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CACNA1H variants are not a cause of monogenic epilepsy.

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Abstract

CACNA1H genetic variants were originally reported in a childhood absence epilepsy cohort. Subsequently, genetic testing for *CACNA1H* became available and is currently offered by commercial laboratories. However, the current status of *CACNA1H* as a monogenic cause of epilepsy is controversial, highlighted by ClinGen's recent re-classification of *CACNA1H* as disputed. We analyzed published *CACNA1H* variants and those reported in ClinVar and found none would be classified as pathogenic or likely pathogenic per the ACMG classification criteria. Moreover, *Cacna1h* did not modify survival in a Dravet Syndrome mouse model. We observed a mild increase in susceptibility to hyperthermia-induced seizures in mice with reduced *Cacna1h* expression. Overall, we conclude that there is limited evidence that *CACNA1H* is a monogenic cause of epilepsy in humans and that this gene should be removed from commercial genetic testing panels to reduce the burden of variants of uncertain significance for healthcare providers, families and patients with epilepsy.

Keywords

CACNA1H; epilepsy; genetics; seizure; ion channel

CACNA1H encodes Cav3.2, a member of the T-type calcium channel family. *CACNA1H* variants were originally reported in a cohort of individuals with childhood absence epilepsy (CAE) (Y. Chen et al., 2003). Since the initial report, a number of studies have reported on *CACNA1H* variants in the context of epilepsy, some support this original observation, while others fail to find sufficient evidence for an association with seizures (Y. Chen et al., 2003; Chioza et al., 2006; Heron et al., 2004). However, many of these early supportive studies suffered from small cohort sizes and lack of statistical rigor (Y. Chen et al., 2003). In addition, exome sequencing studies in large epilepsy cohorts including patients with developmental and epileptic encephalopathy (DEE), genetic generalized epilepsy (GGE) and non-acquired focal epilepsy (NAFE) have failed to identify an enrichment of *de novo* or rare

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CACNA1H variants in patients relative to controls (Epi25 Collaborative. Electronic address & Epi, 2019; Epi & Epilepsy Phenome/Genome, 2017; Epi et al., 2013; Heyne et al., 2018).

Reported pathogenic *CACNA1H* variants are primarily missense, though one frameshift variant has been identified (Table 1). Functional studies of these *CACNA1H* missense variants suggested they may lead to altered biophysical properties or protein trafficking (Y. Chen et al., 2003; Heron et al., 2007; Heron et al., 2004; Khosravani et al., 2005; Vitko et al., 2005). However, because these studies were performed in non-excitable heterologous cell systems (i.e. HEK 293), it remains unclear whether the altered channel properties are sufficient to affect firing of individual neurons or result in hyperexcitability at the network level. Further, without assaying a number of missense variants present in the general population, it is difficult to ascertain what level of altered channel function is tolerated. While studies in model organisms can help elucidate a gene's role in seizure susceptibility, there are few such studies of *Cacna1h* in rodent or other models. Powell et al. demonstrate that a *Cacna1h* variant (*gcm*; R1584P) accounts for ~33% of the variance for seizure frequency and time spent seizing in the spontaneously epileptic rat model of absence seizures (GAERS), in support of Cav3.2 contributing to seizure susceptibility as a modifier (Powell et al., 2009). In contrast, *Cacna1h* knockout mice exhibit constitutively constricted coronary arterioles and focal myocardial fibrosis, but not seizures (C. C. Chen et al., 2003).

