



# Calcium Channel Dysfunction in Epilepsy: Gain of *CACNA1E*

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## De Novo Pathogenic Variants in *CACNA1E* Cause Developmental and Epileptic Encephalopathy With Contractures, Macrocephaly, and Dyskinesias

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Developmental and epileptic encephalopathies (DEEs) are severe neurodevelopmental disorders often beginning in infancy or early childhood that are characterized by intractable seizures, abundant epileptiform activity on electroencephalogram (EEG), and developmental impairment or regression. *CACNA1E* is highly expressed in the central nervous system and encodes the  $\alpha_1$ -subunit of the voltage-gated  $\text{Ca}_v2.3$  channel, which conducts high-voltage-activated R-type calcium currents that initiate synaptic transmission. Using next-generation sequencing techniques, we identified de novo *CACNA1E* variants in 30 individuals with DEE, characterized by refractory infantile-onset seizures, severe hypotonia, and profound developmental impairment, often with congenital contractures, macrocephaly, hyperkinetic movement disorders, and early death. Most of the 14, partially recurring, variants cluster within the cytoplasmic ends of all 4 S6 segments, which form the presumed  $\text{Ca}_v2.3$  channel activation gate. Functional analysis of several S6 variants revealed consistent gain-of-function effects comprising facilitated voltage-dependent activation and slowed inactivation. Another variant located in the domain II S4-S5 linker results in facilitated activation and increased current density. Five participants achieved seizure freedom on the antiepileptic drug topiramate, which blocks R-type calcium channels. We establish pathogenic variants in *CACNA1E* as a cause of DEEs and suggest facilitated R-type calcium currents as a disease mechanism for human epilepsy and developmental disorders.

## Commentary

The voltage-gated calcium channels (VGCCs) control the influx of calcium ions in response to membrane depolarization and regulate intracellular signaling and gene expression in the mammalian central nervous system (CNS). The  $\alpha$  subunits are the primary pore-forming subunits of the calcium channel and are encoded by 10 genes (Table 1). These subunits are made up of 4 homologous domains (DI-DIV) with 6 transmembrane domains each, where the S5 and S6 transmembrane segments line the inner pore of the channel. These genes have cell- and tissue-specific expression patterns and many are implicated in human diseases, including epilepsy (*CACNA1A*, *CACNA1D*, *CACNA1G*).<sup>3,5,11</sup>

Previously, *CACNA1E* was identified as a candidate gene for neurodevelopmental disorders in a meta-analysis of exome sequencing data from 6753 individuals.<sup>16</sup> *CACNA1E* encodes  $\text{Ca}_v2.3$ , an R-type VGCC implicated in both presynaptic neurotransmitter release and postsynaptic somatodendritic integration and long-term potentiation.  $\text{Ca}_v2.3$  is also known to play a role in epileptogenesis in rodents, and its deletion reduces susceptibility to chemically induced seizures.<sup>17,18</sup> In a recent study, Helbig et al present de novo gain-of-function missense

variants in *CACNA1E* as a cause of a developmental and epileptic encephalopathy (DEE) in 30 individuals. Developmental and epileptic encephalopathies are a group of severe epilepsies characterized by refractory seizures and developmental impairment; de novo disease-causing variants are the primary genetic contributors to DEE. The 30 patients with pathogenic *CACNA1E* variants presented with a variable DEE characterized by refractory epilepsy (87% of patients) with median seizure onset at 4.5 months, movement disorders (60%), spastic quadriplegia (53%), congenital joint contractures (43%), macrocephaly (43%), and profound developmental impairments (ie, nonverbal and nonambulatory) (88%).

Helbig and colleagues identified 14 missense variants in 30 individuals by gene panel, exome or genome sequencing through a large international collaboration. The overwhelming majority of missense variants were identified in the S6 helices that form the channel's inner pore. Several interesting genotype-phenotype correlations also emerged based on which of the 4 domains (DI-DIV) variants were located in. Ten individuals carried missense variants in the DI-S6 domain, including 9 individuals with the recurrent p.Gly352Arg variant; all 10 individuals presented with hyperkinetic movement disorders,



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**Table 1.** The Calcium Channel  $\alpha$  Subunits in Human Disease.

Type	Subunit	Gene	Human Disease (Inheritance)	Epilepsy	Pathogenic Mechanism in Epilepsy
L-type	Ca <sub>v</sub> 1.1	<i>CACNA1S</i>	Hypokalemic periodic paralysis (AD, de novo) <sup>1</sup>	N	Gain of function <sup>3</sup>
	Ca <sub>v</sub> 1.2	<i>CACNA1C</i>	Timothy syndrome (de novo, AD) <sup>2</sup>	N (seizures very rare)	
	Ca <sub>v</sub> 1.3	<i>CACNA1D</i>	Autism, ID, epilepsy (de novo) <sup>3</sup>	Y	
	Ca <sub>v</sub> 1.4	<i>CACNA1F</i>	Congenital stationary night blindness (XLR) <sup>4</sup>	N	
P/Q-type	Ca <sub>v</sub> 2.1	<i>CACNA1A</i>	DEE <sup>5</sup> , episodic ataxia, <sup>6</sup> hemiplegic migraine <sup>7</sup> (de novo, AD)	Y	Haploinsufficiency <sup>8</sup>
N-type	Ca <sub>v</sub> 2.2	<i>CACNA1B</i>	Dystonia (disputed) <sup>9,10</sup>	N	
R-type	Ca <sub>v</sub> 2.3	<i>CACNA1E</i>	DEE (de novo)	Y	Gain of function
T-type	Ca <sub>v</sub> 3.1	<i>CACNA1G</i>	Cerebellar atrophy with epilepsy (de novo) <sup>11</sup> , GGE (complex) <sup>12</sup>	Y	Gain of function <sup>11</sup>
	Ca <sub>v</sub> 3.2	<i>CACNA1H</i>	Epilepsy (disputed) <sup>13,14</sup>	?	
	Ca <sub>v</sub> 3.3	<i>CACNA1I</i>	Schizophrenia (candidate) <sup>15</sup>	N	

