✖ Close

Evidence Summary

ALG13 – undetermined early-onset epileptic encephalopathy – *X-linked inheritance*

Classification owner: Epilepsy EP
Calculated classification: Definitive
Modified classification: No Modification
Reason for modified classification: None

Classification status: APPROVED
Date classification saved: 2018 Apr 26, 3:22 pm
Replication Over Time: Yes
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: undetermined early-onset epileptic encephalopathy

Evidence Summary

A precuration for the ALG13 gene, performed by the Epilepsy Gene Curation Expert Panel, lead to the curation of ALG13 in association with X-linked 'Undetermined Early-onset Epileptic Encephalopathy'. The gene-disease association of ALG13 and X-linked Undetermined Early-onset Epileptic Encephalopathy has been classified as Definitive. A p.Asn107Ser variant in ALG13 has been observed de novo in at least ten female probands, with onset of seizures within one to eight months of age. Additional maternally inherited missense variants in ALG13 have been reported in males with a variety of phenotypes, including seizures, bilateral optic nerve atrophy, and intellectual disability. One male individual (Timal et al. 2012, PMID 22492991) was reported to have an ALG13 missense variant and Congenital disorder of glycosylation, type 1, however no other papers besides Timal 2012 test for enzyme activity by way of serum transferrin isofocusing in males with ALG13 variants. One female proband with the de novo p.Asn107Ser variant was tested by transferrin isoform analysis and found to have normal results, indicating that the mechanism of disease is different between males and females (Dimassi et al. 2016; PMID 26138355). At this time no functional data exists to propose a mechanism of disease.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	2	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	10	20	12
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						12
Experimental Evidence	Functional			Biochemical Functions	0	0	0
				Protein Interactions	0	0	
				Expression	0	0	
	Functional Alteration			Patient cells	0	0	0
				Non-patient cells	0	0	
	Models			Non-human model organism	0	0	0
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						0
Total Points							12

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
T22647		NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Epi4K Consortium, **2016**, PMID: 27476654	Female	**Age of Onset:** 1 Months		**HPO term(s):** Generalized tonic-clonic seizures; Infantile spasms; Infantile muscular hypotonia; Choreoathetosis	-	-	-	-		**Method 1:** Exome sequencing	Review		Inheritance is unknown
Patient 11	Proband with other variant type with some evidence of gene impact	NC_000023.11:g.111711720C>T (GRCh38)	Hino-Fukuyo N, et al., **2015**, PMID: 25877686	Male	**Age of Onset:** 5 Months		**HPO term(s):** Optic atrophy; Intellectual disability, severe; Hypsarrhythmia; Generalized myoclonic seizures; Generalized seizures	-	-	-	-		**Method 1:** Exome sequencing; **Method 2:** Sanger sequencing	Score	**0** (0.5)	not scored
Farwell Proband	Proband with other variant type with some evidence of gene impact	NM_001099922.2(ALG13):c.2106T>G (p.Ser702Arg)	Farwell KD, et al., **2015**, PMID: 25356970	Unknown			**HPO term(s):** Generalized seizures; Intellectual disability	-	-	-	-		**Method 1:** Exome sequencing	Score	**0** (0.5)	not scored because sex of the proband is unknown
Trio 37	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	de Ligt J, et al., **2012**, PMID: 23033978	Female			**HPO term(s):** Feeding difficulties; Generalized hypotonia; Global developmental delay; Hydrocephalus; Delayed myelination; Seizures; Self-mutilation; Sleep disturbance; Hypertelorism; Broad face; Coarse facial features; Low-set ears; Wide mouth; Microretrognathia; Scoliosis; Flexion contracture	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Case 1	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Epi4K Consortium, et al., **2013**, PMID: 23934111	Female	**Age of Onset:** 4 Months		**HPO term(s):** Myoclonic atonic seizures; Absent speech; Hypsarrhythmia **free text:** tonic seizures, generalized paroxysmal fast activity	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	
Smith-Packard Proband	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Smith-Packard B, et al., **2015**, PMID: 25732998	Female	**Age of Onset:** 8 Months		**HPO term(s):** Uncontrolled eye movements; Developmental regression; Infantile spasms; Hypsarrhythmia; Feeding difficulties; Global developmental delay; Intellectual disability	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	
Patient 4	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Bastaki F, et al., **2018**, PMID: 28940310	Female	**Age of Onset:** 8 Months		**HPO term(s):** Infantile spasms; Infantile muscular hypotonia; Global developmental delay; Gastroesophageal reflux; Cortical visual impairment; Cerebral cortical atrophy	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	
Patient 6	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Ortega-Moreno L, et al., **2017**, PMID: 29190809	Female	**Age of Onset:** 5 Months		**free text:** Lennox-Gastaut syndrome	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** Epilepsy panel	Score	**2** (2)	
Patient 2	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Kobayashi Y, et al., **2016**, PMID: 26482601	Female	**Age of Onset:** 6 Months		**HPO term(s):** Chorea; Dyskinesia; Hypsarrhythmia; Cerebral atrophy	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Michaud Proband	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Michaud JL, et al., **2014**, PMID: 24781210	Female	**Age of Onset:** 4 Months	Not Hispanic or Latino	**HPO term(s):** Focal seizures, afebril; Severe global developmental delay; Hypsarrhythmia	-	-	-	-		**Method 1:** Exome sequencing **Description of genotyping method:** CNV analysis	Score	**2** (2)	
Case 2	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Epi4K Consortium, et al., **2013**, PMID: 23934111	Female	**Age of Onset:** 1 Months		**HPO term(s):** Seizures; Hypsarrhythmia; Global developmental delay	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	
Patient Troina 2679	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Geisheker MR, et al., **2017**, PMID: 28628100	Female	**Age of Onset:** 3 Months		**HPO term(s):** Intellectual disability, severe; Seizures; Generalized tonic-clonic seizures; Global developmental delay	-	-	-	-			Review	**2** (2)	
Hamici Proband	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Hamici S, et al., **2017**, PMID: 28778787	Female	**Age of Onset:** 6 Months		**HPO term(s):** Infantile spasms; Gastroesophageal reflux; Delayed gross motor development; Abnormality of eye movement; Infantile muscular hypotonia; Feeding difficulties	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	
10A1280	Variant is de novo	NM_001257235.2(ALG13):c.8A>G (p.Asn3Ser)	Dimassi S, et al., **2016**, PMID: 26138355	Female	**Age of Onset:** 2 Months		**HPO term(s):** Hypsarrhythmia; Hypertelorism; Anteverted nares; Long philtrum; Adducted thumb; Mild global developmental delay	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	
														Total points:	**20.00**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

No Experimental evidence was found.

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

CACNA1H – generalised epilepsy – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Limited
Modified classification: Disputed
Reason for modified classification:
Moved from Limited to Disputed given lack of evidence for support as a monogenic AD cause of generalized epilepsy.

Classification status: APPROVED
Date classification saved: 2018 Jul 31, 1:08 pm
Replication Over Time: No
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: generalised epilepsy

Evidence Summary

CACNA1H was evaluated for evidence supporting and refuting its relationship with the broad phenotype of generalized epilepsy (MONDO:0005579) as it has been reported with a variety of phenotypes ranging from childhood absence epilepsy, idiopathic generalized epilepsy and myoclonic astatic epilepsy. CACNA1H has also been described in association with Hyperaldosteronism, familial, type IV, an AD disorder, but evidence for this gene-disease relationship is not considered here. CACNA1H is a voltage-sensitive calcium channel that gives rise to T-type calcium current thought to modulate firing patterns of neurons. CACNA1H has a higher than expected tolerance for missense variants in ExAC and the majority of reported variants have a higher than expected maximum allele frequency for a pathogenic variant. There are no reported de novo pathogenic variants for CACNA1H. Targeted sequencing studies of CACNA1H suggested that missense variants were identified more frequently in patients with childhood absence epilepsy (Chen 2003) and idiopathic generalized epilepsy (Heron 2004, Heron 2007) as compared to control. In Chen 2003, all missense variants were inherited from unaffected parents disputing a clear AD inheritance pattern. In Heron 2004 and Heron 2007, none of the variants segregated consistently with the generalized epilepsy phenotype. CACNA1H is expressed in multiple organ systems and is not unique to the brain (Williams 1999). Whole-cell patch clamp recordings in transfected HEK293 cells with site-directed mutagenesis of identified human CACNA1H missense variants showed inconsistent alteration of channel function (Khosravani 2004, Khosravani 2005, Heron 2007) and no variants were able to be scored as variants with evidence of gene impact. The Genetic Absence Epilepsy Rat from Strausborg (GAERS) has been shown to have a variant in CACNA1H that segregates with seizure expression, however, this is thought to likely represent a polygenic disease model as some rats are null for the variant and still have seizures (Powell 2009). The variant reported in the GAERS model is not reported in human disease. In conclusion, the available genetic and experimental evidence for an AD gene-disease relationship with CACNA1H and generalized epilepsy is insufficient. Variants in CACNA1H are not likely to be causative of epilepsy alone and the gene-disease relationship is Disputed. This curation did not formally examine the hypothesis that CACNA1H is a susceptibility gene, which remains a consideration.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	0	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	0	0	0
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						**0**
Experimental Evidence	Functional			Biochemical Functions	0	0	0.5
				Protein Interactions	0	0	
				Expression	1	0.5	
	Functional Alteration			Patient cells	0	0	1
				Non-patient cells	2	1	
	Models			Non-human model organism	3	3	3
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						4.5
Total Points							**4.5**

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

No scored Case Level evidence was found.

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

Label	Reference	Family ethnicity	Family phenotypes	Number of affected individuals	Number of unaffected individuals	LOD score	LOD score counted
Family B	Heron SE, et al., **2007**, PMID: 17696120		**free text:** Includes idiopathic generalized epilepsy, myoclonic astatic epilepsy, childhood absence epilepsy, unclassified focal seizures	4		**Calculated:** 0.9	No
Family B	Heron SE, et al., **2004**, PMID: 15048902		**free text:** Febrile seizures, Febrile seizures plus, Myoclonic-astatic epilepsy	3		**Calculated:** 0.6	No
					Total LOD score:	**0.00**	

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
CACNA1H tissue expression: Western Blot	**Expression** A	Williams ME, et al., **1999**, PMID: 9930755	Isolated and characterized cDNA for CACNA1H from a human medullary thyroid cardcinoma cell line. Northern blot of RNA transcripts indicated that it is expressed throughout the brain, primarily in the amygdala, caudate nucleus and putamen as well as non neuronal tissues.	Score	**0.5** (0.5)	
CACNA1H channel functional alteration	**Functional Alteration** Non-patient cells	Heron SE, et al., **2007**, PMID: 17696120	Patch-clamp recordings showed 9 of 11 variants resulted in a gain-of-function consistent with increased activity.	Score	**0.5** (0.5)	Data similar to Khosravani 2004
In vitro functional alteration of CACNA1H channel	**Functional Alteration** Non-patient cells	Khosravani H, et al., **2005**, PMID: 15852375	Variant results in more rapid channel activation as well as more rapid inactivation leading to greater channel availability as compared to the wildtype model. These are small changes but are postulated to be significant.	Score	**0.5** (0.5)	Note that the 3rd variant they analyzed, A480T, had no alteration of channel activity.
Genetic Absence Epilepsy Rats from Strasbourg model	**Model Systems** Non-human model organism	Cain SM, et al., **2018**, PMID: 29468672	Variant results in a gain of function in the CACNA1H gene in the Genetic Absence Epilepsy Rats from Strasbourg model. Thalamic reticular neurons sustain oscillatory burst-firing. In vivo knock-down normalized thalamic burst-firing.	Score	**2** (2)	

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
CACNA1H mRNA expression in rat model of Absence Epilepsy	**Model Systems** Non-human model organism	Talley EM, et al., **2000**, PMID: 10648900	Genetic Absence Epilepsy Rats from Strausbourg (GAERS) are neurotypical rats apart from their intermittent absence seizures that occur during adulthood. Increased levels of alpha1H mRNA (15-25%) were found in the thalamic relay nucleus of both juvenile and adult rats as compared to wild type mice. The paper does not conclude how increased mRNA levels would contribute to the absence seizure phenotype.	Score	**0.5** (2)	Unclear if increased mRNA expression of CACNA1H directly affects phenotype
Missense variant in CACNA1H in rat model of absence epilepsy	**Model Systems** Non-human model organism	Powell KL, et al., **2009**, PMID: 19144837	This rat model has adult onset absence epilepsy in an otherwise neuro-typical rat.	Score	**0.5** (2)	Variant not seen in humans
				Total points:	**4.50**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

CACNB4 – epilepsy – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Limited
Modified classification: Disputed
Reason for modified classification:
There is no clinical evidence to support a role for variants in this gene in human disease. There is some limited functional evidence, suggesting this is a candidate gene for epilepsy.

Classification status: APPROVED
Date classification saved: 2018 Jun 22, 5:54 am
Replication Over Time: No
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: epilepsy

Evidence Summary

No summary is provided.

