
Link Between Absence Seizures and T-Type Calcium Channels

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Abstract

An animal model of human absence epilepsy containing a G to C mutation of the $\text{Ca}_v3.2$ T-type Ca^{2+} channel gene (*Cacnalh*) ties together the gene mutation, increased T-type Ca^{2+} channel activity and the epileptic phenotype. Mice lacking a related gene (*Cacn1a*) also show enhanced T-type Ca^{2+} current and increased susceptibility to absence seizures. On the other hand, mutations that decrease T-type Ca^{2+} channel activity in thalamocortical relay neurons display no spike–wave discharges associated with absence seizures. These animal models are supported by genetic studies showing defects in T-type Ca^{2+} channel function in humans suffering from epilepsy. Thus, in both human and animal studies, T-type Ca^{2+} channel antagonists show promise in the treatment of absence seizures.

7.1 Introduction

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizure and by the neurobiological, cognitive, psychological, and social consequences of this condition (Fisher et al. 2005). An epileptic seizure is defined as a transient symptom of abnormal excessive or synchronous neuronal activity in the brain (Fisher et al. 2005). Absence seizures are ascribed to a brief, sudden lapse of consciousness and are considered one of the more common epileptic seizures. Impairment of consciousness and EEG generalized spike–slow wave discharges (SWDs) are two essential characteristics of absence seizure (Panayiotopoulos et al. 1989).

The neurons in the reticular thalamic nucleus (RT), thalamic relay neurons (thalamocortical (TC) neurons), and neocortical pyramidal cells comprise a circuit

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that participates in the formation of sleep. An alteration in this circuitry is believed to be involved in the mechanism of spindles and spike-wave discharges (SWD) and absence seizures (Steriade 1974; Steriade and Contreras 1995; Futatsugi and Riviello 1998; Timofeev et al. 1998; Fuentealba and Steriade 2005; Pinault and O'Brien 2005). Studies have shown that thalamocortical circuits govern the rhythm of cortical excitation by the thalamus and underlie normal physiologic patterns such as those that occur during sleep. The thalamic relay neurons can activate the cortical pyramidal neurons either in a tonic mode, which occurs during wakefulness and rapid-eye-movement (REM) sleep, or in a burst mode, which occurs during non-REM sleep (Steriade et al. 1993). The burst mode is created by T-type calcium channels, which allow for low-threshold depolarization that generates bursts of action potential when voltage-gated sodium channels are generated (Perez-Reyes et al. 1998). The character of tonic or burst firing in the thalamocortical circuit is primarily controlled by input from the thalamic reticular neurons, which hyperpolarize the relay neurons, allowing them to fire in bursts (Chang and Lowenstein 2003). In the normal awake state, the thalamic relay neurons fire in the tonic mode, and thalamocortical projections transfer sensory information to the cortex in a nonrhythmic manner. However, in absence seizures, the abnormal circuit causes rhythmic activation of the cortex during wakefulness and results in SWD and clinical manifestations of impairment of consciousness (Kostopoulos 2001).

