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Review

Genes and molecular mechanisms involved in the epileptogenesis of idiopathic absence epilepsies

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ABSTRACT

Idiopathic absence epilepsies (IAE), that have high prevalence particularly among children and adolescents, are complex disorders mainly caused by genetic factors. Childhood absence epilepsy and juvenile absence epilepsy are among the most common subtypes of IAEs. While the role of ion channels has been the primary focus of epilepsy research, the analysis of mutation and association in both patients with absence epilepsies and animal models revealed the involvement of GABA receptors and calcium channels, but also of novel non-ion channel proteins in inducing spike wave discharges (SWD). Functional studies on a mutated variant of these proteins also support their role in the epileptogenesis of absence seizures. Studies in animal models point to both the thalamus and cortex as the origin of SWDs: the abnormalities in the components of these circuits leading to seizure activity. This review examines the current research on mutations and susceptibility alleles determined in the genes that code for the subunits of GABA receptors (*GABRG2*, *GABRA1*, *GABRB3*, *GABRA5*, $GABA_{(B1)}$ and $GABA_{(B2)}$), calcium channels (*CACNA1A*, *CACNA1G*, *CACNA1H*, *CACNA1I*, *CACNAB4*, *CACNAG2* and *CACNG3*), and novel non-ion channel proteins, taking into account the results of functional studies on these variants.

1. Introduction

Idiopathic absence epilepsies (IAE) are types of generalized epilepsies (IGE) that are not preceded or occasioned by other disorders, but are instead, caused by complex genetic factors.[1] The most common forms of IAEs are childhood absence epilepsy (CAE) and juvenile absence epilepsy (JAE). However, absence seizures can be associated with other subtypes of IGEs, such as juvenile myoclonic epilepsy (JME). The clinical features of CAE are non-convulsive epileptic seizures characterized by the brief (5–20 s) and sudden loss of consciousness with a generalized synchronous, bilateral, 2.5–4 Hz spike and slow wave discharge (SWD) in the electroencephalogram (EEG).[2,3] The onset of the absence seizures having a frequency of up to 200 per day (pyknoleptic), begins between the ages of 3 and 8, with seizures disappearing spontaneously at adolescence. JAE, on the other hand, is characterized by non-pyknoleptic absence seizures, primarily occurring after 10 years of age. Unlike CAE, these seizures are associated frequently with other epileptic symptoms, such as generalized tonic-clonic seizures (GTCS) and myoclonic jerks.

Experiments on animal models revealed that the thalamus and the cortex are both involved in the generation of SWDs.[4,5] There are three main components of a thalamocortical network: (1) Thalamocortical relay neurons which transfer inputs from a large number of sources to pyramidal neurons in the III–IV and V–VI layers of the cortex through excitatory synaptic connections. (2) Layer VI pyramidal cells of the cortex which send back excitatory inputs to the thalamus. (3) Inhibitory GABAergic interneurons in the thalamic reticular nucleus (nRT), which receive excitatory inputs from axon collaterals of the reciprocal thalamocortical and corticothalamic pathways. When activated, these GABAergic neurons send inhibitory inputs to the thalamus and also to each other, but not to the cortex.[6]

When a thalamocortical circuit works properly, a burst of synchronized oscillations with a frequency of ~10 Hz will occur. In this circuit, thalamocortical neurons induce excitatory post-synaptic potentials (EPSPs) in GABAergic nRT neurons via NMDA and non-NMDA receptors. Low-threshold calcium channels in nRT neurons are activated leading to the opening of Na channels, initiating action potentials. The activation of these inhibitory neurons induces inhibitory postsynaptic potentials (IPSPs) in thalamocortical neurons via $GABA_A$ receptors. This inhibitory phase abolishes the burst firing in the circuit for a time. During this hyperpolarized state, low-threshold calcium channels in thalamocortical neurons recover from inactivation. The calcium channels open, depolarizing the membrane, making the cell available for the next burst of action potentials. However, abnormal activity in this circuit would disrupt the alternating

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cycles of excitatory and inhibitory activity, leading to 3–4 Hz spike wave discharges. For example, increased excitatory activity in the cortex or thalamocortical region would lead to a longer burst of nRT neurons firing, causing longer IPSPs mediated by $GABA_B$ receptors via G-protein coupled K channels in the thalamocortical region. During this long hyperpolarized state, calcium channels open, initiating several action potentials in every cycle, resulting in paroxysmal spikes and slow wave discharges.

In addition to possible abnormal activity in the cortex or thalamocortical regions, there are other suggested mechanisms explaining the generation of abnormal spike wave discharges. For example, the loss of $GABA_A$ receptor mediated inhibition between thalamic reticular cells causes these cells to produce longer IPSPs on thalamocortical cells mediated by $GABA_B$ receptors and enhancement of low threshold Ca^{2+}.[7]

This review summarizes not only the mutations and susceptibility alleles determined in patients and animal models in the candidate ion channel and the novel non-ion channel genes but also examines the functional results of these variations through the mechanism of SWDs in the thalamocortical network.