At The Northwestern Adult Epilepsy Genetics clinic, we encountered an individual with an inherited p.Arg295Ter *CACNA1H* truncating variant. This patient has myoclonic epilepsy and difficult to control tonic-clonic seizures as well as mild intellectual disability. The *CACNA1H* variant was identified through clinical genetic testing on a commercially available epilepsy gene panel and was reported as a variant of uncertain significance (VUS). As part of our internal process for variant interpretation, we performed a more thorough evaluation of the evidence that *CACNA1H* is a monogenic cause of epilepsy. Overall, we identified a total of 37 *CACNA1H* variants reported either in the literature or ClinVar in individuals with epilepsy (Table 1). These 37 patients were reported to have a range of epilepsies, including CAE, febrile seizures (FS), myoclonic-astatic epilepsy (MAE), temporal lobe epilepsy (TLE), symptomatic generalized epilepsy (SGE), juvenile myoclonic epilepsy (JME), epilepsy with generalized tonic-clonic seizures (EGTCS), GGE and juvenile absence epilepsy (JAE). We used the American College of Medical Genetics (ACMG) criteria for variant classification of these 37 reported variants (Richards et al., 2015). Overall, 17/37 (45.9%) were reclassified as VUSs and 20/37 (54.1%) as likely benign or benign, none were classified as likely pathogenic. In particular many of these variants were present in a sampling of >140,000 individuals in the general population (gnomAD - see URLs) (Lek et al., 2016). Overall, 28/37 (75%) of these published 'epilepsy-associated' *CACNA1H* variants are present in gnomAD, with slightly over 50% (18/37) being present in 10 or more individuals. For instance, *CACNA1H* p.Ala876Thr is present in over 250 individuals in gnomAD.

URLs
gnomAD population database [v2.1.1 (non-neuro); accessed 28 Jun 2019]: https://gnomad.broadinstitute.org/
Protter: http://wlab.ethz.ch/protter/start/
Clinvar [accessed 28 Jun 2019]: https://www.ncbi.nlm.nih.gov/clinvar/

We also used Combined Annotation Dependent Depletion (CADD) scores to compare published and ClinVar missense *CACNA1H* variants to missense *CACNA1H* variants present in gnomAD. CADD is an *in silico* measure of the likelihood that a genetic variant may be deleterious, higher scores are more likely deleterious and CADD ≥ 20 represents the top 1% of predicted deleterious variants (Kircher et al., 2014; Rentzsch, Witten, Cooper, Shendure, & Kircher, 2019). CADD scores for published *CACNA1H* variants (mean=12.8; n=35) showed the same distribution of CADD scores as the variants present in the general population (gnomAD) (mean=12.9; n=1938) (Figure 1A). Moreover, the 'epilepsy associated' and population variants were both located throughout the protein and showed no clustering in the pore loops, transmembrane domains, or gating brake (Figure 1D–E). For contrast, we performed the same CADD score analysis for two bona-fide calcium channel genes implicated in epilepsy, *CACNA1A* and *CACNA1E*. For both of these genes pathogenic or likely pathogenic missense variants have significantly higher CADD scores compared to missense variants present in gnomAD (Figure 1B–C). Finally, *CACNA1H* is not under functional constraint (Z_{mis}=−2.36; pLI=0), suggesting that missense and predicted loss-of-function alleles are tolerated. Conversely, as above with the CADD score analysis, *CACNA1A* (Z_{mis}=5.78; pLI=1) and *CACNA1E* (Z_{mis}=5.81; pLI=1) are among the most constrained genes in the human genome (Z_{mis}>3.09; pLI≥0.9).

A valid argument could be made that these other monogenic causes, *CACNA1A* and *CACNA1E,* are generally associated with much more severe epilepsy disorders, including DEE; while the *CACNA1H* associated variants are reported in individuals with milder epilepsies. In this instance genetic association is more appropriate, and indeed *CACNA1H* variants may convey some risk to these milder epilepsies. However, as outlined above, this association is disputed in the literature and original reports were not sufficiently statistically rigorous. Moreover, a recent GWAS in 15,000 individuals with various epilepsy syndromes, including CAE, GGE and JME failed to identify any risk loci in or near *CACAN1H* (International League Against Epilepsy Consortium on Complex, 2018). Moreover, exome sequencing in the common epilepsies, including GGE, have failed to identify *CACNA1H* as being enriched for rare variants using a gene burden analysis (Epi25 Collaborative. Electronic address & Epi, 2019; Epi & Epilepsy Phenome/Genome, 2017). Conversely, *CACNA1G* is the most highly enriched gene for deleterious variants in individuals with GGE and is the only calcium channel gene found to be putatively associated with epilepsy in this large exome sequencing study.