Calculated Classification Matrix

			Evidence Type		Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	2	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	0	0	0
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						0
Experimental Evidence	Functional			Biochemical Functions	0	0	0.5
				Protein Interactions	2	0.5	
				Expression	0	0	
	Functional Alteration			Patient cells	0	0	0
				Non-patient cells	2	0	
	Models			Non-human model organism	1	1.5	1.5
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						2
Total Points							2

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Pedigree A I-1	Proband with other variant type with some evidence of gene impact	NM_000726.4(CACNB4):c.1444C>T (p.Arg482Ter)	Escayg A, et al., **2000**, PMID: 10762541	Female			**HPO term(s):** Myoclonus; Absence seizures; Generalized tonic-clonic seizures	-	-	-	-		**Method 1:** Genotyping **Description of genotyping method:** Testing was done by CSGE followed by manual sequencing of the exons were variants were identified, so this method has a lower detection rate than direct sequencing methodologies. Only the CACNB4 gene was tested.	Score	**0** (0.5)	Gene appears tolerant of LOF variants overall based on pLI and presence of LOF variants in ExAC/gnomAD; No familial segregation studies performed
Patient No. 56	Proband with other variant type with some evidence of gene impact	NM_000726.4(CACNB4):c.610delC (p.Gln204Lysfs)	Posey JE, et al., **2017**, PMID: 27959697	Female	**Age of Report:** 9 Years		**HPO term(s):** Profound global developmental delay; Failure to thrive; Dysphagia; Hip dislocation **free text:** brain volume loss	-	-	-	-		**Method 1:** Exome sequencing **Description of genotyping method:** performed in clinical diagnostic lab	Score	**0** (0.5)	Variant in TANGO2 gene is consistent to fully explain phenotype
														Total points:	**0.00**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

Label	Reference	Family ethnicity	Family phenotypes	Number of affected individuals	Number of unaffected individuals	LOD score	LOD score counted
Pedigree B	Escayg A, et al., **2000**, PMID: 10762541		**HPO term(s):** Generalized tonic-clonic seizures; Atypical absence seizures **free text:** idiopathic generalized epilepsy, “an unusual cognitive trigger of seizure initiation”	2		**Calculated:** 0.3	No
Family A	Ohmori I, et al., **2008**, PMID: 18755274		**HPO term(s):** Complex febrile seizures	2		**Calculated:** 0.3	No
					Total LOD score:	**0.00**	

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Electrophysiological analysis in BHK cells	**Functional Alteration** Non-patient cells	Ohmori I, et al., **2008**, PMID: 18755274	No significant differences between WT and mutant channels in the activation and inactivation curves. R468Q-CACNB4 showed greater Ba2+ current density compared with the wild-type CACNB4. Mutant channels had significantly greater peak Ba2+ current densities at voltages between 0 mV and 40 mV in comparison to WT-CACNB4 (Pb0.05) (Fig. 3B). The peak current amplitudes (Pb0.01), cell capacitance (Pb0.05), and current densities (Pb0.05) exhibited by cells expressing R468Q-CACNB4 were significantly greater than WT-CACNB4 (Fig. 3C). R468Q-CACNB4 showed a small increase in the time constant for activation at −10 mV in comparison to WT-CACNB4 (Fig. 4B). There were no significant differences between WT-CACNB4 and R468QCACNB4 with regard to the inactivation fast (τfast) and slow (τslow) time constants (Fig. 4D).	Score	**0** (0.5)	Results from heterologous expression systems can't be generalized to determine effects in vivo. Unclear whether/how much the R468Q-CACNB4 mutation alters phenotype in patient.
Nuclear targeting of truncated B4 protein	**Functional Alteration** Non-patient cells	Etemad S, et al., **2014**, PMID: 24875574	Evidence does not support altered function. Nuclear targeting of β4b(1–481) was not reduced compared with full-length β4b in any of three cell systems, indicating the β4 distal C-terminus does not play an essential role of in nuclear targeting and bringing into question whether nuclear function of calcium channel β4 subunits is critically involved in etiology of epilepsy and ataxia in patients and mouse models with CACNB4 variants	Score	**0** (0.5)	Contradicts prior studies that suggested nuclear localization might be explanation for the functional effects but doesn't exclude another possible mechanism for change in electrophysiological properties in prior studies.
Ataxia and seizures in lh/lh mouse model	**Model Systems** Non-human model organism	Burgess DL, et al., **1997**, PMID: 9039265	Both human and homozygous mouse model can exhibit ataxia and seizures	Score	**1.5** (2)	Downgraded because mouse model is homozygous
Electrophysiological analysis of CACNB4/CACNA1A interaction	**Protein Interactions** physical association (MI:0915)	Escayg A, et al., **2000**, PMID: 10762541	The carboxyl-terminal region of CACNB4 contains an interaction site with CACNA1A and plays a role in the regulation of channel inactivation kinetics. Expression of the wildtype b4 subunit together with the a1A subunit produces a slowly inactivating inward Ba21 current that is not seen in the absence of the b4 subunit. The wild-type b4 subunit produces biphasic inactivation with a fast-inactivating component (F), a slower inactivating component (S), and a noninactivating component (NI). Expression of the R482X variant did not alter voltage dependence of activation but did change the kinetics of inactivation by decreasing the time constant for the fast component of inactivation compared to wild-type	Score	**0** (0.5)	Confirmed findings in prior publication and showed R482X variant behaves similarly to the C-terminal deletion in Walker et al. 1998 but doesn't add any additional new evidence to support relationship between CACNB4 and seizures.

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Interaction between CACNB4 and CACNA1A	**Protein Interactions** physical association (MI:0915)	Walker D, et al., **1998**, PMID: 9442082	The carboxyl-terminal region of CACNB4 contains an interaction site with CACNA1A and plays a role in the regulation of channel inactivation kinetics. Constructs with deletions of the C-terminus of the b4 subunit from rat brain eliminate binding to the C-terminus of the a1A subunit from rabbit brain and decreased the time constant for the fast component of inactivation when co-expressed in Xenopus oocytes	Score	**0.5** (0.5)	
				Total points:	**2.00**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

DNM1 – infantile epilepsy syndrome – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Definitive
Modified classification: No Modification
Reason for modified classification: None

Classification status: APPROVED
Date classification saved: 2018 May 31, 4:42 pm
Replication Over Time: Yes
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: infantile epilepsy syndrome

Evidence Summary

Approved by the ClinGen Neurodevelopmental CDWG 12/13/16.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	0	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	8	16	12
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						12
Experimental Evidence	Functional			Biochemical Functions	0	0	1
				Protein Interactions	1	1	
				Expression	0	0	
	Functional Alteration			Patient cells	0	0	1
				Non-patient cells	1	1	
	Models			Non-human model organism	2	2.5	2.5
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						4.5
Total Points							16.5

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
LGSkj	Variant is de novo	NM_001005336.2(DNM1):c.529G>C (p.Ala177Pro)	EuroEPINOMICS-RES Consortium, et al., **2014**, PMID: 25262651	Female	**Age of Report:** 15 Years		**HPO term(s):** Epileptic encephalopathy **free text:** EIEE	-	-	-	-	not specified		Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Isg	Variant is de novo	NM_001005336.2(DNM1):c.618G>C (p.Lys206Asn)	EuroEPINOMICS-RES Consortium, et al., **2014**, PMID: 25262651	Male	**Age of Report:** 8 Years		**HPO term(s):** Epileptic encephalopathy **free text:** EIEE	-	-	-	-	not specified		Score	**2** (2)	
LGSaix	Variant is de novo	NM_001005336.2(DNM1):c.1076G>C (p.Gly359Ala)	EuroEPINOMICS-RES Consortium, et al., **2014**, PMID: 25262651	Male	**Age of Report:** 6 Years		**HPO term(s):** Epileptic encephalopathy	-	-	-	-	not specified		Score	**2** (2)	
NLES16	Variant is de novo	NM_004408.3(DNM1):c.709C>T (p.Arg237Trp)	EuroEPINOMICS-RES Consortium, et al., **2014**, PMID: 25262651	Female	**Age of Report:** 13 Years		**HPO term(s):** Epileptic encephalopathy	-	-	-	-	not specified		Score	**2** (2)	
Male 1	Variant is de novo	NM_004408.3(DNM1):c.127G>A (p.Gly43Ser)	Nakashima M, et al., **2016**, PMID: 26611353	Male	**Age of Report:** 16 Years		**HPO term(s):** Epileptic encephalopathy	-	-	-	-	not specified		Score	**2** (2)	
Male 2	Variant is de novo	NM_004408.3(DNM1):c.709C>T (p.Arg237Trp)	Nakashima M, et al., **2016**, PMID: 26611353	Male	**Age of Report:** 7 Years		**HPO term(s):** Epileptic encephalopathy	-	-	-	-	not specified		Score	**2** (2)	
Male 1	Variant is de novo	NM_001005336.2(DNM1):c.865A>T (p.Ile289Phe)	Allen NM, et al., **2016**, PMID: 26648591	Male	**Age of Report:** 12 Years		**HPO term(s):** Infantile spasms	-	-	-	-	CGH, POCN		Score	**2** (2)	
112	Variant is de novo	NM_004408.3(DNM1):c.1190G>A (p.Gly397Asp)	Helbig KL, et al., **2016**, PMID: 26795593	Unknown			**HPO term(s):** Infantile spasms	-	-	-	-	not specified		Score	**2** (2)	
															Total points:	**16.00**

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
FA Nonpatient Cells	**Functional Alteration** Non-patient cells	Dhindsa RS, et al., **2015**, PMID: 27066543	Expression of mutant proteins decrease endocytosis activity in dominant negative manner. The G359A variant showed disrupted higher-order DNM1 oliogomerization. EM of mutation DNM1-transfected HeLa cells and DNM1 mutate mice showed vesicle defects indicating vesicle scission activity	Score	**1** (0.5)	Expression of mutant proteins decrease endocytosis activity in dominant negative manner.

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Asinof Rescue	**Model Systems** Non-human model organism	Asinof SK, et al., **2015**, PMID: 26125563	Used “fitful” mice with an EE disorder that carry a spontaneous mutation in DNM1. They demonstrated that seizure activity is independent from dev delay.	Score	**0.5** (2)	Used “fitful” mice with an EE disorder that carry a spontaneous mutation in DNM1. They demonstrated that seizure activity is independent from dev delay.
Boumil Rescue	**Model Systems** Non-human model organism	Boumil RM, et al., **2010**, PMID: 20700442	1st report of "fitful" mice with recurrent, non-lethal seizures; phenotype is semidominant; homozygous mice have more severe phenotype	Score	**2** (2)	
VDB Protein Interaction	**Protein Interactions** physical association (MI:0915)	van der Bliek AM, et al., **1993**, PMID: 8101525	Functions in receptor-mediated endocytosis and required at an intermediate stage in coated vesicle formation. Demonstrated the DNM1 protein expression was at least 30-fold higher in the brain than other tissues	Score	**1** (0.5)	Functions in receptor-mediated endocytosis and required at an intermediate stage in coated vesicle formation. Demonstrated the DNM1 protein expression was at least 30-fold higher in the brain than other tissues
				Total points:	**4.50**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

EFHC1 – juvenile myoclonic epilepsy – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Limited
Modified classification: Disputed
Reason for modified classification:
We have disregarded the very limited functional evidence in light of the complete lack of genetic evidence connecting EFHC1 and JME. In summary, there is convincing evidence disputing the association between EFHC1 and juvenile myoclonic epilepsy (JME). All variants in EFHC1 associated with JME have contradictory evidence for disease association (too common in ExAC/gnomAD, with minor allele frequencies (MAF) of 2.857e-5 to 0.05973). More evidence is needed to either support or refute the role EFHC1 plays in this disease.

Classification status: APPROVED
Date classification saved: 2018 Jul 27, 4:06 pm
Replication Over Time: No
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: juvenile myoclonic epilepsy

Evidence Summary

Numerous probands with juvenile myoclonic epilepsy (JME) and variants in EFHC1 have been reported; however, many of these variants are observed in population databases at frequencies too high to be consistent with JME [PMID: 17634063, 22690745, 22727576, 16839746, 17159113, 15258581]. There were additional variants reported that did not add valuable evidence to the gene disease assertion that may be found in PMID: 18505993.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	0	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	0	0	0
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						**0**
Experimental Evidence	Functional			Biochemical Functions	0	0	0.5
				Protein Interactions	0	0	
				Expression	1	0.5	
	Functional Alteration			Patient cells	0	0	0
				Non-patient cells	0	0	
	Models			Non-human model organism	1	0.5	0.5
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						**1**
Total Points							**1**

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

No scored Case Level evidence was found.

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

Label	Reference	Family ethnicity	Family phenotypes	Number of affected individuals	Number of unaffected individuals	LOD score	LOD score counted

Label	Reference	Family ethnicity	Family phenotypes	Number of affected individuals	Number of unaffected individuals	LOD score	LOD score counted
Family 1	Suzuki T, et al., **2004**, PMID: 15258581		**free text:** Myoclonic seizures (II-5, II-6), Grand-mal tonic clonic seizures (II-5, II-6), Absence seizures (II-6)	5		**Calculated:** 1.51	No
Family 2	Suzuki T, et al., **2004**, PMID: 15258581			3		**Calculated:** 0.9	No
Family 3	Suzuki T, et al., **2004**, PMID: 15258581			3		**Calculated:** 0.9	No
Family 4	Suzuki T, et al., **2004**, PMID: 15258581			5		**Calculated:** 1.2	No
Family 5	Suzuki T, et al., **2004**, PMID: 15258581			3		**Calculated:** 0.9	No
Family 6	Suzuki T, et al., **2004**, PMID: 15258581			2		**Calculated:** 0.6	No
Family 7	Suzuki T, et al., **2004**, PMID: 15258581			11		**Calculated:** 3.61	No
Family 1	Ma S, et al., **2006**, PMID: 16839746			2		**Calculated:** 0.6	No
Family 13	Annesi F, et al., **2007**, PMID: 17634063			3		**Calculated:** 0.6	No
Family 19	Annesi F, et al., **2007**, PMID: 17634063			1		**Calculated:** 0.6	No
Family 25	Annesi F, et al., **2007**, PMID: 17634063			3		**Calculated:** 0.6	No
					Total LOD score:	**0.00**	

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Northern blot in human adult tissues	**Expression** A	Suzuki T, et al., **2004**, PMID: 15258581	Northern blot shows some expression in brain, but lower than other tissues such as heart, lung, liver, kidney	Score	**0.5** (0.5)	
Efhc1 knockout mouse	**Model Systems** Non-human model organism	Suzuki T, et al., **2009**, PMID: 19147686	Myoclonus and increased susceptibility to induced seizures may be similar to early stages of juvenile myoclonic epilepsy phenotype. However, mice are older than 4-12 months of age when phenotypes occur, and they do not develop tonic clonic seizures, absence seizures, or spike-wave complexes like JME patients.	Score	**0.5** (2)	Mouse model shows involvement of Efhc1 in neurological function, but does not model JME.