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; DEE, developmental and epileptic encephalopathy; GGE, genetic generalized epilepsy; ID, intellectual disability; N, no; XLR, X-linked recessive; Y, yes.

compared with only (2/19) individuals with variants outside this domain. In the DII-S6 domain, 13 individuals carried missense variants, including 6 with the p.Ala702Thr variant. The majority of these patients presented with all clinical features of the *CACNA1E*-associated DEE. In contrast, patients with missense variants located in the DIII-S6 presented with a milder phenotype, as 2 patients never developed seizures and one has been seizure-free for 5 years, spoke single words, and walked independently. A single individual with the full *CACNA1E*-associated DEE was identified with a missense variant in DIV-S6. Although validation of these genotype–phenotype correlations in a larger cohort are needed, they may provide novel insights into channel function. Future studies in patient-derived induced pluripotent stem cells are likely to shed light on pathogenic mechanisms and potential therapeutic targets.

As a first step toward assessing the effect of these missense variants on channel function, 3 of the DII-S6 variants, associated with the full spectrum of clinical features of *CACNA1A*-associated DEE, were transiently transfected into a heterologous expression system (tsA201). Electrophysiological recordings showed a hyperpolarizing shift in voltage-dependent activation, slowed kinetics of inactivation, and increased current density. These findings are consistent with a gain-of-function mechanism akin to de novo pathogenic *CACNA1D* and *CACNA1G* variants in other individuals with epilepsy.<sup>3,11</sup> Moreover, the overwhelming majority of pathogenic de novo variants in epilepsy-associated VGCCs (*CACNA1A*, *CACNA1D*, *CACNA1G*) are missense variants located in the S6 segments of the transmembrane domains. All 4 genes are also highly intolerant to missense variation with  $z$ -scores which place them in the first percentile (*CACNA1A*: 7.23, *CACNA1D*: 5.57, *CACNA1G*: 4.97, *CACNA1E*: 6.61).<sup>19</sup> Collectively, these studies highlight the preponderance of missense VGCC variants in patients with epilepsy, and that, to date, gain-of-function is the likely prevailing pathogenic mechanism that underpins severe VGCC-associated epilepsy.

The role of loss-of-VGCC-function in epilepsy is less well established. In the report by Helbig et al, an additional 3 individuals with truncating *CACNA1E* variants were identified,

including 1 somatic mosaic (27% of cells), 1 inherited from an unaffected parent, and 1 with unknown inheritance. All 3 individuals presented with a much milder phenotype including epilepsy in 2 of 3 individuals as well as mild developmental delays, though all 3 could walk and had single words. Although patients with episodic ataxia and epilepsy carry loss-of-function (truncating) *CACNA1A* variants, reports of truncations in the other epilepsy-associated VGCCs are rare and further evidence is needed to establish the role of loss-of-function variants in VGCC-associated epilepsy, including *CACNA1E*. It should be noted, however, that there are examples of other ion channels implicated in DEE (eg, *KCNQ2*, *SCN2A*) that cause seizures by both loss and gain of function mechanisms, with some preliminary evidence of genotype–phenotype correlations.<sup>20,21</sup> For instance, individuals with loss of function *KCNQ2* variants present with benign familial neonatal epilepsy where seizures generally resolve within the first few months of life, while missense variants are associated with DEE with neonatal onset, refractory seizures, and severe to moderate ID. These loss-of-function variants result in reduction of potassium current up to 50%, while both dominant-negative and gain-of-function effects have been reported for DEE-associated variants.<sup>20</sup> As genetic testing becomes more widespread, in particular in milder forms of neurodevelopmental disorders with and without epilepsy, similar genotype–phenotype correlations will likely begin to emerge. Large studies, first in heterologous systems, complemented by patient-derived induced pluripotent stem cell (iPSC) studies are needed to understand how genetic variants give rise to specific phenotypes.

To date, gene discovery in epilepsy has been dominated by the identification of disease genes characterized by haploinsufficiency, mostly due to the relative ease with which truncating variants can be interpreted. This study, as well as similar studies in *SCN8A*, highlight the role of recurrent missense variants in this condition. This discovery is being rapidly facilitated by the increased numbers of patients undergoing genetic testing, particularly within the clinical setting. Indeed, many of the patients identified in the Helbig study were identified through clinical

genetic testing (11/30) and the use of matchmaker exchange networks such as Genematcher.<sup>22</sup> Greater awareness in the clinical setting of matchmaker exchange networks will accelerate gene discovery and our understanding of genotype–phenotype correlations, and functional annotation will continue to enhance our understanding of protein function and dysfunction with the ultimate goal of precision medicine for every individual.

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