Three different T-type calcium channels express T-type currents: alpha 1G (*Cacna1g*, Cav3.1), alpha 1H (*Cacna1h*, Cav3.2), and alpha 1I (*Cacna1i*, Cav3.3) (Cribbs et al. 1998; Perez-Reyes et al. 1998, 1999; Lee et al. 1999; Perez-Reyes 2003). Considerable data suggest that abnormal T-type current may be the primary culprit in the formation of absence seizures: (1) mutations of the human and mouse *Cacna1a* calcium channel gene, mutations of the *Cacna1a*'s ancillary genes, and knockout of the *Cacna1a* initiate a complex absence-associated epileptic condition that is associated with an ataxia phenotype. In these mutated mice, elevated neuronal low-voltage-activated T-type calcium currents influence thalamocortical network activity and contribute to the generation of cortical spike-wave discharges (SWDs) associated with absence seizures (Zhang et al. 2002, 2004; Ernst et al. 2009). (2) Genetic absence epileptic rats from Strasbourg (GAERS), which present with recurrent generalized nonconvulsive seizures characterized by bilateral and synchronous spike-wave discharges (SWDs) and accompanied with behavioral arrest and staring (Marescaux and Vergnes 1995), exhibit elevated thalamic T-type current and increased *cacna1g* and *Cacna1h* mRNA expression (Tsakiridou et al. 1995; Talley et al. 2000). More recently, data have shown that a Cav3.2 T-type calcium channel point mutation mediates splice-variant-specific effects on function, and the mutation correlates directly with the epileptic phenotype in the GAERS animal model (Powell et al. 2009). (3) Overexpression of *Cacna1g* in mice results in spike-wave discharges and pure absence seizures (Ernst et al. 2009), while mice lacking *Cacna1g* T-type calcium channels exhibit no burst firings in thalamic relay neurons and are resistant to absence seizures (Kim et al. 2001; Song et al. 2004). (4) Gain-of-function polymorphisms in the human *Cacna1h* gene prevail in patients with absence seizures (Chen et al. 2003a;

Khosravani et al. 2004, 2005; Lu et al. 2005; Vitko et al. 2005; Peloquin et al. 2006; Heron et al. 2007). Moreover, functional polymorphisms in the *CACNA1G* gene have been also detected in a related juvenile absence syndrome (Singh et al. 2007). (5) Full antagonism of a T-type calcium channel can inhibit absence seizures and reduce the duration and cycle frequency of SWD (Barton et al. 2005; Tringham et al. 2012).

7.2 Low-Threshold Calcium Current from T-Type Calcium Channels Gene Is Involved in the Formation of Absence Seizures

The major characterizations of T-type channels include low-voltage thresholds for activation and inactivation, fast inactivation, and small single channel conductance in isotonic Ba^{2+} (Perez-Reyes et al. 1999). In neurons, calcium influx through T-type calcium channels triggers low-threshold spikes, which in turn activates a burst of action potentials mediated by sodium channels (burst firing). Burst firing is thought to play an important role in the synchronized activity of the thalamus observed in absence epilepsy (Perez-Reyes 2003).

7.2.1 Calcium-Dependent Burst Firing, Driven by T-Type Calcium Currents, Is Necessary for the Genesis of Spike-Wave Discharge of Absence Seizures

Cortical neurons directly innervate reticular and thalamocortical neurons, whereas the reticular neurons (RT) provide GABAergic projections onto each other and onto thalamocortical neurons, which in turn synapse onto neocortical neurons. The thalamus receives most sensory input; the primary source of excitatory synapses projecting to the thalamus is from the cortex (Liu et al. 1995; Erisir et al. 1997a, b; Khosravani and Zamponi 2006). Both neocortical and thalamocortical cells have excitatory projections back to reticular neurons. The activity of the circuit is further regulated via thalamic local circuits and neocortical interneurons (Steriade et al. 1993). The circuit containing the reticular thalamic nucleus (RT), thalamic relay neurons, and neocortical pyramidal cells has been implicated in the formation of sleep spindles and spike-wave discharges and is involved in the mechanism of absence epilepsy (Futatsugi and Riviello 1998). Thalamic neurons fire in two different modes, burst or tonic (Steriade et al. 1993; Ramcharan et al. 2000; Sherman 2001). The state of the neurons determines the mode of firing: inhibition-induced hyperpolarization leads to burst firing, while excitation-mediated depolarization leads to tonic firing (Steriade et al. 1993). T-type calcium channels underlie burst firings (Cain and Snutch 2013). The entry of calcium ions through T-type calcium channels leads to the depolarization of the membrane, allowing T-type current to generate low-threshold spikes (LTS) that trigger bursts of sodium-dependent action potentials (Perez-Reyes 2003; Cain and Snutch 2010).