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
				Total points:	1.00	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

GRIN2D – infantile epilepsy syndrome – *Autosomal dominant inheritance (primarily or exclusively de novo)*

Classification owner: Epilepsy EP
Calculated classification: Limited
Modified classification: No Modification
Reason for modified classification: None

Classification status: APPROVED
Date classification saved: 2018 Jul 06, 7:34 am
Replication Over Time: No
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: infantile epilepsy syndrome

Evidence Summary

Approved by the ClinGen Epilepsy Expert Panel 7/3/2018.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	0	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	2	3	3
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						3
Experimental Evidence	Functional			Biochemical Functions	1	0.5	0.5
				Protein Interactions	0	0	
				Expression	0	0	
	Functional Alteration			Patient cells	0	0	1
				Non-patient cells	1	1	
	Models			Non-human model organism	0	0	0
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						1.5
Total Points							4.5

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Proband 1	Variant is de novo	NM_000836.2(GRIN2D):c.1999G>A (p.Val667Ile)	Li D, et al., **2016**, PMID: 27616483	Female	**Age of Onset:** 4 Months	Unknown	**HPO term(s):** Seizures; Profound global developmental delay **free text:** intractable epilepsy, global developmental delay, static encephalopathy	-	-	-	-	Metabolic screening studies negative, blood and urine based screening studies negative, CSF normal, muscle biopsy mildly abnormal, clinical diagnostic genetic testing unremarkable	**Method 1:** Exome sequencing; **Method 2:** Sanger sequencing **Description of genotyping method:** Exome sequencing of proband and mother, father was unavailable initially. Sanger confirmation in proband and both parents.	Score	**1.5** (2)	Missense mutation
Proband 2	Variant is de novo	NM_000836.2(GRIN2D):c.1999G>A (p.Val667Ile)	Li D, et al., **2016**, PMID: 27616483	Female	**Age of Onset:** 2 Months	Not Hispanic or Latino	**HPO term(s):** obsolete Severe neonatal hypotonia in males; Seizures; Profound global developmental delay **free text:** congenital hypotonia, focal epilepsy, global developmental delay	-	-	-	-	metabolic screening unremarkable, no evidence of congenital disorders of glycosilation, CSF normal, microcephaly	**Method 1:** Genotyping **Description of genotyping method:** gene panel analysis, Sanger confirmation in proband and both parents	Score	**1.5** (2)	Missense mutation
														Total points:	**3.00**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
NMDA receptor	**Biochemical Function** A	Li D, et al., **2016**, PMID: 27616483	Lesca et al., 2013; Lemke et al., 2013; Carvill et al., 2013	Score	**0.5** (0.5)	There is strong evidence for the involvement of other NMDA receptor subunits in epilepsy (GRIN2A, epilepsy aphasia spectrum) or neurodevelopmental disorders with or without epilepsy (GRIN2B, GRIN1) including gain-of-function mutations. Therfore, it is very convincing that gain-of-function mutations in GRIN2D cause epilepsy as well.
pharmacological properties	**Functional Alteration** Non-patient cells	Li D, et al., **2016**, PMID: 27616483	activation by lower concentrations of co-agonists, increased current of open channel, slower deactivation	Score	**1** (0.5)	Multiple well-performed experiments supporting gain of function.
transfected rat cortical neurons	**Model Systems** Cell culture model	Li D, et al., **2016**, PMID: 27616483	Assay shows excitotoxic cell death triggered by excessive influx of calcium due to the gain-of-function mutation. Effect is reduced by adding memantine (NMDA antagonist).	Review	**1** (1)	Not included as it is not confirmed that developmental delay in humans is caused by exitotoxic cell death
				Total points:	**1.50**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

KCNA2 – infantile epilepsy syndrome – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Strong
Modified classification: No Modification
Reason for modified classification: None

Classification status: APPROVED
Date classification saved: 2018 May 31, 1:55 pm
Replication Over Time: No
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: infantile epilepsy syndrome

Evidence Summary

No summary is provided.

Calculated Classification Matrix

			Evidence Type		Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	0	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	10	16	12
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						12
Experimental Evidence	Functional			Biochemical Functions	1	1	2
				Protein Interactions	0	0	
				Expression	2	1	
	Functional Alteration			Patient cells	0	0	1
				Non-patient cells	4	1	
	Models			Non-human model organism	1	0.5	1
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	1	0.5	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						4
Total Points							16

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Female 1	Variant is de novo	NM_002354.2(EPCAM):c.392C>A (p.Thr131Asn)	Syrbe S, et al., **2015**, PMID: 25751627	Female	**Age of Report:** 8 Years		**HPO term(s):** Epileptic encephalopathy; Focal seizures, afebril; Intellectual disability, moderate; Ataxia **free text:** epileptic encephalopathy - FS at 17m (hemiclonic) Other sz types: FS, myoclonic, focal motor seizures, focal dyscognitive seizures, secondary GTCS Seizure free since 7.5y Mild-moderate ataxia, constant myoclonus Mild-moderate ID Delayed speech development Subclinical hypothyroidism IGF-1 -0.7sd EEG: multifocal sharp waves and sharp slow waves, accentuated over left frontocentral region with marked increase during sleep Normal MRI	-	-	-	-	SCN1A and UBE3A sequencing; 15q11 FISH; 485 gene panel (see PMID: 22612257 Lemke et al. 2012)		Score	**1** (2)	Parental relationships not confirmed Absent in gnomAD/ExAC
Male 2	Variant is de novo	NM_003227.3(TFR2):c.2033G>C (p.Arg678Pro)	Syrbe S, et al., **2015**, PMID: 25751627	Male	**Age of Report:** 7 Years		**HPO term(s):** Epileptic encephalopathy; Intellectual disability, moderate; Myoclonic atonic seizures **free text:** epileptic encephalopathy - myoclonic seizures at 11m Other sz types: myoclonic, myoclonic-atonic (24m) Seizure free since 4y Normal devt prior to sz onset followed by stagnation Mild-moderate ID Normal MRI EEG: multifocal sharp waves and polyspikes (normal since 6y)	-	-	-	-	Karyotype; subtelomeric FISH; whole exome sequencing		Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Male 5	Variant is de novo	NM_002354.2(EPCAM):c.392C>A (p.Thr131Asn)	Syrbe S, et al., **2015**, PMID: 25751627	Male	**Age of Report:** 19 Years		**HPO term(s):** Epileptic encephalopathy; Focal seizures, afebril; Intellectual disability, moderate **free text:** epileptic encephalopathy - Febrile status epilepticus at 8m Other sz types: FS, focal motor, secondary GTCS Seizure free since 15y Moderate ID with speech delay Severe scoliosis EEG: at 4y suggestive of ESES; multifocal, activated by sleep (normal since 17y) MRI: normal	-	-	-	-	300 gene panel; no other past genetic testing indicated		Score	**1** (2)	Parental relationships not confirmed Absent in gnomAD/ExAC
Male 6	Variant is de novo	NM_000535.6(PMS2):c.1819G>A (p.Val607Ile)	Syrbe S, et al., **2015**, PMID: 25751627	Male	**Age of Report:** 36 Years		**HPO term(s):** Epileptic encephalopathy; Generalized seizures; Intellectual disability, severe; Ataxia **free text:** epileptic encephalopathy - GTCS at 6m Other sz types: myoclonic, atypical absence Severe ID; non-verbal Moderate ataxia, occasional myoclonus EEG at 22y: frequent GSW on slow background MRI "gross cerebellar atrophy" Mild dysmorphic facial features Born at 34wk due to maternal toxemia	-	-	-	-	array CGH; WES		Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Male 7	Variant is de novo	NM_000251.2(MSH2):c.1922G>A (p.Cys641Tyr)	Syrbe S, et al., **2015**, PMID: 25751627	Male	**Age of Report:** 26 Years		**HPO term(s):** Epileptic encephalopathy; Generalized seizures; Intellectual disability, moderate; Ataxia **free text:** epileptic encephalopathy - Febrile status epilepticus at 5m Other sz types: GTCS, absences Moderate ID Moderate-severe ataxia; hyperreflexia Broad based ataxic gait; impaired fine motor skills dysdiadochokinesis, dysmetric knee-shin-slide EEG at 6y: GSW and GPSW MRI normal	-	-	-	-			Score	**2** (2)	
Male 7	Variant is de novo	NM_000251.2(MSH2):c.1922G>A (p.Cys641Tyr)	Pena SD, et al., **2015**, PMID: 25477152	Male	**Age of Report:** 7 Years		**free text:** Febrile seizure 15m Other seizure types: afebrile GTCS, myoclonic, absence hypotonia, ataxia, tremor, hyperkinetic movements, developmental delay EEG: 3Hz GSW; 2-2.5Hz GSW	-	-	-	-		**Method 1:** Exome sequencing	Score	**2** (2)	
Male 11	Variant is de novo	NM_004974.3(KCNA2):c.869T>C (p.Leu290Pro)	Allen NM, et al., **2016**, PMID: 26648591	Male	**Age of Report:** 5 Years		**free text:** 7 weeks, nonspecific events at 3 months, absences 9 months, ongoing frequent absences (typical and atypical), occasional GTCS	-	-	-	-	Karyotype, CGH, POLG, SCN1A, GLUT1, TITF1; WES	**Method 1:** Exome sequencing	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Female 4	Variant is de novo	NM_000217.2(KCNA1):c.1223T>C (p.Val408Ala)	Allou L, et al., **2017**, PMID: 27062609	Female	**Age of Report:** 16 Years		**free text:** 4m: seizure onset (extension jerky movements of all four limbs) and strabismus noted Pyramidal signs, loss of visual contact at 7m Refractory sz in childhood: atonic seizures, myoclonic seizures, partial tonic – clonic seizures with episodes of status epilepticus Devt regression at 9m Feeding difficulties Non-verbal, wheelchair dependent Severe scoliosis Acquired microcephaly Stereotyped hand movements MRI: progressive cerebellar atrophy	-	-	-	-	negative for Angelman syndrome and array CGH; WES	**Method 1:** Exome sequencing	Score	**2** (2)	
Male H	Variant is de novo	NM_000217.2(KCNA1):c.*1522C>G	Hundallah K, et al., **2016**, PMID: 27117551	Male	**Age of Report:** 1 Months		**free text:** Day of life 1: clonic, myoclonic seizures Other sz type: focal, tonic, secondary generalized Encephalopathic Axial and appendicular hypotonia Severe devt delay; microcephaly developmental delay; axial hypotonia; and appendicular spasticity; compatible with spastic cerebral palsy. There was no eye contact or interaction. Repeated brain MRI showed atrophy of the supratentorial and inferior part of brain with delayed myelination Negative metabolic work up EEG: frequent independent bilateral temporal discharges MRI: normal	-	-	-	-	WES but unclear if trio	**Method 1:** Exome sequencing	Score	**1** (2)	Parental relationships not confirmed Absent in gnomAD/ExAC

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Proband B	Variant is de novo	NM_000251.2(MSH2):c.1922G>A (p.Cys641Tyr)	Corbett MA, et al., **2016**, PMID: 27733563	Female	**Age of Report:** 9 Years		**free text:** Onset 12m: tonic sz Other sz types: myoclonic and absences Sz initially triggered by minor head injury, then by infection/fever Ataxia; cerebellar signs on examination; normal eye movement Initial normal devt, slowed at 12m (sz onset) Progressive cerebellar atrophy on MRI	-	-	-	-	Sequencing of KCNA2		Score	**1** (2)	Variant allegedly de novo, but no indication of parental testing/confirmed relationships
														Total points:	**16.00**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Biochemical Function	**Biochemical Function** B	Shen W, et al., **2004**, PMID: 13679409	Kv1.2 channels shown to be expressed in medium spiny neurons. Currents attributable to Kv1.2 channels activated rapidly, inactivated slowly, and recovered from inactivation slowly. Study showed a role for Kv1.2 channels in regulating state transitions and repetitive discharge in these neurons.	Score	**1** (0.5)	Kv1.2 channels shown to be expressed in medium spiny neurons. Currents attributable to Kv1.2 channels activated rapidly, inactivated slowly, and recovered from inactivation slowly. Study showed a role for Kv1.2 channels in regulating state transitions and repetitive discharge in these neurons.
Functional Expression	**Expression** A	Lorincz A, et al., **2008**, PMID: 19118165	Widely expressed in CNS. Increased expression in AIS along with Nav1.6 and Kv1.1. Expressed in glutamatergic pyramidal cells and GABAergic interneurons. High expression in juxtaparanodal axons. Co-expression with Kv1.1 in AIS of PCs, suggesting heteromeric channels.	Score	**0.5** (0.5)	
Functional Expression	**Expression** A	Wang H, et al., **1994**, PMID: 8046438	Widely expressed in mouse brain; colocalized with Kv1.1 See also Gtex for brain specific expression of KCNA2	Score	**0.5** (0.5)	
Cell Culture Model System.1	**Functional Alteration** Non-patient cells	Syrbe S, et al., **2015**, PMID: 25751627	p.Pro405Leu variant expressed in Xenopus oocytes; co-expressed with WT Kv1.2 channels in increasing ratios (1:1; 1:2; 1:4). Current amplitudes recorded via patch clamp. Showed increasing loss-of-function, consistent with dominant-negative effect	Score	**0.5** (0.5)	

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Cell Culture Model System.2	**Functional Alteration** Non-patient cells	Syrbe S, et al., **2015**, PMID: 25751627	p.Ile263Thr variant expressed in Xenopus oocytes; co-expressed with WT Kv1.2 channels in increasing ratios (1:1; 1:2; 1:4). Current amplitudes recorded via patch clamp. Showed increasing loss-of-function, consistent with dominant-negative effect	Score	**0.5** (0.5)	
Cell Culture Model System.3	**Functional Alteration** Non-patient cells	Syrbe S, et al., **2015**, PMID: 25751627	p.Arg297Gln variant expressed in Xenopus oocytes; co-expressed with WT Kv1.2 channels. Current amplitudes measured via patch clamp. Mean current amplitudes significantly increased compared to WT. Activation curve of WT + Arg297Gln channels significantly shifted to more hyperpolarized potentials. Resting membrane potential ~40mV more negative than WT. Consistent with constituitively open channels and GOF.	Score	**0** (0.5)	Previously Counted
Cell Culture Model System.4	**Functional Alteration** Non-patient cells	Syrbe S, et al., **2015**, PMID: 25751627	p.Leu298Phe variant expressed in Xenopus oocytes; co-expressed with WT Kv1.2 channels. Current amplitudes measured via patch clamp. Mean current amplitudes significantly increased compared to WT. Activation curve of WT + Leu298Phe channels significantly shifted to more hyperpolarized potentials. Resting membrane potential ~40mV more negative than WT. Consistent with constituitively open channels and GOF.	Score	**0** (0.5)	Previously counted
Animal Model	**Model Systems** Non-human model organism	Brew HM, et al., **2007**, PMID: 17634333	Kcna2 knock out mice have spontaneous seizures with increased seizure susceptibility compared to WT and heterozygous mice. Seizure onset in neonatal period; 50% died post-seizure (stopped breathing). Neurons of the medial nucleus of the trapezoid body were hyperexcitable.	Score	**0.5** (2)	0.5 point since this animal model is a knock out (not consistent with human disorder which is autosomal dominant); however some human mutations are clearly LOF with dominant-negative effect
Models and Rescue	**Rescue** Non-human model organism	Xie G, et al., **2010**, PMID: 20696761	Homozygous and heterozygous for p.I402T Kcna2 variant. Considered model for cerebellar ataxia; abnormal gait and behavior, severe post natal growth retardation (homozygous worse than heterozygous). Heterozygotes had normal life span (similar to WT). 50% of homozygotes died between 3rd and 6th weeks of life. No spontaneous seizures; heterozygotes showed no significant differences on rotarod test. Heterozygotes and homozygotes showed severe tremors, myoclonic jerks, ataxic movements. Decreased grip strength.Treated with acetazolamide; partially rescued phenotype. I402T reduces amount of functional channels but only induces subtle changes in channel properties (CHO cell transfection); reduces density of Kv1 channel current. Increased expression of Kv1.2 partially rescues gait abnormality/coordination impairment in mouse	Score	**0.5** (2)	0.5 for the animal model and rescue combined (since the tremors, myoclonic jerks, and gait abnormalities are similar to patients but no spontaneous seizures)
				Total points:	**4.00**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)

Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest

Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)

Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)

Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state

Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

KCNT1 – childhood-onset epilepsy syndrome – *Other*

Classification owner: Epilepsy EP
Calculated classification: Limited
Modified classification: Definitive
Reason for modified classification:
In the original evidence summary the variants (ClinVar IDs: 39594, 39593, 39595, 39596, 126421) were all accounted for as pathogenic and scored as such under de novo gene evidence = a total of 18 points, with a final maximum score of 12 points for genetic evidence. These cannot be accounted for in the GCI due to this error message from the GCI "The gene impact for each variant associated with this proband must be specified in order to score this proband (see variant(s) and links to curating their gene impact in variant section for this Individual, above)." In order to account for these variants in the evidence summary, these variants must be curated and as of current time of this review they have not been evaluated for pathogenicity. The final total score is then 14 points with replication over time = Definitive. This curation is displayed on the ClinGen website as such.