We previously published *Cacna1g* as a modifier of both *Scn1a* and *Scn2a* mouse models of epilepsy (Calhoun, Hawkins, Zachwieja, & Kearney, 2016, 2017). Given that *Cacna1g* and *Cacna1h* are both members of the T-type calcium channel gene family and *CACNA1G* is enriched for rare variants in GGE, we sought to determine whether altered *Cacna1h* expression would also modulate phenotype severity in a mouse model of Dravet syndrome. *Cacna1h*$^{KO/+}$ (1H$^{KO/+}$) mice were crossed with *Scn1a*^{tm1Kea} (1A$^{KO/+}$) mice to generate double transgenic (1A$^{KO/+}$;1H$^{KO/+}$) mice and single mutant littermate controls. We found that mice with reduced *Cacna1h* expression (1A$^{KO/+}$;1H$^{KO/+}$) exhibited similar survival over 8 weeks relative to their control littermates (1A$^{KO/+}$) (Fig. 2A). 1A$^{KO/+}$;1H$^{KO/+}$ mice were slightly more susceptible to hyperthermia-induced seizures relative to control 1A$^{KO/+}$ littermates (Fig. 2B). However, the magnitude of this effect is much smaller than the effect

of antiepileptic drugs (AEDs) such as clobazam, levetiracetam, phenobarbital, or lamotrigine suggesting that, while statistically significant, the result is unlikely to be biologically relevant (Hawkins et al., 2017). Based on a lack of effect on survival and a small but statistically significant increase in hyperthermia-induced seizure susceptibility, we conclude that *Cacna1h* is not a robust modifier of *Scn1a*-associated seizures.

In summary, there is currently no human genetic evidence to support the association of *CACNA1H* with epilepsy, nor does *Cacna1h* meaningfully modify seizure susceptibility in an *Scn1a* mouse model. Similar to our findings here, the ClinGen working group has undertaken an effort to curate genes appearing on panels (I. Helbig et al., 2018) and has classified *CACNA1H* as a disputed gene based on poor genetic support, moderate experimental support, and lack of replication over time. Despite minimal evidence for *CACNA1H* as a monogenic cause of epilepsy, it currently appears on at least 20 independent commercial clinical genetic tests. While *CACNA1H*-associated variants could potentially be a risk factor for milder epilepsies, the inclusion of 'risk factor' genes is completely inappropriate for current clinical genetic testing, as identification of variants has no diagnostic value. New *CACNA1H* variant epilepsy case reports continue to appear in the literature, which may lead to confusion among clinicians and genetic counselors when interpreting VUS in *CACNA1H*, as was the case initially in our Adult Epilepsy Genetic Clinic (Chourasia, Osso-Rivera, Ghosh, Von Allmen, & Koenig, 2019). There a large number of VUS being reported in this gene, data from one commercial company alone has reported 407 *CACNA1H* VUSs in 4859 individuals (almost 10% of reports) in recent years from their epilepsy panel (Truty et al., 2019). These VUSs place a large burden on clinicians and genetic counselors to properly counsel patients regarding variants that are unlikely to be a primary contributor to their phenotype. Our findings here are not limited to *CACNA1H*, and the ClinGen working group are reevaluating genes to ensure they are removed from clinical testing panels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in gnomAD and Clinvar (please see URLs below). Mouse data (hyperthermia & survival) will be provided upon reasonable request.