However, T-type calcium channels remain inactive when the membrane potential is in its resting state. The channels must first recover from inactivation by membrane hyperpolarization below the resting potential. They then can be activated by small depolarizations driven by hyperpolarization-activated current (IH currents). When activated, T-type channels produce low-threshold calcium currents which induce rebound excitation above threshold that in turn triggers the generation of a burst of action potentials. Therefore, inhibitory inputs are essential for burst firings by neurons, which play a central role in the pathogenesis of absence epilepsy (McCormick and Bal 1997; Perez-Reyes 2003).

RT neurons are regarded as pacemakers of spindles and play a central role in spindle oscillations involved in early sleep (Steriade et al. 1985, 1986, 1987; Fuentealba and Steriade 2005). They are also thought to inhibit thalamocortical neurons during cortically generated spike-wave (absence) seizures, which may explain the obliteration of input from external stimuli and unconsciousness during epileptic fits (Fuentealba and Steriade 2005). More recent data have shown that an alteration in the firing properties of thalamocortical relay (TC) nuclei, caused by a disruption of a *Cacna1g* single gene in mice, is sufficient to induce absence seizures (Kim et al. 2001; Song et al. 2004; Ernst et al. 2009).

7.2.2 Absence Seizure Animal Model GAERS Has Alternative Alpha1 H Transcripts and Selectively Increases T-Current in RT Neurons

In genetic absence epileptic rats from Strasbourg (GAERS), 100 % of the rats present with recurrent generalized nonconvulsive seizures characterized by bilateral and synchronous spike-wave discharges (SWD) accompanied by behavioral arrest, staring, and sometimes twitching of the vibrissae. Spontaneous SWD (7–11 cps) start and end abruptly on a normal background EEG at a mean frequency of 1.5 per minute. In GAERS, drugs effective against absence seizures in humans suppress the SWD in a dose-dependent manner, whereas drugs specific for convulsive or focal seizures are ineffective (Marescaux and Vergnes 1995). The many similarities between GAERS and human absence seizures support using this type of genetic rodent model, as it likely plays an important role in understanding the causes of human absence seizures. Early research showed that T-type current is selectively increased in the RT neurons of GAERS (Tsakiridou et al. 1995). A further study using a quantitative *in situ* hybridization technique demonstrated a significant, albeit small, elevation in T-type calcium channel mRNA (alpha 1G and alpha 1H) in the thalamus of GAERS (Talley et al. 2000). Moreover, a mutation of alpha 1H gene in GAERS was discovered, which correlates with the number and frequency of seizures in progeny of an F1 intercross. This mutation, R1584P, is located in a portion of the III–IV linker region in Cav3.2 at Exon24. There are two major thalamic *Cacna1h* splice variants in GAERS, either with or without Exon25 introduced into the splice. The variants act “epistatically” and require the presence of Exon25 to produce significantly fast recovery from channel inactivation and

great charge transference during high-frequency bursts. Of particular interest is that the ratio of Cav3.2 (+25 Exon) mRNA to Cav3.2 (−25 Exon) mRNA is greater in the thalamus of GAERS animals compared with non-epileptic controls, which suggests that the relative proportion of Cav3.2 (+25) to Cav3.2 (−25) is subject to transcriptional regulation (Powell et al. 2009).

7.2.3 The Absence Seizures Associated with Mutations of Cacna1a, and Cacna1a's Ancillary Calcium Channel Subunits in Mice Are the Results of Indirect Potentiating T-Type Calcium Current

