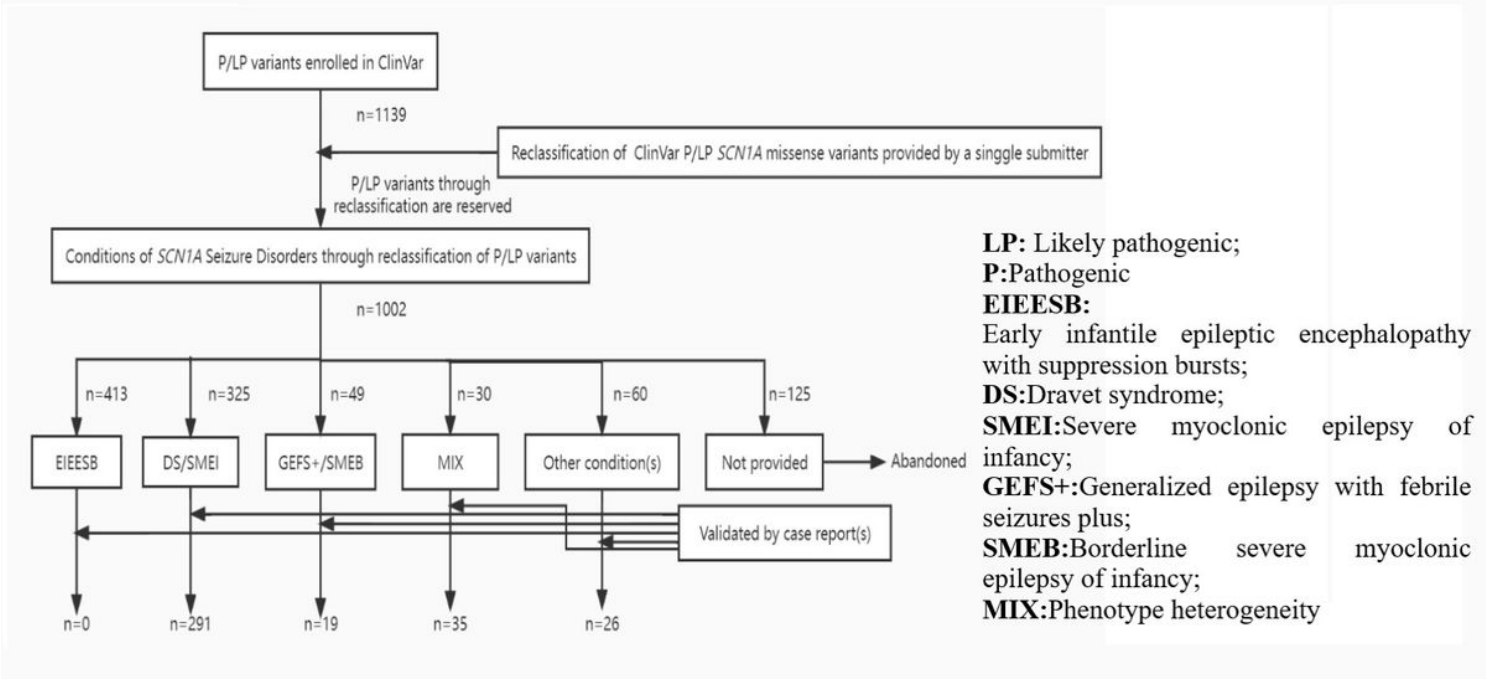

Figure 1

Flowchart for reclassification of condition(s) of SCN1A Seizure Disorder

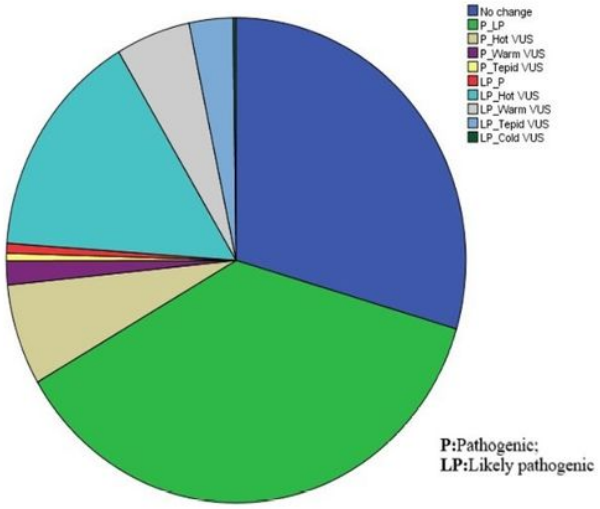

Figure.2A Distribution of 383 *SCN1A* gene missense variants between ClinVar classification and reclassification

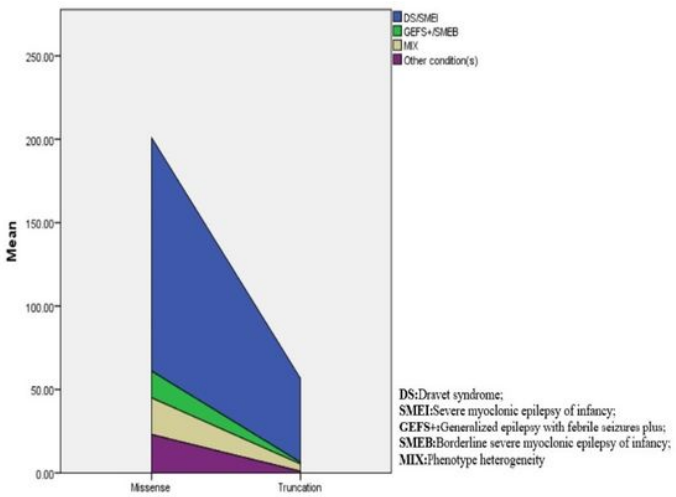

Figure.2B Distribution of variant types of conditions of *SCN1A* Seizure Disorder in ClinVar by validation

Figure 2

Distribution of SCN1A gene variants and variant types

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1Rawdataofthereclassificationoutcomesof383ClinVarPLPSCN1Amissensevariantsprovidedbyasingglesubmitter.xlsx
- SupplementaryTable2Rawdatafor291SCN1AvariantsvalidatedbycasereportsassociatedwithDSorSMEI.xlsx

- SupplementaryTable3Rawdatafor19SCN1AvariantsvalidatedbycasereportsassociatedwithGEFSorSMEB.xlsx
- SupplementaryTable4Rawdatafor35SCN1AvariantsvalidatedbycasereportsassociatedwithMIX.xlsx
- SupplementaryTable5Rawdatafor26SCN1AvariantsvalidatedbycasereportsassociatedwithOtherconditions.xlsx