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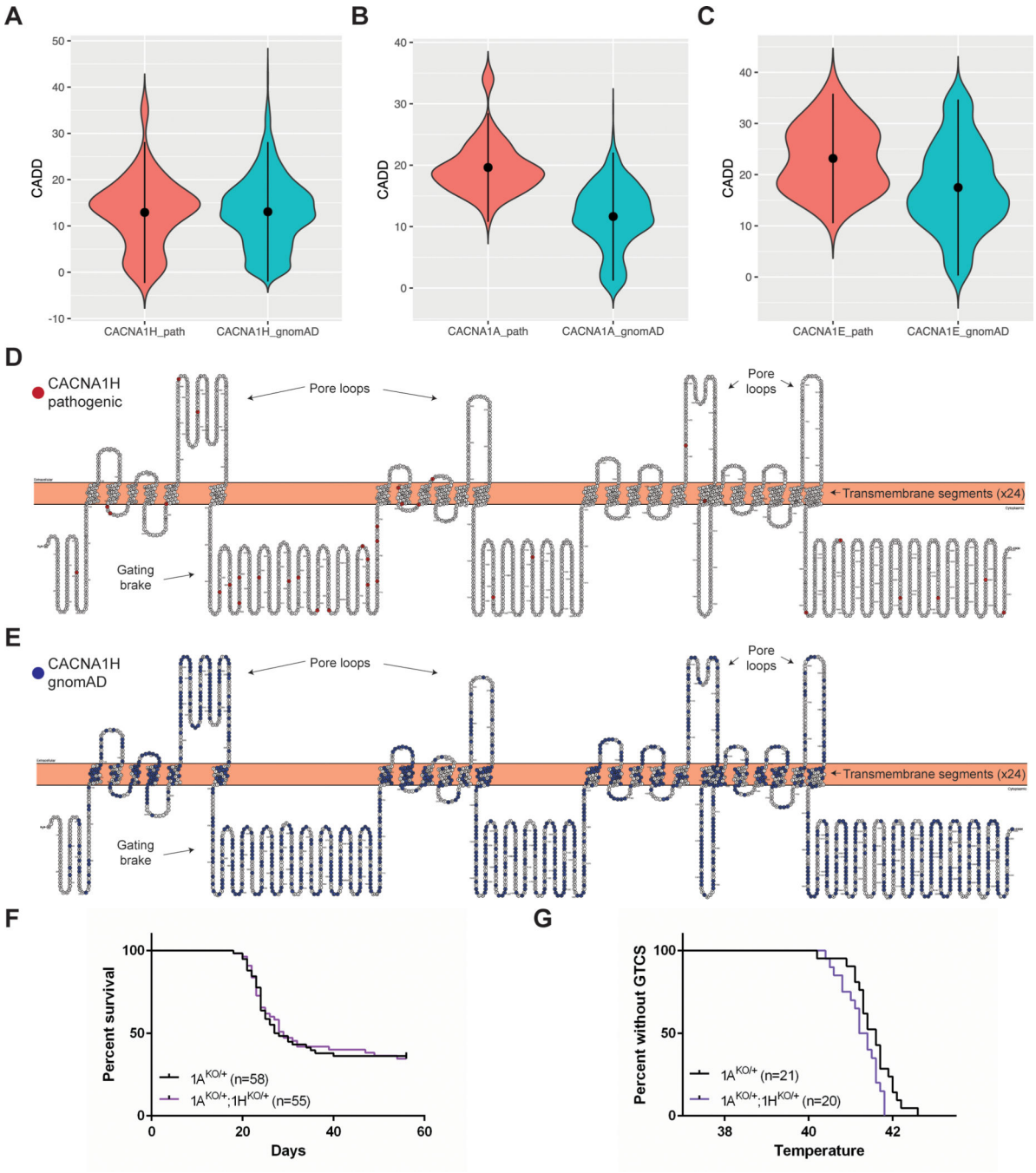

Figure 1. Analysis of published or Clinvar 'pathogenic' missense calcium channel variants and effect of reduced *Cacna1h* expression in a *Scn1a* mouse model of Dravet syndrome.