The Cacna1a gene encodes the transmembrane pore-forming subunit of the P-/Q-type or CaV2.1 voltage-gated calcium channel, which are the principal channels supporting neurotransmitter release in the mammalian central nervous system (Westenbroek et al. 1995). Cacna1a genes are profoundly expressed in the cell bodies and dendrites of cerebellar Purkinje and granule cells (Westenbroek et al. 1995). Dominant mutations in Cacna1a are associated with episodic ataxia type 2, familial hemiplegic migraine type 1, and spinocerebellar ataxia type 6, and 7 % have absence epilepsy (Rajakulendran et al. 2010, 2012). Several spontaneously occurring homozygous mouse mutants of Cacna1a are good research models of human absence epilepsy, having provided information on tottering, leaner, rocker, rolling Nagoya, lethargic, ducky, and stargazer (Fletcher et al. 1996; Burgess and Noebels 1999a, b; Fletcher and Frankel 1999). These strains exhibit episodes of motor arrest with spike-wave EEG similar to that seen in human absence epilepsy but also show cerebellar degeneration, ataxia, and dystonia. It has been observed that a 45 % increase in peak current densities of T-type calcium channel currents is evoked at −50 mV from −110 mV in tottering (Cav2.1/alpha 1A subunit), lethargic (β4 subunit), and stargazer (γ4 subunit) mice compared with wild type. The half-maximal voltages for steady-state inactivation of T-type calcium channel currents were shifted in a depolarized direction by 7.5–13.5 mV in these three mutants (Zhang et al. 2002); these data demonstrate that a mutation in Cacna1a or in its regulatory subunit genes increases intrinsic membrane excitability in thalamic neurons by potentiating T-type calcium channel currents. Another example of Cacna1a mutations that correlate with absence seizure comes from the Cacna1a knockout mice. Mice with a null mutation of Cacna1a (alpha1A−/−) are susceptible to absence seizures characterized by typical spike-wave discharges (SWDs) and behavioral arrests. Isolated thalamocortical relay (TC) neurons from these knockout mice show increased T-type calcium currents in vitro (Song et al. 2004).

7.2.4 Mice with Genetically Modified T-Type Calcium Channels Show Aberrant t-Current Implicated in the Genesis of Absence Seizures

7.2.4.1 Cav3.1 Genetically Modified Mice

Mice with a null mutation of the alpha 1G subunit of the T-type calcium channel lack the ability to generate burst mode action potentials but show the normal pattern of tonic mode firing. The thalamus in these alpha 1G-deficient mice is specifically resistant to the generation of spike-wave discharges in response to GABA (B) receptor activation (Kim et al. 2001). Therefore, alpha 1G T-type calcium channels are thought to play a critical role in the genesis of absence seizures in the thalamocortical pathway by modulation of the intrinsic firing pattern (Kim et al. 2001). This idea was further supported by *Cacna1a* (−) and *Cacna1g* (−) double mutation mice, which demonstrate that generation of SWDs in mutant *Cacna1a* is suppressed by the deletion of the *Cacna1g* gene. Cross-breeding alpha 1A−/− mice with mice harboring a null mutation of alpha 1G show a complete loss of T-type calcium current in TC neurons and display no SWDs. Similar results were obtained using double-mutant mice harboring the alpha 1G mutation plus another mutation such as lethargic (*beta4(lh/lh)*), tottering (*alpha1A(tg/tg)*), or stargazer (*gamma2(stg/stg)*) (Song et al. 2004). More importantly, two BAC transgenic murine lines which overexpress the *Cacna1g* gene induce pure absence epilepsy through genetic enhancement of the thalamocortical network (Ernst et al. 2009).

7.2.4.2 Knockout of Cav3.2

In *Cav3.2* knockout (KO) mice, the burst of RT neurons has a lower spike frequency and less prominent acceleration-deceleration change. In contrast, ventroposterior neurons (VP) of *Cav3.2* KO mice showed a higher ratio of bursts and a higher discharge rate within a burst than those of the wild-type (WT) control. In addition, the long-lasting tonic episodes in RT neurons of the *Cav3.2* KO have less stereotypic regularity than episodes in their WT counterparts (Liao et al. 2011). Another example of *cacna1h* gene's implication in epileptogenesis originated from the observation of the pilocarpine model of epilepsy. According to one report, there is a transient and selective upregulation of *Cav3.2* subunits at the mRNA and protein levels after pilocarpine-induced status epilepticus. These functional changes are absent in mice lacking *Cav3.2* subunits. Essentially, the development of neuropathological hallmarks of chronic epilepsy, such as subfield-specific neuron loss in hippocampal formation and mossy fiber sprouting, is almost completely absent in *Cav3.2* knockout mice (Becker et al. 2008).