Classification status: APPROVED
Date classification saved: 2018 May 31, 3:32 pm
Replication Over Time: Yes
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: childhood-onset epilepsy syndrome

Evidence Summary

Reviewed and approved by the ClinGen Epilepsy Expert Panel 10/20/2017.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	0	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	0	0	0
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						0
Experimental Evidence	Functional			Biochemical Functions	0	0	1
				Protein Interactions	0	0	
				Expression	1	1	
	Functional Alteration			Patient cells	0	0	1
				Non-patient cells	1	1	
	Models			Non-human model organism	0	0	0
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						2
Total Points							2

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Male 1	Variant is de novo	NM_020822.2(KCNT1):c.2800G>A (p.Ala934Thr)	Barcia G, et al., **2012**, PMID: 23086397	Male	**Age of Onset:** 1 Months		**HPO term(s):** Focal motor seizures; Generalized hypotonia; Infantile axial hypotonia; Microcephaly; Absent speech; Cerebral cortical atrophy; Delayed CNS myelination **free text:** Focal, generalized, and tonic seizures once per day. Normal psychomotor developemnet noted before epilepsy, regression after seizure onset.	-	-	-	-	negative for SCN1A mutations. De novo mutations in MCM9 (V613M, benign) and CUX1 (R864Q, gnomAD freq= 4.075e-6). Normal metabolic workup.	**Method 1:** Exome sequencing	Supports	()	
Male 2	Variant is de novo	NM_020822.2(KCNT1):c.1283G>A (p.Arg428Gln)	Barcia G, et al., **2012**, PMID: 23086397	Male	**Age of Onset:** 2 Months		**HPO term(s):** Focal motor seizures; Generalized hypotonia; Infantile axial hypotonia; Microcephaly; Cerebral cortical atrophy; Delayed CNS myelination; Aplasia/Hypoplasia of the corpus callosum; Delayed gross motor development **free text:** Focal, generalized, and tonic or clonic seizures once per day. Normal psychomotor developemnet noted before epilepsy, regression after seizure onset.	-	-	-	-	negative for SCN1A mutations. De novo mutations in MYH14 (Q1191R, associaed with hearing loss) and PLXNB2 (R131H, gnomAD freq= 1.81e-5). Normal metabolic workup.	**Method 1:** Exome sequencing	Supports	()	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Male 3	Variant is de novo	NM_020822.2(KCNT1):c.1283G>A (p.Arg428Gln)	Barcia G, et al., **2012**, PMID: 23086397	Male	**Age of Onset:** 1 Days		**HPO term(s):** Focal autonomic seizures; Infantile axial hypotonia; Microcephaly; Cerebral cortical atrophy; Delayed CNS myelination; Aplasia/Hypoplasia of the corpus callosum; Morphological abnormality of the pyramidal tract; Delayed gross motor development **free text:** Focal, generalized, and tonic seizures once per day. Autonomimc seizures included bradycardia and cyanosis.	-	-	-	-	negative for SCN1A mutations. Normal metabolic workup.	**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** de novo confirmed from parents Sanger sequencing KCNT1 PCR and Sanger sequencing	Supports	()	
Male 4	Variant is de novo	NM_020822.2(KCNT1):c.1283G>A (p.Arg428Gln)	Barcia G, et al., **2012**, PMID: 23086397	Male	**Age of Onset:** 1 Days		**HPO term(s):** Focal motor seizures; Infantile axial hypotonia; Microcephaly; Morphological abnormality of the pyramidal tract; Aplasia/Hypoplasia of the corpus callosum **free text:** Focal motor seizures once per day.	-	-	-	-	negative for SCN1A mutations. Normal metabolic workup.	**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** de novo confirmed from parents Sanger sequencing KCNT1 PCR and Sanger sequencing	Supports	()	Variant Evidence: Xenopus oocytes transfected with rat R409Q (homolog of hR428Q) had 2-3 fold greater current amplitude than wildtype (Figure 1), and were unresponsive to PKC activitation (Figure 2).

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Male 5	Variant is de novo	NM_020822.2(KCNT1):c.1421G>A (p.Arg474His)	Barcia G, et al., **2012**, PMID: 23086397	Male	**Age of Onset:** 2 Weeks		**HPO term(s):** Focal motor seizures; Infantile axial hypotonia **free text:** Focal motor seizures once per day. MRI was normal at 1 mo.	-	-	-	-	negative for SCN1A mutations. Normal metabolic workup.	**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** de novo confirmed from parents Sanger sequencing KCNT1 PCR and Sanger sequencing	Supports	()	
Female 6	Variant is de novo	NM_020822.2(KCNT1):c.2280C>G (p.Ile760Met)	Barcia G, et al., **2012**, PMID: 23086397	Female	**Age of Onset:** 3 Days		**HPO term(s):** Focal autonomic seizures; Focal motor seizures; Infantile axial hypotonia **free text:** Focal motor seizures once per day. Autonomic includes cyanosis. MRI was normal at 1 and 2 months old)	-	-	-	-	negative for SCN1A mutations. Normal metabolic workup.	**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** de novo confirmed from parents Sanger sequencing KCNT1 PCR and Sanger sequencing	Supports	()	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Female 1	Variant is de novo	NM_020822.2(KCNT1):c.862G>A (p.Gly288Ser)	Ishii A, et al., **2013**, PMID: 24029078	Female	**Age of Onset:** 2 Months		**HPO term(s):** Focal motor seizures; Generalized hypotonia; Delayed gross motor development; Developmental regression **free text:** Dx resistent seizures. Upwards of 30 seizures/ day, 20 or more were focal seizures. Epileptic spasms never observed. Brain MRI at 3mo was normal. Interictal EEGs showed asynchronous high-voltage slow activites with multifocal spkies. Vagus nerve stimulation commnsed at 83 months and markedly reduced seizures. Gross de velopmental delay noted at 97 mo.	-	-	-	-	Blood gas analysis, blood cell counts and chemistry, metabolic screening or amino and organic acids, aconventional chromosomal analysis and chromosomal microarray were unremarkable. Negative for SCN1A, SLC2A1, ARX, CDKL5, STXBP1, and SLC25A22 mutations.	**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** Bidirectional KCNT1 PCR and Sanger sequencing	Supports	()	Predicted pathogenic by Poly-Phen-2
Female 2	Variant is de novo	NM_020822.2(KCNT1):c.862G>A (p.Gly288Ser)	Ishii A, et al., **2013**, PMID: 24029078	Female	**Age of Onset:** 2 Months		**HPO term(s):** Generalized hypotonia; Developmental regression **free text:** During seizure had loss of responsiveness, apnea, eye deviation, grimacing, and tongue protrusion. Psychomoter delay happened post seizure onset. Seizure frequency= 20-30/day. Interictal EEGs showed asynchronous high-voltage slow activites with multifocal spkies. Rx with clorazepate decreased frequency of seizures.	-	-	-	-	Blood gas analysis, blood cell counts and chemistry, metabolic screening or amino and organic acids, aconventional chromosomal analysis and chromosomal microarray were unremarkable. Negative for CDK5L mutations.	**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** Bidirectional KCNT1 PCR and Sanger sequencing	Supports	()	Predicted pathogenic by Poly-Phen-2
														Total points:	**0.00**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Summary of Functional Expression Studies (3)	**Expression** A	Fagerberg L, et al., **2014**, PMID: 24309898	NCBI Gene RNA-seq analysis data shows KCNT1 having the highest expression in the brain, with lower expression in the ovary, spleen, and testis. RPKM in brain~ 4.2 Performed immunostaining (IMS) on rat brain to show the expression and subcellular localization of KCNT1. KCNT1 was expressed thoruhgout several brain regions with the highest expression noted in the brain stem. Northern blot of rat tissue showed high expression of KCNT1 in the brain (4.5kB transcript) and the kidney (7.5kB transcript). ISH of rat brain showed KCNT1 expression in neurons, and no expression was detected in glia (Figure 3) IMS of KCNT1 in isolated embryonic cortical neurons and hippocamapal neurons	Score	**1** (0.5)	Combined studies
FA Non-patient cells	**Functional Alteration** Non-patient cells	Tang QY, et al., **2016**, PMID: 26725113	Xenopus oocytes were transienty transfected with constructs to rat KCNT1 and 12 mutations associated with epilepsy. Consistently 7 variants (V525F, G269S, R409Q, R455H, Y775H, R907C, and F911I), showed altered sensitivity to Na2+ concentration for activation (Figure 2). tey show that one variant R379Q consistently shows reduced sensistivty to Na2+, compared to the other 7 that show enhanced sensitivty. Channel open probability is also enhanced in the R379Q, Y775H and R455H variants) Note: 4 of these mutations are associated with nocturnal epilepsy (R379Q, Y775H, R907C, and M875I). The authors also note that all the mutations are GOF!!	Score	**1** (0.5)	Given the number of potenitally pathogenic mutations they evaluated for Na sensitivity and channel opening probability I increased the to the maximum.
				Total points:	**2.00**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

MAGI2 – infantile epilepsy syndrome – *X-linked inheritance*

Classification owner: Epilepsy EP
Calculated classification: Limited
Modified classification: Disputed
Reason for modified classification:
There are at least 4 individuals that have been identified who have a deletion on the same locus with infantile spasms but are not missing MAGI2

Classification status: APPROVED
Date classification saved: 2018 Jun 26, 10:28 am
Replication Over Time: No
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: infantile epilepsy syndrome

Evidence Summary

MAGI2 was evaluated for evidence supporting and refuting its relationship with infantile epilepsy (MONDO:0020071). Of note, variation in MAGI2 has also been described in association with Nephrotic Syndrome, an AR disorder; evidence for this gene-disease relationship is not considered here. MAGI2 was initially thought to be involved in infantile epilepsy after reports of large deletions including MAGI2 (and other genes) in individuals with infantile epilepsy (Marshall 2008 18565486, Peterson 26030165). MAGI2 was thought to be the causative gene because it has been found to interact with stargazin, a protein that has been associated with epilepsy in mice (Deng 2006 16870733), and it was the region with the most overlap amongst patients with documented infantile spasms (Marshall 2008 18565486). However, no single-gene deletions or single nucleotide variants have been reported in this gene in individuals with infantile epilepsy; additionally, the Marshall 2008 and Rothlisberger 2010 studies each contained one individual with a deletion at the 7q11.23-7q2.11 locus that did not encompass MAGI2. There has also been an individual with a deletion at this locus that spanned part of MAGI2 who did not have a a diagnosis of epilepsy though it had not been ruled out (Marshall 2008 18565486). Additionally, there have been 4 other individuals with deletions at this locus that do not include MAGI2 who presented with infantile spasms suggesting that there is a different candidate gene for this disorder (Paciorkowski 21694734, Morimoto 2003 14636357, Komoike 20146355). Furthermore, the MAGI2 null mouse displayed renal abnormalities consistent with patients with intragenic null frameshift variants who had no reports described of seizures indicating that the absence of MAGI2 may cause nephrotic syndrome instead of infantile epilepsy (Ihara 2014 25108225, Bierzynska 2017 27932480). In conclusion, given that there have been no variants impacting only MAGI2 in patients with infantile spasms, patients with MAGI2 variants have expressed a different phenotype, and patients with deletions at this locus that do not include MAGI2 have reported seizures, this gene-disease association is Disputed.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	0	0	0
				Proband with predicted or proven null variant	2	0	0
				Variant is *de novo*	0	0	0
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						**0**
Experimental Evidence	Functional			Biochemical Functions	0	0	0.5
				Protein Interactions	2	0	
				Expression	2	0.5	
	Functional Alteration			Patient cells	0	0	0
				Non-patient cells	0	0	
	Models			Non-human model organism	2	0	0
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						**0.5**
Total Points							**0.5**