(A) Distribution of CADD scores in *CACNA1H*. The CACNA1H_path distribution (n=35) is from published missense variants or Clinvar pathogenic or likely pathogenic missense variants as listed in Table 1. The CACNA1H_gnomAD distribution (n=1938) includes all *CACNA1H* missense variants in gnomAD. Error bars represent standard deviation. p = 0.9758 (Mann-Whitney ranksum). **(B)** Distribution of CADD scores in *CACNA1A*. The CACNA1A_path distribution (n=46) includes all Clinvar pathogenic or likely pathogenic

missense variants. The CACNA1A_gnomAD distribution (n=910) includes all *CACNA1A* missense variants in gnomAD. Error bars represent standard deviation. p < 2.2e-16 (Mann-Whitney ranksum). **(C)** Distribution of CADD scores in *CACNA1E*. The CACNA1E_path distribution (n=17) is from published missense variants (K. L. Helbig et al., 2018) or Clinvar pathogenic or likely pathogenic missense variants. The CACNA1E_gnomAD distribution (n=821) is from *CACNA1E* missense variants in gnomAD. Error bars represent standard deviation. p = 0.003252 (Mann-Whitney ranksum). **(D)** Distribution of published or Clinvar 'pathogenic' missense variants in *CACNA1H* (Protter; see URLs). **(E)** Distribution of gnomAD missense variants in *CACNA1H* (Protter; see URLs). **(F)** Reduced *Cacna1h* expression does not affect survival in a mouse model of Dravet syndrome. p = 0.978 (Mantel-Cox logrank). **(G)** Reduced *Cacna1h* expression increases hyperthermia-induced seizure susceptibility. GTCS = generalized tonic-clonic seizure. p = 0.0332 (Mantel-Cox logrank).

Table 1:

Reanalysis of *CACNA1H* missense variants from the literature and present in Clinvar.

					gnomAD				
Chr-Pos-Ref-Alt	**cDNA**	**Protein**	**Cons**	**CADD**	**AF_Popmax**	**Num_Het_Indiv**	**Num_Homoz_Indiv**	**ACMG**	**Study**
16–1250335-C-T	c.883C>T	p.Arg295Ter	truncation	N/A	NF	0	0	VUS	This study
16–1245503-C-G	c.483C>G	p.Phe161Leu	missense	18.24	0.00001961	2	0	Likely benign	(Y. Chen et al., 2003)
16–1250296-G-A	c.844G>A	p.Glu282Lys	missense	35	0.0002268	4	0	VUS	
16–1251816-T-A	c.1366T>A	p.Cys456Ser	missense	24.9	NF	0	0	VUS	
16–1251945-G-A	c.1495G>A	p.Gly499Ser	missense	13.14	0.0105	255	3	Likely benign	
16–1252393-C-T	c.1943C>T	p.Pro648Leu	missense	15.87	0.00001422	1	0	Likely benign	
16–1254238-G-A	c.2231G>A	p.Arg744Gln	missense	0.062	0.00006633	6	0	Likely benign	
16–1254250-C-T	c.2243C>T	p.Ala748Val	missense	3.376	0.0006849	16	0	Likely benign	
16–1254325-G-A	c.2318G>A	p.Gly773Asp	missense	2.789	0.00297	59	0	Likely benign	
16–1254357-G-A	c.2350G>A	p.Gly784Ser	missense	1.238	0.0002643	8	0	Likely benign	
16–1255153-G-A	c.2491G>A	p.Val831Met	missense	21.2	0.0001537	4	0	VUS	
16–1255204-G-A	c.2542G>A	p.Gly848Ser	missense	17.91	0.0002485	20	0	Likely benign	
16–1261517-G-A	c.4387G>A	p.Asp1463Asn	missense	15.74	0.001373	46	0	Likely benign	
16–1251888-G-A	c.1438G>A	p.Ala480Thr	missense	9.555	0.00001704	1	0	Likely benign	(Heron et al., 2004) *
16–1252303-C-T	c.1853C>T	p.Pro618Leu	missense	18.95	0.0009646	123	0	Likely benign	
16–1252306-CAG-C	N/A	p.Val621GlyfsTer34	frameshift	N/A	0.0002159	13	0	VUS	
16–1254271-G-A	c.2264G>A	p.Gly755Asp	missense	7.817	0.0004536	66	0	Likely benign	
16–1245509-G-C	c.489G>C	p.Gln163His	missense	17.02	0.01677	1258	14	Likely benign	(Chourasia et al., 2019)
16–1252062-A-T	c.1612A>T	p.Arg538Trp **	missense	13.5	NF	0	0	VUS	
16–1255229-C-T	c.2567C>T	p.Pro856Leu	missense	14.07	0.0001025	12	0	Likely benign	
16–1268643-C-T	c.5879C>T	p. Thr1960Ile ***	missense	9.512	NF	0	0	VUS	
16–1270254-G-A	c.6322G>A	p.Ala2108Thr	missense	15.33	0.002292	340	2	VUS	
16–1250392-C-T	c.940C>T	p.Pro314Ser	missense	4.308	0.0005333	10	0	Likely benign	(Liang et al., 2006)
16–1251924-C-T	c.1474C>T	p.Pro492Ser	missense	12.85	0.0005567	6	0	Likely benign	
16–1248668-G-C	c.697G>C	p.Val233Leu	missense	14.99	NF	0	0	VUS	Clinvar ****