7.2.4.3 Knockout of Cav3.3

There are two T-type calcium channel genes expressed in the nucleus reticularis thalami (RT), *Cav3.2* and *Cav3.3*, with the *Cav3.3* protein being more abundantly expressed. In the transgenic *CaV3.3*−/− murine line, the absence of *Cav3.3* channels in RT cells prevented oscillatory bursting in the low-frequency (4–

10 Hz) range but spared tonic discharge. In contrast, adjacent TC neurons expressing Cav3.1 channels retain low-threshold bursts (Astori et al. 2011).

7.2.5 Antiseizure Drugs and T-Type Calcium Channel

T-type calcium channels play an important role in the generation and maintenance of SWDs in absence seizures. Decreasing the functions of alpha 1G, alpha 1H, and alpha 1I through the use of antiepileptic drugs may play a role in reducing absence seizures by decreasing excitability in thalamic circuits and the ability to recruit network oscillatory activity in thalamic and thalamocortical circuits. Ethosuximide (ETX), a known antiepileptic drug effective in the treatment of generalized absence seizures, was shown to block T-type calcium channels in thalamic relay neurons (Leresche et al. 1998; Barton et al. 2005; Broicher et al. 2007). A new study identified two T-type calcium channel blockers, Z941 and Z944, with attenuated burst firing of thalamic reticular nucleus neurons in GAERS. Z941 and Z944 suppress absence seizures by 85–90 % and reduce both the duration and the cycle frequency of SWDs in GAERS. It has been suggested that Z941 and Z944 likely target the predominant neural circuitry involved in SWDs by inhibiting the ictogenic properties of the cortical neurons, as well as by disrupting the resonant circuitry of the thalamocortical and RT neurons (Tringham et al. 2012). The ability of the T-type calcium channel antagonists to inhibit absence seizures and reduce the duration and cycle frequency of spike–wave discharges also suggests that T-type current generated by T-type calcium channels is a key component in the formation of absence seizures.

7.3 Genetics of Absence Seizure

Twin studies have provided convincing evidence implicating genetic influences in the development of absence seizures. The concordance for the presence of childhood absence epilepsy is 70–85 % and 33 % in monozygotic twins and first-degree relatives, respectively (Crunelli and Leresche 2002). Therefore, idiopathic epilepsy is regarded as primarily genetic but may be polygenic, with different variant alleles working together to contribute to the symptoms of epilepsy (Mulley et al. 2005). Over the past decade, a number of genes have been associated with rare monogenic and idiopathic epilepsies that have relatively simple inheritance. These studies revealed the importance of ion channel genes in epilepsy, but these mutations occur only in rare monogenic epilepsy syndrome (Lu and Wang 2009) and therefore may not be applicable to common seizures. The complex genetics of idiopathic epilepsy, which involves multiple alleles working together, means that the traditional method of seeking pathological genes through genetic linkage may not be a good strategy in this type of disease. Direct sequencing of candidate genes for rare functional variants based, focusing on selected mechanisms to identify the susceptibility genes of absence seizure, is a better option (Lowenstein and Messing 2007).