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Proband 175	Proband with predicted or proven null variant	NM_012301.3(MAGI2):c.3998delG (p.Gly1333Alafs)	Bierzynska A, et al., **2017**, PMID: 27932480	Female	**Age of Onset:** 4 Years			2	-	**Calculated:** 0.3	No		**Method 1:** Exome sequencing **Description of genotyping method:** Whole exome sequencing performed on patients 175 and 180 The MAGI2 homozygous frameshift deletion p.(Gly1333Alafs*141) was verified by Sanger sequencing, and this was the only homozygous variant present in both 175 and 175S. It was also present in the mother (175M) as a heterozygote but could not be verified in the father, because DNA was not available	Score	**0** (1.5)	These patients were not described to have epilepsy and the animal models for LOF of just the MAGI2 gene only implicates renal disease.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Sporadic case 180	Proband with predicted or proven null variant	NM_012301.3(MAGI2):c.3526_3533dup (p.Glu1178Aspfs) NM_012301.3(MAGI2):c.64_71delAGGAACCC (p.Arg22Glyfs)	Bierzynska A, et al., **2017**, PMID: 27932480	Male	**Age of Onset:** 3 Months	Not Hispanic or Latino	**free text:** nephrotic syndrome, there was no report of seizures	-	-	-	-		**Method 1:** Exome sequencing	Score	**0** (1.5)	This individual has a sporadic case of congenital nephrotic syndrome with a molecular basis of 2 compound het LOF variants identified by WES. Confirmed the variants were in trans in the parents. The null animal models for this gene provide support for the nephrotic syndrome association as opposed to the epilepsy suggesting that this gene is not related to infantile epilepsy.
														Total points:	**0.00**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
MAGI2 expression in kidney implicates function	**Expression** A	Balbas MD, et al., **2014**, PMID: 25271328	MAGI-2 is exclusively expressed in podocytes, specialized cells forming part of the glomerular filter, where it interacts with the slit diaphragm protein nephrin. Our results reveal that MAGI-2 is required for the integrity of the kidney filter and podocyte survival. Moreover, we demonstrate that PECs can be activated to form glomerular lesions resembling a noninflammatory glomerulopathy with extensive extracapillary proliferation, sometimes resembling crescents, following rapid and severe podocyte loss.	Score	**0** (0.5)	This doesn't provide support for epilepsy, in fact a few of the intragenic LOF variants described in cases have been found in patients with kidney disease.
Expression of MAGI2 in the brain of mice	**Expression** A	Deng F, et al., **2006**, PMID: 16870733	We performed coimmunoprecipitation experiments to test whether TARPs interacted with MAGI-2 in vivo in mouse brain (Fig. 4). The anti-TARP antibody coimmunoprecipitated with two prominent MAGI-2 isoforms from wild-type and stargazer cerebral cortex but not from cerebellum or hippocampus from either mouse. These isoforms correspond to MAGI-2 ⏐ and ┐ based on their sizes. In wild-type cerebral cortex, ┌2, ┌3, and ┌8 were also immunoprecipitated so any of these, or some combination, might be responsible for interacting with MAGI-2.	Score	**0.5** (0.5)	This evidence does suggest that the gene could be involved in epilepsy as it is showing that the gene is expressed in the CNS
MAGI2 Null mouse	**Model Systems** Non-human model organism	Ihara K, et al., **2014**, PMID: 25108225	Membrane-associated guanylate kinase inverted 2 (MAGI-2) is a tight junction protein in epithelial tissues	Score	**0** (2)	This mouse model supports the evidence indicating that the MAGI2 gene is associated with abnomalities of the renal system as opposed to abnormalities of the CNS causing early infantile spasms
YWHAG1	**Model Systems** Non-human model organism	Komoike Y, et al., **2010**, PMID: 20146355	The gene YWHAG1 is identified by this paper to be a candidate gene for the cause of the ISS phenotype in the patients. One of the patients has haploinsufficiency of this gene adn they found that the heart was malformed with arrhythmia in the morphant zebrafish. They don't say anything about whether the zebrafish had epilepsy but they do suggest that the protein is critical for development of the nervous system.	Score	**0** (2)	This identified a different candidate gene in the locus that was identified to have deletions in the patients with ISS. These individuals did not have a deletionn of the MAGI2 gene and therefore this paper was evidence against the association.

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Stargazin interaction with MAGI2	**Protein Interactions** physical association (MI:0915)	Deng F, et al., **2006**, PMID: 16870733	. We used yeast two-hybrid screening to identify MAGI-2(membrane associated guanylate kinase,WW and PDZ domain containing 2) as a novel candidate interactor with the cytoplasmic C termini of the TARPs. MAGI-2 [also known as S-SCAM (synaptic scaffolding molecule)] is a multi-PDZ domain scaffolding protein that interacts with several different ligands in brain, including PTEN (phosphatase and tensin homolog), dasm1(dendrite arborization and synapse maturation 1), dendrin, axin, ┐- and └-catenin, neuroligin, hyperpolarization-activated cation channels,┐1-adrenergic receptors, and NMDA receptors. We confirmed that MAGI-2 coimmunoprecipitated with stargazin in vivo from mouse cerebral cortex and used in vitro assays to localize the interaction to the C-terminal -TTPV amino acid motif of stargazin and thePDZ1, PDZ3, and PDZ5 domains of MAGI-2. Expression of stargazin recruited MAGI-2 to cell membranes and cell– cell contact sites intransfected HEK-293T cells dependent on the presence of the stargazin -TTPV motif. These experiments identify MAGI-2 as a strong candidate for linking TARP/AMPA receptor complexes to a wide range of other postsynaptic molecules and pathways and advance our knowledge of protein interactions at mammalian CNS synapses.	Score	**0** (0.5)	Though the Stargazin CACNG2 gene has been implicated with intellectual disability in humans (Hamdan 2010 21376300) and epilepsy in mice (Letts 1998 9697694), , there doesn't seem to be enough genetic evidence to consider this association definitive, and therefore, this interaction shouldn't be scored as evidence that this gene causes the epilepsy
USH1G interacts with MAGI2	**Protein Interactions** physical association (MI:0915)	Bauß K, et al., **2014**, PMID: 24608321	identified Magi2 (membrane-associated guanylate kinase inverted-2) as a new component of the USH protein interactome, binding to the multifunctional scaffold protein SANS (USH1G). We showed that the SANS-Magi2 complex assembly is regulated by the phosphorylation of an internal PDZ-binding motif in the sterile alpha motif domain of SANS by the protein kinase CK2. We affirmed Magi2's role in receptormediated, clathrin-dependent endocytosis and showed that phosphorylated SANS tightly regulates Magi2-mediated endocytosis. Furthermore, we demonstrated the localization of the SANSMagi2 complex in the periciliary membrane complex facing the ciliary pocket of retinal photoreceptor cells in situ.	Score	**0** (0.5)	This interaction, though they implicate that the MAGI2 protein may be involved in neuron endo/exocytosis, doesn't directly implicate the phenotype because they only tested neurons in the retina. This study cites several other studies showing that MAGI2 participates in endocytosis, however these studies are out of the scope of this curation.
				Total points:	**0.50**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

✖ Close

Evidence Summary

RYR3 – undetermined early-onset epileptic encephalopathy – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Limited
Modified classification: No Modification
Reason for modified classification: None

Classification status: APPROVED
Date classification saved: 2018 Jun 28, 2:15 pm
Replication Over Time: No
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: undetermined early-onset epileptic encephalopathy

Evidence Summary

An association with epileptic encephalopathy was made made in a single publication in 2014 that reported 2 probands with different de novo missense variants. While there is reasonable biological plausibility, there is no experimental support for gene-disease association. In summary, there is limited evidence to support this gene-disease association, though no evidence has emerged to contradict it. Approved by the ClinGen Epilepsy Expert Panel 6/19/2018.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	0	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	2	1	1
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						1
Experimental Evidence	Functional			Biochemical Functions	0	0	0
				Protein Interactions	0	0	
				Expression	0	0	
	Functional Alteration			Patient cells	0	0	0
				Non-patient cells	0	0	
	Models			Non-human model organism	0	0	0
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	0
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						0
Total Points							1

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
NLES14-1	Variant is de novo	NM_001036.4(RYR3):c.14104G>A (p.Asp4702Asn)	Passa I, et al., **2015**, PMID: 25262652	Unknown			**HPO term(s):** Epileptic spasms	-	-	-	-	Prior testing was not an inclusion criterion for the study. Prior testing for each proband is not given.	**Method 1:** Exome sequencing	Score	**0.5** (2)	Prior testing of known epileptic encephalopathy genes not reported. Gene generally tolerant of missense variation (ExAC z = 0.75)
Lgsnd24647gu-1	Variant is de novo	NM_001036.4(RYR3):c.9608_9610del (p.Ile3203del)	Passa I, et al., **2015**, PMID: 25262652	Unknown			**HPO term(s):** Epileptic encephalopathy	-	-	-	-	Prior testing not an inclusion criterion for study entry and no testing reported by proband.	**Method 1:** Exome sequencing	Score	**0.5** (2)	Prior testing of known epileptic encephalopathy genes not reported. Gene generally tolerant of missense variation (ExAC z = 0.75)
														Total points:	**1.00**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

No Experimental evidence was found.

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

Close

Evidence Summary

SCN8A – complex neurodevelopmental disorder – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Definitive
Modified classification: No Modification
Reason for modified classification: None

Classification status: APPROVED
Date classification saved: 2018 Jul 18, 7:50 am
Replication Over Time: Yes
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: complex neurodevelopmental disorder

Evidence Summary

Approved by the ClinGen Epilepsy Expert Panel 10/20/2017.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	3	0.2	0.2
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	17	30.5	12
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						12
Experimental Evidence	Functional			Biochemical Functions	0	0	2
				Protein Interactions	1	0.5	
				Expression	1	2	
	Functional Alteration			Patient cells	0	0	0.5
				Non-patient cells	1	0.5	
	Models			Non-human model organism	1	4	4
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						6
Total Points							18

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
T3929 and Patient H	Proband with other variant type with some evidence of gene impact	NM_014191.3(SCN8A):c.3991C>G (p.Leu1331Val)	Carvill GL, et al., **2013**, PMID: 23708187	Male	**Age of Diagnosis:** 18 Months		**free text:** EIEE started at 18 months of age. Part of a large case control, phenotypes included: EE was defined by refractory seizures and congnitive slowing or regression associated with frequent, ongoing epileptiform activity. Patient H in Larsen et al., 2015: Seizure types experienced focal dyskinesia, myoclonic, status epilepticus and tonic. HAd developmental delay prior to seizures, delayed regression in development following seizure onset. Diagnosed as unclassified EE. HAd ataxia and autistic features	-	-	-	-	negative for 64 other EE causing gene mutations., or genes associated with epilepsy in neurodevelopmental disorders	**Method 1:** Exome sequencing **Description of genotyping method:** High-throughput targeted sequencing of 65 candidate genes in 500 EIEE cases, using MIP technology	Score	**0.1** (0.5)	Because this variant is inherited from a mosaic father it was classified as non-LOF, which requires variant evidence for default points. In the absence of variant evidence the score is for VUS
T2657	Proband with other variant type with some evidence of gene impact	NM_014191.3(SCN8A):c.1984C>T (p.Arg662Cys)	Carvill GL, et al., **2013**, PMID: 23708187	Male			**free text:** no information other than having EIEE was listed. Subjects had refractory seizures and congnitive slowing or regression associated with frequent, onging epiletoform activity.	-	-	-	-	negative for 64 other EE causing gene mutations., or genes associated with epilepsy in neurodevelopmental disorders	**Method 1:** Exome sequencing **Description of genotyping method:** High-throughput targeted sequencing of 65 candidate genes in 500 EIEE cases, using MIP technology	Score	**0.1** (0.5)	No variant evidence to support dysfunction due to mutation, therefore classified as VUS.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
T20187	Proband with other variant type with some evidence of gene impact	NM_014191.3(SCN8A):c.5615G>A (p.Arg1872Gln)	Carvill GL, et al., **2013**, PMID: 23708187	Male			**free text:** no information other than having EIEE was listed. Subjects had refractory seizures and congnitive slowing or regression associated with frequent, onging epiletoform activity.	-	-	-	-	negative for 64 other EE causing gene mutations., or genes associated with epilepsy in neurodevelopmental disorders	**Method 1:** Exome sequencing **Description of genotyping method:** High-throughput targeted sequencing of 65 candidate genes in 500 EIEE cases, using MIP technology	Score	**0** (0.5)	Given that there is no variant evidence, and a frequency in gnomAD, I will not score pending expert review. There is a chance the one allele found in the Latino population could be a somatic mosaicism. Variant Evidence: Wagnon 2015, functional data.
Proband-N1768D	Variant is de novo	NM_014191.3(SCN8A):c.5302A>G (p.Asn1768Asp)	Veeramah KR, et al., **2012**, PMID: 22365152	Female	**Age of Diagnosis:** 6 Months		**free text:** At 6 mo presented with unexplained refractory epilepsy, with early-onset, brief (2-10s) generalized seizures. At 4 yo, seizures changed ot epileptic spasms. She also presented with intellectual disability, developmental delay, hypotonia, and motor and balance coordination deficits. Walked independently at 3 yo, and could speak 5-6 words at 4yo. Speech regressed after epliletic spasms commensed. she also developed OCD and repetitive behaviors, which lead to classification of autism (Authors noted no period of normal	-	-	-	-	WGS was performed. Two compound heterozygous mutations in NRP2 and UNC13C wre found. Authors noted functionally relevant, but neither is associated with disease in humans. Per gnomAD NRP2 R334C= 0.001615, R428W= 1.372e-4 (0.0012 Ashkenazi Jews). UNC13C D304E= 0.005833 (0.04178 A-Jewish). V2196A= 0.002491 (0.01237 A-Jewish).	**Method 1:** Whole genome shotgun sequencing; **Method 2:** Sanger sequencing **Description of genotyping method:** Peformed CNV microarray (neg), then WGS on quartet, confirmed mutation by Sanger seq.	Score	**3** (2)	The WGS of the parents (and sibling) confirmed maternity and paternity through looking at common haplotypes, therefore I can increase the varient evidence points. Variant Evidence: The N1768D variant was cloned into the TTX-resistant NAv1.6. The clones was then transfected into the ND7/23 DRG neuron-derived cell line. Electrophysiological recordings were performed, and the N1768D expressing cells showed enhanced neuronal excitability, suggesting GoF mechanism for the mutation in SCN8A. Gave maximum points, but question as authors mentioned the NRP2 and UNC13 mutations being contributory, but all the mutations are

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
							developemnt was observed in the proband). EEG of initial seizures showed no association with illness of fever and occured up to 40-50 times per day. MRI scans from 6 mo-15yo were normal, and PET scan failed to show any focal abnormalities. Proband died at 15yo from SUDEP, autopsy showed pulmonary edema and only mild temporal-lobe subpial gliosis.									too high in the general population (gnomAD) to be considered causitive (and most found in homozygous state).
Proband 1	Variant is de novo	NM_014191.3(SCN8A):c.2300C>T (p.Thr767Ile)	Estacion M, et al., **2014**, PMID: 24874546	Male	**Age of Onset:** 1 Days		**free text:** Exhibited profound developmental delay, intellectual disability and intractable seizures. Myoclonic jerks and sitffness were observed. EEG confirmed hyperekplexia. Seizures began at 2 wo, EEG showed multifocal epileptiform activity, with normal backgroun and normal MRI. Daily tonic and myoclonic seizures continued, with rare tonic-clonic seizures. At 2 yo EEG revealed multifocal epileptiform activity with background slowing, and MRI revealed delayed myelination.	-	-	-	-	negative for GLRA1, GLRB and MeCP2. Metabolic tests were normal.	**Method 1:** Exome sequencing **Description of genotyping method:** WES sequencing	Score	**2.5** (2)	Variant Evidence: Voltage clamp performed in transfected ND7/23 neuron cell line revealed a 2.5-fold reduction in peak current density for the T761I channels vs. WT. A shift in the Boltzman curve for conduction vs voltage to the left was observed in the T761I variant vs. WT, indicating hyperpolarization, while the fast-inactivation fit showed no changes. The 10mV shift in voltage suggests signifcantly increased excitability. Response to depolarization revealed a 3-fiold increase in the T761I over WT for average ramp response, further indicating increased