					gnomAD				
Chr-Pos-Ref-Alt	**cDNA**	**Protein**	**Cons**	**CADD**	**AF_Popmax**	**Num_Het_Indiv**	**Num_Homoz_Indiv**	**ACMG**	**Study**
16–1252243-C-T	c.1793C>T	p.Ala598Val	missense	9.282	NF	0	0	VUS	
16–1254058-C-A	c.2051C>A	p.Pro684His	missense	12.5	NF	0	0	VUS	
16–1258106-C-T	c.3248C>T	p.Thr1083Met	missense	21.2	NF	0	0	VUS	
16–1262024-A-G	c.4645A>G	p.Met1549Val	missense	21	NF	0	0	VUS	
16–1270830-A-G	c.6898A>G	p.Ile2300Val	missense	0.064	0.0002785	16	0	VUS	
16–1203912-G-T	c.175G>T	p.Gly59Cys	missense	14.23	0.001147	5	0	Likely benign	(Lee, Lee, & Lee, 2018)
16–1254370-G-A	c.2363G>A	p.Arg788His	missense	14.67	0.0003706	11	0	VUS	
16–1270518-C-A	c.6586C>A	p.Pro2196Thr	missense	14.83	0.0001351	3	0	Likely benign	
16–1270936-A-G	c.7004A>G	p.Lys2335Arg	missense	10.16	NF	0	0	VUS	
16–1259140-G-A	c.3472G>A	p.Gly1158Ser	missense	0.199	0.0002341	13	0	Likely benign	(Becker et al., 2017)
16–1254369-C-T	c.2362C>T	p.Arg788Cys	missense	7.372	0.1249	20068	1083	Benign	(Heron et al., 2007)
16–1256126-G-A	c.2626G>A	p.Ala876Thr	missense	13.09	0.002548	263	0	VUS	
16–1268439-G-A	c.5675G>A	p.Arg1892His	missense	15.63	0.001531	55	1	VUS	

Abbreviations: cDNA = coding DNA, Cons = consequence, AF_Popmax = allele frequency in the population with highest allele frequency, Num_Het_Indiv = number of heterozygous individuals with this variant present in gnomAD, Num_Homoz_Indiv = number of homozygous individuals with this variant present in gnomAD, ACMG = ACMG reclassification.

*Manuscript notes none of these four variants segregate with phenotype.

**Manuscript lists as c.1612C>T / p.Leu538Phe, but amino acid 538 is actually Arg.

***manuscript lists as c.5879C>T / p. Ala1960Val, but amino acid 1960 is actually Thr.

****Clinvar pathogenic or likely pathogenic variants.