Patients with childhood absence epilepsy (CAE) have the following specific characteristics: (1) onset in childhood, which may remit in the adult; (2) brief absence seizures (~4–20 s) that occur frequently, sometimes hundreds per day, characterized by 3 Hz spike wave on their EEG; (3) the symptoms of CAE can be activated by hyperventilation or light stimulation; (4) it is genetically predisposed, with a 16–45 % positive family history; (5) T-type calcium channel blockers, which attenuate thalamic burst firing and suppress absence seizures, are very effective drugs in treating patients with CAE; and (6) no abnormal radiological finding, and patients are otherwise neurologically normal (Porter 1993; Crunelli and Leresche 2002). These unique characteristics of CAE suggest that there are some genes that correlate with the maintenance of thalamocortical synchronization and both cognitive and brain development may be improperly expressed. The gene's expression may be increased in the early developmental stage of the brain but decrease or become silent in the adult. Environmental factors may also affect gene expression. Research information indicates that the circuit neurons of the reticular thalamic nucleus (RT), thalamic relay cells, and neocortical pyramidal cells comprise a circuit implicated in the formation of sleep spindles and spike-wave discharges and the mechanisms of absence epilepsy. Therefore, factors that interfere with this circuit may lead to absence seizures. The mice of alpha (1G)-deficient thalamus are specifically resistant to the generation of spike-wave discharges in response to GABA (B) receptor activation (Kim et al. 2001). On the other hand, transgenic murine lines overexpressing the *Cacna1g* gene induce pure absence epilepsy through genetic enhancement of the thalamocortical network (Ernst et al. 2009). One can infer from these findings that pure absence seizures in human beings, such as typical CAE, may originate from enhanced T-type current due to mutations or alternative transcripts of the three T-type calcium channel genes. The identification of T-type variants implicated in common complex epilepsy provides an important step for us in understanding this complex genetic disease.

7.3.1 CACNA1H Is a Susceptibility Gene of Idiopathic Epilepsy

7.3.1.1 Variants of CACNA1H Gene Detected in Patients with CAE

We have conducted direct sequencing of exons 3–35 and the exon–intron boundaries of the *Cacna1h* gene in 118 patients with CAE of Han ethnicity recruited from Northern China. Sixty-eight variations have been detected in the *Cacna1h* gene, and, among the variations identified, 12 were missense mutations and found in only 14 of the 118 patients in a heterozygous state but none in the 230 unrelated controls. The identified missense mutations occurred in highly conserved residues of the T-type calcium channel gene. These mutations were further introduced into human *Cav3.2* cDNA and transfected into HEK-293 cells for whole-cell patch-clamp recordings. Computer simulations predicted that some mutations favor burst firings. More experimental evidence revealed that many mutant channels are activated in response to small voltage changes, an alteration in the rate of recovery of channels from their inactivated state, or an increase in the

surface expression of the channels (Chen et al. 2003a; Khosravani et al. 2004, 2005; Lu et al. 2005; Vitko et al. 2005; Peloquin et al. 2006).

7.3.1.2 Variants Detected in Other Types of Idiopathic Epilepsy

Research results from Heron et al. (2007) further support the original concept that the *Cacna1h* gene is a susceptibility gene in absence seizure, which is also associated with an extended spectrum of idiopathic generalized epilepsies in the Caucasian population. In their study, 240 epilepsy patients and 95 control subjects were tested. More than 100 variants were detected, including 19 novel variants involving amino acid changes in subjects with phenotypes including childhood absence seizures, juvenile absence seizures, juvenile myoclonic, and myoclonic astatic epilepsies, as well as febrile seizures and temporal lobe epilepsy. Electrophysiological analysis of 11 variants showed that 9 had altered channel properties, generally in ways that should increase calcium current (Heron et al. 2007).

7.3.1.3 Variations in *CACNA1G* and *CACNA1I* in Patients with CAE

To evaluate the *CACNA1G* (alpha 1G) gene's contribution to the pathological mechanism of common children absence epilepsy, we also sequenced all of the exons in the *CACNA1G* gene in 48 patients within a Chinese population with CAE but failed to find a link between alpha 1G and human absence epilepsy (Chen et al. 2003b). However, a recent report described several putative functional variants of the *CACNA1G* gene in patients with idiopathic generalized epilepsy (IGE). The Ala570Val variant was found in one of 123 IGE patients but was not observed in a pool of 360 healthy controls. The Ala1089Ser substitution segregated into three juvenile myoclonic epilepsy (JME) affected members of a two-generation Japanese family and in one healthy control. In addition, an Asp980Asn substitution was found in two JME patients and three control individuals (Singh et al. 2007). To date, no report has shown a link between patients with idiopathic epilepsy and mutations of *CACNA1I* (Wang et al. 2006).