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
							Longest seizure-free period was two weekd. Rx with levetiracetam, with addition of phenobarbital, and lacosamide (which showed improement). After increased seizure activity oxcarbazepine was added. At 3 yo, patient is minimally responsive with no obvious dysmorphic features. Profound central hypotonia with some spasticity in the extremities. Patient does not roll over, has complete head lag, and does not fixate or track. Feeding is by gastric tube. Multiple hospital admissions for pneumonia.									channel activity. (Suggesting GOF mutation) Current clamp recording were performed in neonatla rat hippocampal neurons transfected to express T761I and WT SCN8A constructs. Cells expressing the T761I variant exihibt increased spontaneuous activity, suggestive of enhanced channel function. The threshold for the first action potential in T761I expressing cells was 25pA vs 64pA in WT, almost half the threshold. Due to variant evidence provided, I will increase to 2.5 points.
Patient E	Variant is de novo	NM_014191.3(SCN8A):c.2668G>A (p.Ala890Thr)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 9 Months		**free text:** First seizure was nocturanl bilateral convulsive with cyanosis. No other seizure types oted. Seizure-free for 6 months on valporic acid and oxcarbazepine. Development delayed prior to seizure onset, continued delayed post seizures. Moderate ID, speaks single words, moderate hypotonia, ADHD.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient G	Variant is de novo	NM_014191.3(SCN8A):c.5614C>T (p.Arg1872Trp)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 4 Months		**free text:** First seizure was tonic with status epilepticus and cyanosis. Other seizure types include generalized tonic-cloni with Status epilepticus, tonic with cyanosis, absence with myoclonic jerks, and epileptic spasms. Development noted as normal prior to seizures and delayed post seizure onset. Severe ID, no speech, loss of eye contact, hypertonia, generalized hyperreflexia, wheelchair bound. Normal MRI at seizure onset and followup. Dx with EIEE. Died at 5yo from SUDEP.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient L	Variant is de novo	NM_014191.3(SCN8A):c.1228G>C (p.Val410Leu)	Larsen J, et al., **2015**, PMID: 25568300	Male	**Age of Onset:** 4 Months		**free text:** First seizure was epileptic spasms, and has continued to just be this tpe. Delayed development noted prior to seizures, with ocntinued delayed post seizure onset. Noted with Severe ID. MRI at 5mo showed delayed myelination, residual superficial hemosiderin with frontal predominance. No speech, loss of eye contact, severe hypotonia, secondary microcephaly.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	
Patient N	Variant is de novo	NM_014191.3(SCN8A):c.4948G>A (p.Ala1650Thr)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 3 Months		**free text:** First Seizure focal clonic evolving to bilateral convulsive. Other seizure types include statue epilepticus, asymmetric tonic, and atypical absence. Delayed development noted prior to seizure onset, continued delay post seizures onset. Severe ID, quadriparesis with dystonic posturing, dystonic-dyskinateic movement disorder, hypotonia, wheelchair bound.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient P	Variant is de novo	NM_014191.3(SCN8A):c.4774G>C (p.Val1592Leu)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 5 Months		**free text:** First seizure focal with eye deviation, lip smacking, apnea, perioral cyanosis. Seizure-free for 6 weeks on oxcarbazepine. Normal development prior to seizure, delayed post seizure onset. No mention on intellet, probably due to young age. MRI at 5mo showed mild diffuse white matter volume loss with prominent frontal arachnoid spaces. Noted to have hypotinia.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	
Patient Q	Variant is de novo	NM_014191.3(SCN8A):c.5614C>T (p.Arg1872Trp)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 1 Months		**free text:** First seizure prolonged tonic. Other seizure types include focal evolving to bilateral convulsive, myoclonic, tonic-clonic, statue epilepticus, nonconvulsive statue epilepticus. Development noted as normal prior to seizure onset, delayed with regression post seizure. SEvere ID, dystonia, intention tremor with some spaticity. Normal MRI.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient A	Variant is de novo	NM_014191.3(SCN8A):c.4850G>A (p.Arg1617Gln)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 5 Months		**free text:** At seizure onset had a generalized tonic-clonic seizure during afebrile gastroenteritis. Other seizure types noted are clonic, tonic, atypical absence, and myoclinic. Noted as normal development before seizure onset, with delayed and regression in developemnt post seizure. Severe ID. Normal MRI at onset. No speech, loss of eye contact from 30mo, hypotonia, dystonia, wheelchair-bound. Death at 3yo, from seizure (SUDEP?).	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	
Patient F	Variant is de novo	NM_014191.3(SCN8A):c.4435A>G (p.Ile1479Val)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 1 Days		**free text:** First seizure was nocturanl myoclonic. Other seizure types noted status epilepticus, perioral cyanosis, and tonic. No information on develoment prior to seizures due to onset at 1 day old. Development was delayed, moderate ID, hypotonic, movement disorder. MRI at 2.5mo showed circumscript hypoplasia or corpus callosum.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient B	Variant is de novo	NM_014191.3(SCN8A):c.2879T>A (p.Val960Asp)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 2 Months		**free text:** First seizure was focal clonic. Other seizure types include, clonic, generalized tonic-clonic, atonic, eplipetic spasms, and absence seizures. Tonic, and Focal clonic evolved to bilaterl convulsive seizures. Normal development prior to seizure, but delayed development with regression noted post seizure onset. Severe ID, no speech, loss of eye contact from 2yo on, hypotonia, dystonic cerebral palsy, sterotypies, wheel-chair bound. Normal MRI at onset but at 5yo showed diffuse atrophy.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	
Patient I	Variant is de novo	NM_014191.3(SCN8A):c.779T>C (p.Phe260Ser)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 4 Months		**free text:** First seizure was clusters of generalized tonic-clonic. Other seizure types include tonic, focal dyscognitive, focal myoclonic. Development normal prior to sezures and delayed with regression post seizure onset. Moderate ID, speaks short sentences, ataxic gait. MRI normal at onset and followup.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient C	Variant is de novo	NM_014191.3(SCN8A):c.5401C>G (p.Gln1801Glu)	Larsen J, et al., **2015**, PMID: 25568300	Male	**Age of Onset:** 3 Months		**free text:** First seizure(s) focal, generalized tonic-clonic, epileptic spasms with eye deviation. Other seizures types noted focal, tonic, generalized tonic clonic preceded by apnea and deep cyanosis, focal clinoc evolving to bilateral convulsive, generalized, and status epilepticus. Noted as normal developement prior to seizures, but delayed with regression post seizure onset. Severe ID, no speech, loss of eye contact, generalized hypotonia, fatigablemuscle weakness and ptosis, dyskinesia, sterotypic and movements, not sitting, Autistic features. Dx at clinical was Dravt-like. MRI normal at onset, at 15mo showed cerebral atrophy.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient M	Variant is de novo	NP_055006.1(SCN8A):p.Pro1428_Lys1473del	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 10 Months		**free text:** First Seizure clonic alternating. Other seizure types include bilateral convulsive with tonic posture and/or vomiting. Delayed development noted prior to seizure onset, with continued dealy post onset. Severe ID, no speech, loss of eye contact, severe extrapyramidal movement disorder, severe dystrophy, gastroparesis, microcephaly.MRI at 17mo showed normal. Dx with EIEE	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**2** (2)	
Patient D	Variant is de novo	NM_014191.3(SCN8A):c.5615G>A (p.Arg1872Gln)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 7 Months		**free text:** No information other than having EIEE was listed. Subjects had refractory seizures and congnitive slowing or regression associated with frequent, onging epiletoform activity. Noted that development was normal prior to seizures, and delayed post seizure onset. Moderate ID, repetitive language, macroencephaly, generalized hyperreflexia, clumsiness, autisitc features.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**0** (2)	All parents were tested per the materials and methods section. Some patients had maternity and paternity confirmed, but the specific patients with this were not noted. Because this is in ExAC I worry if this is a pentrant variant. This invididuals parents were negative, but nothing was noted of paternity or maternity for this patient. It is in a hotspot AA position, so likely pathogenic, but without previous testing, I will default to experts for scoring.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient O	Variant is de novo	NM_014191.3(SCN8A):c.643A>G (p.Asn215Arg)	Larsen J, et al., **2015**, PMID: 25568300	Female	**Age of Onset:** 6 Months		**free text:** First Seizure epileptic spasms. Other seizures types include episodes of prolonged staring. Seizure free for 3 years with no treatment. Development noted as normal prior to seizure onset and delayed post seizures. Severe ID, no speech, loss of eye contact, hand stereotypies similar to Rett syndrome but superimposed by general chorea involving arms, legs, and body, wheel-chair boudn. MRI in infancy noted as normal. Dx with Rett-like syndrome.	-	-	-	-		**Method 1:** Sanger sequencing **Description of genotyping method:** MIP capture of SCN8A and/or Sanger sequencing of SCN8A exons	Score	**1** (2)	All parents were tested per the materials and methods section. Some patients had maternity and paternity confirmed, but the specific patients with this were not noted. Dx with Rett-like syndrome, and seizure-free for 3 years with no treatment, which seems out of line with the SCN8A phenotype alone. I will reduce points pending expert review.
Proband 1	Variant is de novo	NM_014191.3(SCN8A):c.3979A>G (p.Ile1327Val)	Vaher U, et al., **2014**, PMID: 24352161	Male	**Age of Diagnosis:** 1 Days		**free text:** Born at term by emergency C-section due to fetal hypoxia. Birth weight 4575g and Apgar scores 5/5/6. Seizures present immediately after birth. Intubated and therapeutical hypothermia in general anesthesia for 72 hours was applied, but seizures continued. Once removed from anesthesia, coarse tremor, myoclonias and voice expression	-	-	-	-	Metabolic tests were normal. GM1- and GM2-gangliosidosis, sailidosis, galatcosialidosis, neuronal ceriod lipofuscinosis-1, and neuronal ceriod lipofuscinosis-2 were excluded by enzymatic analysis. Neuronal transmitter, amino acid and pipecolic acid analysis of CSF were normal. Negative for SLC2A1, POLG, AGCI, and mitochondrial DNA sequncing results.	**Method 1:** Exome sequencing **Description of genotyping method:** trio-WES was performed.	Score	**0** (2)	No variant evidence to support this missense mutation, so categorized as a VUS. Furthermore, given the dysmorphic features and alternate diagnosis (muscle pathology) from other patients, and a half-sister with epilepsythere is a possiblity of a modifying or combinatorial mutation in the family (maternally), I will reduce points to VUS for this variant pending expert review.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
							appeared in addition to seizures. Seizures were generalized tonic with apnea and bradycardia or focal tonic wtih eye squeezing and mouthing in series up to 60 times per day.Seizures were refractory to treatment. He had central hypotonia with stiffness. No active moevement or head control, eye contact occasional. Dysmorphic facial appearance (high frontal hairline, micrognathia, dysmorphic ears, full cheeks), congenital multiple arthrogryposis, hop dysplasia, inguinal hernia, and hydrocele were present. Kidney stones were Dx at 7 mo. Patient died at 1 year 5mo due to progressive respiratory failure during respiratory illness. Autopsy refused.MRI's were normal at 9 days old and 1.5 months, but at 5 mo nad 11 mo, mild frontotemporal atrophy and thinning of the corpus callosum, and delay in myelination were described. At 1.75 months, transitory hypogycorrhacia was seen. EM of									

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
							muscle biopsy showed features of muscle pathology, damaged hyperchromophilic fibers w/ increased lipd deposits and lysosomal enzymes, abnormal mitochondria with loss of inner cristae and inflammation.									
														Total points:	**30.70**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Expression Functional	**Expression** A	Plummer NW, et al., **1998**, PMID: 9828131	Showed the expression of mouse Nav1.6 is high in the brain and spinal cord and extremely low to absent in the heart. Lorincz 2010: Showed imm8unolocalization of Nav1.6 to the AIS in neurons and well as immunogold of Nav1.6/ SCN8A localizing it to the nodes of Ranvier and AIS on neurons. Wagnon et al., 2015: shows expression of the mutant N1768D in brains of the knockin mice.	Score	**2** (0.5)	Given the restricted expression in neurons, and the multiple lines of evidence, assigning max points of 2
FA Non-patient Cells	**Functional Alteration** Non-patient cells	Veeramah KR, et al., **2012**, PMID: 22365152	Hippocampal neurons (rat) transfected with mutant Nav1.6 exhibited increased spontaneous firing and increased frequency of action potentials	Score	**0.5** (0.5)	

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Animal Model	**Model Systems** Non-human model organism	Wagnon JL, et al., **2015**, PMID: 25227913	Generated genetic locus specific knockin mice of the EIEE patient derived mutaiton N1768D, using TALON technology. These mice express the mutation in a heterozygous state, and are not a transgenic overxpression. The heterozygous mice develop seizures after 3 months of age, and eventually die from SUDEP at 14 weeks (on average). The mice also display deficits in the accelrating rotarod compared to WT mice, indicative of motor dysfunction as seen in patient with EIEE. The homozygous N1768D mice show an enhance/ accelerated rate of seizure onset and death, indicating a dosage dependent outcome. Lopez-Santiago et al., 2017 showed: Dissociated hippocampal neurons from mutant mice exhibited increased persistent current; recordings from brain slices from mutant mice revealed early after-depolarizations and spontaneous firing in hippocampal neurons	Score	**4** (2)	no changes to open field, sociability or fear conditioning were observed. Given that the mice express a patient derived mutation and fully recapitulate the disease, I will give full points for the model.
Function PI	**Protein Interactions** physical association (MI:0915)	Wagnon JL, et al., **2016**, PMID: 26900580	C-terminus of Nav1.6 interacted with Navb1 (Scn1b)	Score	**0.5** (0.5)	
				Total points:	**7.00**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

SCN9A – epilepsy – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Limited
Modified classification: No Modification
Reason for modified classification: None

Classification status: APPROVED
Date classification saved: 2018 Jun 15, 1:23 pm
Replication Over Time: No
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: epilepsy

Evidence Summary

The SCN9A gene has been associated with autosomal dominant epilepsy (MONDO:0005027) using the ClinGen Clinical Validity Framework as of 6/5/2018. This association was made using case-level data only. At least 4 missense variants have been reported in humans. SCN9A was first associated with this disease in humans as early as 2009 (Singh et al.). Association is seen in at least 4 probands in 2 publications (Singh et al. 2009, Mulley et al. 2013). Variants in this gene segregated with disease in 21 additional family members. This gene-disease association is weakly supported by a mouse model with a homozygous p.Asn641Tyr variant knock-in. In summary, there is limited evidence to support this gene-disease association. Although more evidence is needed to support a causal role, no convincing evidence has emerged that contradicts the gene-disease association.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	10	0.8	0.8
				Proband with predicted or proven null variant	1	0	0
				Variant is *de novo*	0	0	0
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			1	3 (8.1*)	3
	Case-Control				0	0	0
	Genetic Evidence Total						3.8
Experimental Evidence	Functional			Biochemical Functions	0	0	0
				Protein Interactions	0	0	
				Expression	0	0	
	Functional Alteration			Patient cells	0	0	0
				Non-patient cells	0	0	
	Models			Non-human model organism	2	0.5	0.5
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						0.5
Total Points							4.3

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
34351	Proband with other variant type with some evidence of gene impact	NM_002977.3(SCN9A):c.184A>G (p.Ile62Val)	Singh NA, et al., **2009**, PMID: 19763161	Unknown		Hispanic or Latino	**HPO term(s):** Febrile seizures	-	-	-	-		**Description of genotyping method:** Also assessed for SCN1A variants	Score	**0.1** (0.5)	Not found in 276 ethnically matched Hispanic controls. Present in 2/33582 Latino alleles in gnomAD. Downgraded because there is no family evidence or strong computational prediction of pathogenicity.
33418	Proband with other variant type with some evidence of gene impact	NM_002977.3(SCN9A):c.2215A>G (p.Ile739Val)	Singh NA, et al., **2009**, PMID: 19763161	Unknown		Not Hispanic or Latino	**HPO term(s):** Febrile seizures **free text:** idiopathic generalized epilepsy	-	-	-	-		**Description of genotyping method:** Tested for SCN9A and SCN1A variants	Score	**0** (0.5)	Variant is present in 360/104402 (0.3%) European (non-Finnish) alleles and 2 homozygotes in gnomAD.
40095	Proband with other variant type with some evidence of gene impact	NM_002977.3(SCN9A):c.446C>A (p.Pro149Gln)	Singh NA, et al., **2009**, PMID: 19763161	Unknown		Not Hispanic or Latino	**HPO term(s):** Febrile seizures	-	-	-	-		**Description of genotyping method:** Tested for SCN9A and SCN1A variants	Score	**0.1** (0.5)	Variant is absent from gnomAD and absent from 562 ethnically matched Caucasian controls. Downgraded because there is no family evidence or strong computational prediction of pathogenicity.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
EPD279.1	Proband with other variant type with some evidence of gene impact	NM_002977.3(SCN9A):c.1469G>A (p.Ser490Asn)	Singh NA, et al., **2009**, PMID: 19763161	Unknown		Not Hispanic or Latino	**HPO term(s):** Febrile seizures	-	-	-	-		**Description of genotyping method:** Tested for SCN9A and SCN1A variants	Score	**0** (0.5)	Not scored because the variant is present in 4903/276958 total alleles, as well as 226 homozygotes, in gnomAD
34447	Proband with other variant type with some evidence of gene impact	NM_002977.3(SCN9A):c.1964A>G (p.Lys655Arg)	Singh NA, et al., **2009**, PMID: 19763161	Unknown		Not Hispanic or Latino	**HPO term(s):** Febrile seizures **free text:** generalized spike wave, idiopathic generalized epilepsy	-	-	-	-		**Description of genotyping method:** Tested for SCN9A and SCN1A variants	Score	**0** (0.5)	Not scored because the variant is present in 0.3% (356/125902) European (non-Finnish) alleles and 1 homozygote in gnomAD, as well as 0.3% (101/34262) Latino alleles in gnomAD.
K4425 Proband	Proband with other variant type with some evidence of gene impact	NM_002977.3(SCN9A):c.1921A>T (p.Asn641Tyr)	Singh NA, et al., **2009**, PMID: 19763161	Female			**HPO term(s):** Febrile seizures; Focal seizures, afebril	21	13	**Published:** 8.1	Yes		**Method 1:** Genotyping; **Method 2:** Exome sequencing **Description of genotyping method:** Linkage analysis, sequencing of the following genes in the linkage region: SCN1A, SCN2A, SCN3A, SCN7A, SCN9A. KCNH7, SLC4A10	Score	**0.5** (0.5)	

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Mulley Proband	Proband with other variant type with some evidence of gene impact	NC_000002.12:g.166303195G>T (GRCh38)	Mulley JC, et al., **2013**, PMID: 23895530	Unknown			**HPO term(s):** Febrile seizures	2	-	**Calculated:** 0.3	No		**Method 1:** Sanger sequencing **Description of genotyping method:** Screened for SCN1A, SCN9A, GABRG2, PCDH19, and SCN1B	Score	**0.1** (0.5)	Downgraded because the family was only sequenced for 5 genes, little familial and phenotypic information is available, and the variant is present in 1/834 (0.12%) Latino alleles. Note: Discussion prompted over whether there is a minimum number of alleles that should be assessed for a population, for the allele frequency to be convincing evidence.
Allen Proband	Proband with other variant type with some evidence of gene impact	NM_002977.3(SCN9A):c.1964A>G (p.Lys655Arg)	Allen NM, et al., **2016**, PMID: 27504264	Male	**Age of Onset:** 23 Months			-	-	-	-			Score	**0** (0.5)	Not scored because the variant is present in 356/125902 European (non-Finnish) alleles. Proband also has an SCNM1 and CPA6 variants.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Cen Proband	Proband with other variant type with some evidence of gene impact	NM_002977.3(SCN9A):c.29A>G (p.Gln10Arg)	Cen Z, et al., **2017**, PMID: 28704742	Male	**Age of Report:** 6 Years		**HPO term(s):** Febrile seizures; Focal seizures, afebril	3	-	**Calculated:** 0.6	No		**Method 1:** Exome sequencing **Description of genotyping method:** NGS sequencing panel of 480 epilepsy genes	Score	**0** (0.5)	Variant is present in 33/18832 (0.18%) East Asian alleles and 1 homozygote in gnomAD
Case 1 Proband	Proband with other variant type with some evidence of gene impact	NC_000002.12:g.166293358C>T (GRCh38)	Yang C, et al., **2018**, PMID: 29500686	Male	**Age of Onset:** 7 Days		**HPO term(s):** Febrile seizures; Focal seizures, afebril	2	-	**Calculated:** 0.3	No		**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** "Common epilepsy genes were screened." At least sequenced SCN9A and SCN1A	Score	**0** (0.5)	After expert review, this case was not scored. The variant is present in 15/18440 (0.08%) East Asian alleles. The onset of disease, at 7 days in the proband, suggests a more severe phenotype. The paper provides little information on sequencing methods, so there is doubt as to whether this variant is truly causative.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Case 2 Proband	Proband with predicted or proven null variant	NC_000002.12:g.166198901_166198905del (GRCh38)	Yang C, et al., **2018**, PMID: 29500686	Unknown	**Age of Onset:** 3 Years		**HPO term(s):** Focal seizures, afebril; Febrile seizures; Global brain atrophy	2	-	**Calculated:** 0.3	No		**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** Sequencing of at least SCN1A and SCN9A	Score	**0** (1.5)	After expert review this variant was not scored. The variant is present in 14/17248 (0.08%) East Asian alleles. This gene is not constrained for LoF variant according to ExAC (as of 6/5/2018). The sequencing methods are not well defined, so it is possible the causative variant was not sequenced. Additionally, the proband also has mild diffuse brain atrophy, which is not a consistent phenotype with the gene-disease association.
														Total points:	**0.80**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Singh Animal Model	**Model Systems** Non-human model organism	Singh NA, et al., **2009**, PMID: 19763161	knock-in mice were subjected to corneal electrical stimulation using the staircase method to either clonic seizure endpoint or tonic hindlimb extension seizure endpoint that depolarize the forebrain and hindbrain regions, respectively. Homozygous knockin mice has significantly reduced thresholds to minimal clonic and minimal tonic hindlimb extension seizures relative to wildtype mice. Male and female mice has significantly lower CC(50) value compared to het. knockin and wildtype.	Score	**0.5** (2)	This mouse model was downgraded because the researchers had to stress the model (i.e. homozygous knock-in and corneal electrical stimulation) to elicit a response.
Nassar Model	**Model Systems** Non-human model organism	Nassar MA, et al., **2004**, PMID: 15314237	Analyzed pain behavior	Score	**0** (2)	Not scored because they examine pain behavior
				Total points:	**0.50**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

SRPX2 – rolandic epilepsy-speech dyspraxia syndrome – *X-linked inheritance (dominant)*

Classification owner: Epilepsy EP
Calculated classification: No Classification
Modified classification: Disputed
Reason for modified classification:
Limited genetic evidence and an alternative explanation for 1 of the 2 reported families. No replication in additional cases since the initial report in 2006.

Classification status: APPROVED
Date classification saved: 2018 Jul 19, 5:41 pm
Replication Over Time: No
Contradictory Evidence? Proband: **Yes**, Experimental: **No**
Disease: rolandic epilepsy-speech dyspraxia syndrome

Evidence Summary

The genetic evidence in support of this gene-disease association rests on the report of 2 multiplex families from a single paper in 2006. While some evidence (segregation, initial experiments) supports pathogenicity there is conflicting evidence about the significance of the 2 described variants including mean allele frequency in gnomAD, a high dN/dS ratio, and the presence of another putative cause in the family (GRIN2A). Without significant supportive genetic evidence, experimental evidence should not be used to fortify the association.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	1	0	0
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	0	0	0
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						**0**
Experimental Evidence	Functional			Biochemical Functions	0	0	0
				Protein Interactions	0	0	
				Expression	0	0	
	Functional Alteration			Patient cells	0	0	0
				Non-patient cells	0	0	
	Models			Non-human model organism	0	0	0
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	0	0	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						**0**
Total Points							**0**

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
1883		NM_000833.4(GRIN2A):c.2146G>A (p.Ala716Thr)	Lesca G, et al., **2013**, PMID: 23933820					8	-	-	-			Contradicts		Alternative explanation for the original reported proband. Segregation not perfect but gene-disease association for this gene is more robust.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
III.3	Proband with other variant type with some evidence of gene impact	NM_014467.2(SRPX2):c.980A>G (p.Asn327Ser)	Roll P, et al., **2006**, PMID: 16497722	Female	**Age of Report:** 10 Years		**free text:** Delayed language development, rolandic seizures, oro-facial dyspraxia, FSIQ40, MRI normal, PET bilateral perisylvian hypermetabolism	9	-	**Published:** 3.01	No	Linkage to Xq - ~20cM at Xq21-q22, FISH did not detect any large scale deletions, mutation screen identified change which co-segregated with the phenotype.	**Method 1:** Whole genome shotgun sequencing	Review	**0** (0.5)	Deglycosylation with an enzyme reduced the molecular weight of the intracellular vs extracellular fractions and resulted in co-migration with the WT Retention of mutant protein in the ER in CHO, cultured murine cortical neurons, and in patient fibroblasts. Co-localized with ubiquitin in patient fibroblasts suggesting altered intracellular processing and misfolding. While some evidence (segregation, initial experiments) supports pathogenicity there is conflicting evidence about the significance of this variant including mean allele frequency in gnomAD, a high dN/dSratio, and the presence of another putative cause in the family (GRIN2A).

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
T2472-1	Proband with other variant type with some evidence of gene impact	NM_014467.2(SRPX2):c.215A>C (p.Tyr72Ser)	Roll P, et al., **2006**, PMID: 16497722	Male	**Age of Onset:** 12 Years	Not Hispanic or Latino	**HPO term(s):** Perisylvian polymicrogyria; Focal seizures with impairment of consciousness or awareness; Hyperreflexia **free text:** Perisylvian polymicrogyria (bilateral, posterior, L>R), focal seizures, generalized hyperreflexia, delayed walking (19m), FSIQ = 86	-	-	-	-	Karyotype		Score	**0** (0.5)	Questionable non-segregations or incomplete penetrance. MAF= 0.00003747 in gnomAD without homozygotes, but in 4 hemizygotes.
														Total points:	**0.00**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Electroporation of layer V/VI cortical neurons in E12.5 mic	**Model Systems** Non-human model organism	Sia GM, et al., **2013**, PMID: 24179158	The isolation-induced infant pup ultrasonic vocalization task has been used to characterize mouse models of human language, social, and arousal disorders. In addition to this phenotype, cultured transfected neurons showed a decrease in dendritic spine density and a decrease in the miniature excitatory post-synaptic currents (mEPSCs) measured during whole-cell voltage clamp recording.	Review	**0** (2)	Given that there are only 2 affected families reported with one having conflicting evidence, experimental evidence was not scored to prevent skewing of the final category.
In utero Srpx2 silencing	**Rescue** Non-human model organism	Salmi M, et al., **2013**, PMID: 23831613		Review	**2** (2)	While this experimental evidence reported a potential role for this gene in neuronal migration and abnormal firing patterns, it was not included in scoring of this gene-disease association because of limited clinical genetic evidence.
				Total points:	**0.00**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF

✖ Close

Evidence Summary

STXBP1 – infantile epilepsy syndrome – *Autosomal dominant inheritance*

Classification owner: Epilepsy EP
Calculated classification: Definitive
Modified classification: No Modification
Reason for modified classification: None

Classification status: APPROVED
Date classification saved: 2018 Jun 01, 1:09 pm
Replication Over Time: Yes
Contradictory Evidence? Proband: **No**, Experimental: **No**
Disease: infantile epilepsy syndrome

Evidence Summary

Approved by the ClinGen Epilepsy Expert Panel 10/20/2017.