7.4 Alternative Transcripts of *Cacna1h* Gene May Be Major Factors Implicated in the Formation of Complex Absence Seizures

Of the three T-type calcium channels, alpha 1G subunits are the predominant source of T-type channels in thalamocortical relay (TC) nuclei; 1H and 1I subunits are localized to the thalamic reticular nucleus (RT), and all three forms are found in distinct but overlapping layers of the neocortex (Talley et al. 1999). The characteristic of age-dependent remission in 70 % of the patients with CAE suggests that gene dynamic expression is involved in the mechanism of CAE. Evidence has shown that subtle modifications in T-type channel gating have profound consequences for synaptically evoked firing dynamics in native neurons (Tschertter et al. 2011). All three T-type calcium channel genes have multiple transcripts, and these isoforms likely have different biophysical properties, accounting for the

complex genetic characteristics of absence seizure. For example, both alpha 1H (Cav3.2) and alpha 1I (Cav3.3) transcripts are expressed in RT neurons; it has been established that Cav3.3-mediated currents generate bursts that are most closely correlated with typical RT bursts (Cain and Snutch 2010). However, Cav3.2 channels are also expressed in RT an observation consistent with the presence of Cav3.2-like currents in these neurons. The fast activation kinetics and the lower threshold of activation of the Cav3.2 channels may play a critical role in the initiation of bursts as well as the appearance of important physiological contributions that become apparent during a series of multiple bursts (Cain and Snutch 2010). Individuals may have diverse T-type calcium isoforms; if some of these unique isoforms increase T-type current, they may facilitate aberrant cortical synchronization and possibly initiate absence seizures. Therefore, the inherited complexity of multiple variants of the T-type calcium gene may be expressed through different T-type calcium channel genes to initiate CAE development.

The *Cacna1h* gene (Cav3.2) is located on chromosome 16p13.3 and expressed in the thalamic reticular nucleus. It is often alternatively spliced and generates a family of variant transcripts. Results on the website of National Center for Biotechnology Information (NCBI)'s AceView, which analyzed 115 GenBank accessions from 104 alpha 1H cDNA clones, illustrate that the human *CACNA1H* gene contains 38 distinct introns. Transcription produces 10 different mRNAs, 7 alternatively spliced variants, and 3 unspliced forms. There are 3 probable alternative promoters, 2 non-overlapping alternative last exons, and 2 validated alternative polyadenylation sites. The mRNAs appear to differ by truncation at the 5' and 3' ends, the presence or absence of a cassette exon, and overlapping exons with different boundaries. Structural changes created by missense mutations may differentially affect the activity of alternative gene products, whereas missense, silent, and noncoding mutations may alter developmental regulation of splice-variant expression. Zhang's research (Zhang et al. 2002, 2004) illustrates that the *Cacna1h* gene is alternatively spliced at 12–14 sites and is capable of generating both functional and nonfunctional transcripts. Biophysical profiles of different alternative Cav3.2 forms reveal variations in kinetics and steady-state gating parameters, most likely related to altered membrane firing. Zhang et al. (2002, 2004) further examined mutations of *Cacna1h* gene, such as C456S, D1463N, and A1765A, which appear to be unique in Chinese CAE patients but elicit minimal or no changes in Cav3.2 function, in relationship to candidate exonic splicing enhancer sequences. These missense and silent mutations appear to create or change the regulatory specificity of exonic splicing enhancer sequences that control splicing regulation. The *Cacna1h* gene is highly variable. According to our data (Chen et al. 2003a) and analysis of NCBI SNP data, the average SNP density of exons in the *Cacna1h* gene is almost 1 SNP for every 48 base pairs. The average SNP density of introns in the *Cacna1h* gene is almost one SNP for every 64 base pairs. It is 40 times higher in exons and 20 times higher in introns than the average genomic density. Many of the SNP's allele frequency rates are above 10%. A low/high SNP frequency rate involves a complicated profile, where everyone can have their personal unique SNP variant system. Variants of the *Cacna1h* gene may destroy,

create, or alter the regulatory specificity of predicted exonic splicing enhancer sequences that control splicing regulation. For example, the GAERS animal model, which contains a point mutation of the *Cacna1h* gene (R1584P), contain splice-variant-specific effects, requiring the presence of Exon25 to produce significantly faster recovery from channel inactivation and enhanced charge transference during high-frequency bursts (Powell et al. 2009). More research is required to clarify how variants of the *Cacna1h* gene affect its transcripts and how these alternative transcripts relate to the expression of CAE.

One of the main precipitating factors provoking CAE seizures is hyperventilation. As a result, carbon dioxide levels decrease in the blood, which causes the body's pH to become more alkaline. Interestingly, reducing agents such as dithiothreitol (DTT) selectively enhance native T-type current in reticular thalamic (RT) neurons and recombinant Cav3.2 (alpha 1H) current, but not native and recombinant Cav3.1 (alpha 1G) and Cav3.3 (alpha 1I)-based currents. Research shows that only Cav3.2 is unregulated by reducing agents and that reducing agents increase burst firing of RT neurons in wild-type rats and mice, but not Cav3.2 knockout mice; it also revealed an important role for the Cav3.2 isoform in thalamic signaling (Joksovic et al. 2006). More work is required to identify a possible connection between hyperventilation and transient elevation in T-type current, such as alpha 1H's T-type current in the future.

Conclusions and Future Directions

Absence seizures delineate bilaterally synchronous burst firing of an ensemble of reciprocally connected neuronal populations located in the thalamus and neocortex. Extensive research has demonstrated that both thalamic and cortical circuits participate in spike-wave activity. The concept that the thalamocortical circuit can become a widespread 3 Hz oscillator that disrupts either thalamic or cortical portions of the circuit, thereby contributing to seizures, is widely accepted by scientists. Evidence of a link between T-type calcium channel genes and absence seizures has also received considerable support since the introduction of absence seizure animal research models or examination of variations in T-type calcium channel genes in patients with epilepsy. Abnormal components, by enhancing this T-type current-associated circuit, may initiate absence seizures. Mutations of the *Cacna1a* gene and its ancillary calcium current occur in patients and mice with complex phenotypes. However, overexpression of *Cacna1g* using a transgenic mouse model results in spike-wave discharges and "pure" absence seizures. This suggests that increasing some alternative transcripts of T-type calcium channel genes may be enough to initiate pure type absence seizure. Extensive alternative splice isoform profiling of humans containing *Cacna1h*, *Cacna1g*, and *Cacna1i* genes demonstrates highly diversified T-type channel mRNA transcript populations with distinct electrophysiology properties (Cain and Snutch 2010). The genetic complexity of CAE may arise from different genes or one gene with differential alleles that work together to lead to abnormal enhancement of T-type current. As our data illustrate, the *Cacna1h* gene has a high degree of variation in each

individual. Variants of this T-type calcium channel genes may alter channel gate character, increase gene expression levels, or create expressive alternative transcripts. These complexities result in elevations in T-type current, which in turn enhances the bursting activity of thalamic neurons, thereby increasing the ability to recruit network oscillatory actions in the thalamocortical circuits and initiate absence seizures. In the near future, more work is required on the relationship between variants and alternative transcripts in the role of absence seizure. The characteristic remission of absence seizures with age suggests that gene expression is involved in the mechanism of CAE. Valuable information can be gleaned from research examining the dynamic epigenetic process of T-type calcium channels.

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