Calculated Classification Matrix

Evidence Type					Count	Total Points	Points Counted
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact	1	0.25	0.25
				Proband with predicted or proven null variant	0	0	0
				Variant is *de novo*	5	14	12
			Autosomal Recessive Disorder	Two variants (not predicted/proven null) with some evidence of gene impact in *trans*	0	0	0
				Two variants in *trans* and at least one *de novo* or a predicted/proven null variant	0	0	
		Segregation			0	0 (0*)	0
	Case-Control				0	0	0
	Genetic Evidence Total						12
Experimental Evidence	Functional			Biochemical Functions	1	1	2
				Protein Interactions	0	0	
				Expression	2	1.5	
	Functional Alteration			Patient cells	1	0.5	1.5
				Non-patient cells	1	1	
	Models			Non-human model organism	1	2	3
				Cell culture model	0	0	
	Rescue			Rescue in human	0	0	
				Rescue in non-human model organism	0	0	
				Rescue in cell culture model	1	1	
				Rescue in patient cells	0	0	
	Experimental Evidence Total						6
Total Points							18

* – Combined LOD Score

Genetic Evidence: Case Level (variants, segregation)

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Subject 3	Proband with other variant type with some evidence of gene impact	NM_003165.3(STXBP1):c.1631G>A (p.Gly544Asp)	Saitsu H, et al., **2008**, PMID: 18469812	Male	**Age of Diagnosis:** 1 Days		**free text:** born at term with asphyxia. First seizure at 4 days old, eye blinking followed by clonic. Movements of the shoulder. Tonic seizure at 10 days old, with suppression burst pattern on EEG. Treated with vitamin B6 and nitrazepan from 2 months old. Severe developmental delay.Walked at 7y.o., speaks only a few words at 37y.o., feeds self. Has mild spastic diplegia.	-	-	-	-		**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** PCR and Sanger of STXBP1 20 exons	Score	**0.25** (0.5)	Note: not a true de novo as the father was not tested due to death, so you cannot rule out potential segregation and penetrance issues. Variant Evidence: N2A cells transiently transfected with G544D variant show aggregation of the variant and irregular distribution compared to WT (Suppl Figure 1). No other testing for gene mutations though, so cannot rule out the potential of mutations in other EIEE genes from deceased father, score down pending expert review.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Subject 6	Variant is de novo	NM_003165.3(STXBP1):c.539G>A (p.Cys180Tyr)	Saitsu H, et al., **2008**, PMID: 18469812	Male	**Age of Diagnosis:** 1 Days		**free text:** Born at term with asphyxia. Seizures started at 3 weeks, tonic seizure with blinking and grimacing, that spontaneously disappeared after two weeks. Myoclonic seizures noted at 2 montsh old, followed by 10se of crying and facial flushing 3-4x/day. At 3 months oldm left extremities were elevated in a cluster of seizures. MRI showed normal structure but delayed myelination. EEG showed supression burst pattern. Hypotonic, profound ID, and severe spastic quadriplegia. Seizures intractable, daily medication.	-	-	-	-		**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** PCR and Sanger of STXBP1 20 exons	Score	**3** (2)	Maternity and paternity confirmed. Variant Evidence: Circular dichroism shows destabilization of the C180Y variant, and it unfolded at a much lower temperature than WT STXBP1 (Figure 4B,C). The C180Y variant had reduced binding to STX1A (syntaxin) compared to WT (Fig. 4D-E). The C180Y variant also showed abnormal clustering when expessed in N2A cells compared to WT (Suppl Fig 1).

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Subject 7	Variant is de novo	NM_003165.3(STXBP1):c.1328T>G (p.Met443Arg)	Saitsu H, et al., **2008**, PMID: 18469812	Female	**Age of Diagnosis:** 1 Days		**free text:** Born at term with asphyxia. Generalized tonic seizures with flexion upper extremities at 6 weeks old. At 1 mo abnormal upward gazing after downward eye movement. Normal brain MRI at 3 months,. EEG showed suppression burst pattern when awake and asleep. At 4mo, EEG showed hypsarrhythmia-like pattern compatible to West Syndrome. I, TRH-temporally effective (hourly injections). Profound developmental delay. o head control or speech at 13 y.o., mild spastic quadriplegia. MRI at 13y.o. showed mild brain atriphy and delayed myelination.	-	-	-	-		**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** PCR and Sanger of STXBP1 20 exons	Score	**2.5** (2)	Maternity and paternity confirmed. Variant Evidence: the M443R variant showed abnormal clustering in N2A cells compared to WT STXBP1 (Suppl Fig 1).

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Subject 11	Variant is de novo	NM_003165.3(STXBP1):c.251T>A (p.Val84Asp)	Saitsu H, et al., **2008**, PMID: 18469812	Male	**Age of Diagnosis:** 1 Days		**free text:** Born at term. Seizures began at 2mo, tonic spasms or generalized tonic clonic convulsions. EEG at 9mo showed suppression burst pattern at first and evolved to hypsarrhythmia compatible with West syndrome. Intractable seizures, requiring daily medication. Continued choreoathetotic involuntary movements since 18mo. Profound ID, no head control, no words, severe hypotonic quadriplegia. Brain MRI at 8yo showed mild atrophy in frontal lobe, no structureal abnormalities.	-	-	-	-		**Method 1:** PCR; **Method 2:** Sanger sequencing **Description of genotyping method:** PCR and Sanger of STXBP1 20 exons	Score	**2.5** (2)	Maternity and paternity confirmed. Variant Evidence: the V84D variant showed abnormal clustering in N2A cells compared to WT STXBP1 (Suppl Fig 1).

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient 1	Variant is de novo	NM_003165.3(STXBP1):c.169+1G>A	Gerber SH, et al., **2008**, PMID: 18703708	Female	**Age of Diagnosis:** 1 Days		**free text:** Born at term to unremarkable pregnancy. First seizure at 6 weeks old, with loss of visual contact, upward gaze deviatino, and clonic facial movements. Treated with phenobarbital to control seizures. EEG showed epileptic activity in right fronto-temporal region with slow spikes in left fronto-temporal region. Slight slowing in the left central region. Medication was stopped after two years of no seizures., remained seizure free for 8 years, then relapsed with 2 generalized seizures. RTx valporic acid, which did not control the seizures. Seizures now accompanied by loss of vision, tremors and axial hypotonia and head drops, and diffuse myoclonia. Now has intractable seizures with at least 3/ day that are generalized tonic seizures.Shows developmental delay, sat by herself at 2 .5 years and walked at 4 years, and hypotonia At 27 years old she has no speech, walks with assistance, and completely dependent.	-	-	-	-	negative for STX1A, VAMP2, SNAP25, and STY1 mutations (authors tested only SNARE components), none associated with EIEE	**Method 1:** Sanger sequencing	Score	**3** (2)	Splice site mutation. Variant Evidence: Figure 2 shows that the mutation at the splice site causes alteration in the coding sequence, and presumbaly a premature stop codon. qRT_PCR of the patients blood shows 50% reduced STXBP1 expression, suggesting LOF mutation.This is a denovo mutation that is LOF, with cofirmed maternity and paternity, so increase points to max.

Label	Variant type	Variant	Reference	Proband sex	Proband age	Proband ethnicity	Proband phenotypes	Segregations				Proband previous testing	Proband methods of detection	Score status	Proband points (default points)	Reason for changed score
								# Aff	# Unaff	LOD score	Counted					
Patient 2	Variant is de novo	NM_003165.3(STXBP1):c.1162C>T (p.Arg388Ter)	Gerber SH, et al., **2008**, PMID: 18703708	Female	**Age of Report:** 15 Years		**free text:** Born at term form an unremarkable pregnancy. Early development characterized by hypotonia, gross and diffuse tremor, and global delay. Sat by herself at 18months, walked at 4 years, severe sialorrhea suggestive of some orofacial dyspraxia. First seizure was at 2.75 years old. EEG showed epileptic focus in the right tempporal region, diffusing to the occipital left region. RTx carbamazepine and seizures controlled for 2 years. Then she had right versive seizures with bilateral frontal slowing lateralized on the left side and left frontal spkies on the EEgG. RTx clobazam and has only had 1-2 seizures every 6 months, which are motor seizures at night. EEG shows slowing, with some splikes and waves in both posterior regions. She is nonverbal, cannot eat by herself, hypotonic with altered gait when walking.	-	-	-	-	negative for STX1A, VAMP2, SNAP25, and STY1 mutations (authors tested only SNARE components), none associated with EIEE. Normal karyotyping and metabolic tests were normal. Negative for UBE3a mutation and methylation changes. Negative for MECP2 mutations.	**Method 1:** Sanger sequencing	Score	**3** (2)	This is a LOF mutation, but de novo with confirmed maternity and paternity, so increase points to max. This is a LOF mutation, but de novo with confirmed maternity and paternity, so increase points to max.
														Total points:	**14.25**	

Genetic Evidence: Case Level (family segregation information without proband data or scored proband data)

No segregation evidence for a Family without a proband was found.

Genetic Evidence: Case-Control

No scored Case-Control evidence was found.

Experimental Evidence

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
STXBP1 binding to STX1A	**Biochemical Function** B	Pevsner J, et al., **1994**, PMID: 8108429	Show that STXBP1 and STX1A (syntaxin) bind at a 1:1 ratio (glycerol gradients, Fig 4)). They confirmed the binding with in vitro binding assays (Fig 5) , and found that STXBP1 also bound STX2, STX3 but not STX4.	Score	**1** (0.5)	Note: placed in biochemical as this interaction is required for STXBP1 function, but ATX1A is not a EIEE gene, therefore we cannot count as protein interactions per the SOP. Plus several other papers show this association as being important including the Saitsu et al., 2008, so can give increased points.
rSTXBP1 developmental expression	**Expression** A	Pevsner J, et al., **1994**, PMID: 8108429	cloned and characterized the expression of ratSTXBP1 (n-Sec1) in developeing rat tissue. Northrn Blot dot blot analysis showed high STXBP1 expression in the brain and spinal cord, 100x more than in other tissues. STXBP1 was shown to express in other tissues (including heart, spleen, lung, liver, muscle, kidney, and testis) but only after long overexposure of the autoradiogram. They also showed temporal expression of STXBP1 in the developing rat brain. Low levels of STXBP1 are seen at E16 and increase to a maximum level at P7, concomitant with synapse formation. Also performed western blot to show restricted expression of SSTXBP1 in the brain (regions include cerebellum, thalamus, hippocampus, corpus callosum, cerebral cortex, olfactory bulb).	Score	**1** (0.5)	Functional Expression
STXBP1 human expression	**Expression** B	Swanson DA, et al., **1998**, PMID: 9545644	Northern blot analysis of human tissue shows expression of STXBP1 preferentially in the brain, with low levels in other tissues for the "short" isoform (Fig. 1). ID's alternatively spliced "longer" isoform of STXBP1 that has restricted expression in the brain (cerebellum) and retina (Fig. 2).	Score	**0.5** (0.5)	Also mapped STXBP1 to chormosome 9q34.1 by FISH.

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
human iPSCs EIEE model	**Functional Alteration** Patient cells	Yamashita S, et al., **2016**, PMID: 26918652	Generated human STXBP1-R367X iPSC-derived neurons. Karyotyping of iPSCs from patient and control were normal. Both control and R367X iPSCs differentiated into neruons similarly, and expressed the appropriate markers. qRT-PCR and western blot showed 50% reductino of STXBP1 levels in the R367X iPSC-derived neurons compared to the controls (Fig 1D). Interestingly, qRT-PCr analysis showed increased expression of GRIN2A, GRIN2Bm and NKCC1 in the R367X cells compared to controls.WEstern blot showed reduced expression of STX1A in the R367X cells, but IHC showed that the localization of STX1A had changed, and was forming aggregates in the cytoplasm and not at the cell membrane as in controls. STXBP1-R367X cells also showed reduced neurite extension compared to controls. Note: iPSCs derived from the patients father were used as controls. Patient was male (Japanese), presented with focal motor seizures in neonatla perios and eplileptic spasms arain MRI showed hypoplasia of corpus callosum and EEG showed suppression burst pattern. STXBP1 c.1009C>T, p. R367X variant. RTx phenobarbital, ACTH, zonisamide, valporate, and clobazam (ineffective). RTx levetiracetam, seizures stopped, has profound psychomotor retardation.	Score	**0.5** (1)	The reduction in STX1A levels and mislocalization is to be expected, and the change in neurite extension is new, but it would have been nice to show changes in synpatic transmission in these cells, scored down to 0.5
FA Np Cells	**Functional Alteration** Non-patient cells	Patzke C, et al., **2015**, PMID: 26280581	Shows STXBP1 expression is required for neuronal survival. No changes to neuron shape, dendritic aborization, or synapse numbers in STXBP1 het vs. WT cells. Dendritic length and branching were unchanged (opposite effect then what was seen in Yamashita 2016 in patient cells). No changes to the number or size of synapses per dendritic segment were observed.	Score	**1** (0.5)	increased points due to amount of data shown and tested.
Animal Model	**Model Systems** Non-human model organism	Verhage M, et al., **2000**, PMID: 10657302	Generated conventional STXBP1 knockout mice replacing 5 exons (encoding AA 14-144) with a neomycin selection cassette through homologous recombination. Homozygous null STXBP1 mice are completely paralyzed, are born at term but die immediately after birth (respiration problems). STXBP1 null mice lack neurotransmission in E18 neocortical slices. Synaptic events were absent from E15-E18 diaphraghm neuromuscular junctions in STXBP1. nu,, mice compared to WT. Interestingly, brain structure was intact, with no diffreence observed between STXBP1 null and WT mice, but massive cell death was observed in the brains of STXBP1 null mice at E14.5 and progressing to E18.5, and was attributed to apoptosis (TUNEL+ apoptotic bodies present). Northern and Western blot confirm loss of STXBP1 expression in homozygous null mice.	Score	**2** (2)	Unfortunately, they did not study the heterozygus mice in this paper. Twenty three other papers reference this mouse model.

Label	Experimental category	Reference	Explanation	Score status	Points (default points)	Reason for changed score
Rescue in Engineered Equivalent	**Rescue** Cell culture model	Patzke C, et al., **2015**, PMID: 26280581	Co-expressing STXBP1 that is controlled by light allows for rescue of STXBP1 expression. The STXBP1 neurons exhibited ~2-fold decrease in EPSC amplitude. RE-expression of WT-STXBP1 in the het cells 5 days before stimulation completely rescued the reduced EPSC amplitude, suggesting that STXBP1 loss in neurons results in a presynaptic impairment. No changes in coeeficient of variation and Ca2+-triggered release were observed between STBP1 het and WT iNeurons.	Score	**1** (1)	Conclusion: STXBP1 mutation causes ~2 fold decrease in synpatic strength in excitatory neurons.
				Total points:	**7.00**	

Biochemical Function: The gene product performs a biochemical function shared with other known genes in the disease of interest (A), OR the gene product is consistent with the observed phenotype(s) (B)
Protein Interactions: The gene product interacts with proteins previously implicated (genetically or biochemically) in the disease of interest
Expression: The gene is expressed in tissues relevant to the disease of interest (A), OR the gene is altered in expression in patients who have the disease (B)
Functional Alteration of gene/gene product: The gene and/or gene product function is demonstrably altered in cultured patient or non-patient cells carrying candidate variant(s)
Model Systems: Non-human model organism OR cell culture model with a similarly disrupted copy of the affected gene shows a phenotype consistent with human disease state
Rescue: The phenotype in humans, non-human model organisms, cell culture models, or patient cells can be rescued by exogenous wild-type gene or gene product

For best printing, choose "Landscape" for layout, 50% for Scale, "Minimum" for Margins, and select "Background graphics".

Close Print PDF