2. Genetic etiology of idiopathic absence epilepsies

Because the cellular mechanism of absence seizures indicates the involvement of ion channels in the pathogenesis of absence epilepsies; gene analysis carried out both on patients and on animal models, revealed both mutations and susceptibility alleles in genes that code for the subunits of GABA receptors and Ca^{2+} channels (as shown in Table 1). Unfortunately, most of these variations are rare and restricted to a few a patients; thus, the components of the complex genetic factors involved in the triggering of absence seizures are still unclear. However, in recent years, research into these factors has continued, and the focus on using novel non-ion channel genes and approaches, such as studying the copy number variations (CNV), epigenetic modifications, and exome sequencing, has drawn more attention to the importance of understanding the place of genetic components in absence seizures.

2.1. GABA receptors

GABA is the main inhibitory neurotransmitter in the central nervous system. It interacts with two major subtypes of receptors, $GABA_A$ and $GABA_B$, which are involved in the generation of spike wave discharges during absence seizures.

2.1.1. $GABA_A$ receptors

Ionotropic $GABA_A$ receptors mediate fast synaptic inhibition in the central nervous system. Binding of GABA to $GABA_A$ receptors allows an influx of Cl^- ions which causes hyperpolarization of the membrane and inhibition of action potentials.[8] $GABA_A$ receptors are pentameric structures, consisting of five out of at least 18 subunits (α1-6, β1-3, γ1-3, δ, ε, θ, ρ1-3).[9] Each subunit has four transmembrane domains with the second transmembrane of each subunit forming the channel pore.[10] The genes that code for these subunits are localized as clusters on different chromosomes. The most prevalent $GABA_A$ receptor throughout the brain is formed by the α1, β2, and the γ2 subunits, encoded by *GABRA1*, *GABRB2* and *GABRG2* genes localized on 5q34–35. Another GABA gene cluster (*GABRA5*, *GABRB3* and *GABRG3*) resides on 15q11–12, coding for the α5, β3 and γ3 subunits. These receptors can be modulated by steroids, barbiturates and benzodiazepines. The role of $GABA_A$ receptors in epileptic activity has been studied extensively; researchers found that $GABA_A$ receptor agonists, such as barbiturates and benzodiazepines, suppress seizures while the $GABA_A$ blockers, such as bicuculline, penicillin and picrotoxin induce epileptic activity in model animals.

In analysis of $GABA_A$ receptors in absence seizures, the research has for the most part focused on the γ2 subunit, which was the first mutation identified in the *GABRG2* gene in a family with CAE and febrile seizures.[11] The γ2 subunit is known to be responsible for modulation of benzodiazepine and receptor targeting.[8] Mutation in the CAE family, arginine to glutamine substitution at amino acid 43 (Arg43Glu), led to the loss of current through GABA receptors due to an impairment in traffic from the receptor to the membrane. Functional studies in this area pointed out that the subunit with the mutation is stuck in the endoplasmic reticulum, leading to decreased surface expression.[12] Arg43Glu mutation was assessed in a mouse model which was constructed by the insertion of an Arg43Glu mutation in the heterozygous state.[13] The mouse showed a similar phenotype indicating childhood absence epilepsy and confirming the causative role of the γ2 subunit. A reduction of inhibition in the cortex of the mouse was detected, marking the cortex as the start region of SWDs. Further, thalamic bursting is subject to inputs from the cortical region, and thus, inhibition in the cortex would alter these inputs, triggering SWDs.

A second mutation in *GABRG2* was also found in a family with CAE and febrile seizures.[14] The IVS6 + 2T → G mutation disrupted a putative splice site, which probably caused a truncated protein. Despite these findings, contradictory results in Japanese and Chinese populations limited the potential impact of this study because mutation analysis and association studies revealed negative linkage of CAE to *GABRG2* in these populations.[15,16]

The first study to understand the role of *GABRA1* in the pathogenesis of epilepsy included 61 JME, 38 JAE, and 29 CAE patients, revealed no linkage.[17] Further study identified mutations in a JME family[18] and a CAE patient.[19] In the CAE patient, the single base pair deletion (975delC) had caused a premature stop codon. Functional studies showed that the truncated receptor could not integrate into the membrane and thus caused no current. Due to haploinsufficiency there were losses in the function of the inhibitory action of GABA channels in the thalamic neurons. However, in the Japanese population, attempts to find any causative mutation in typical and atypical absence patients revealed negative results.[15]

The *GABRB3* gene codes for the β3-subunit of the $GABA_A$ receptor.[20] Association analysis carried out with different ethnic groups for *GABRB3* gene displayed possible associations in CAE patients.[21–23] In Urak's study, a common promoter haplotype was found to be at a higher frequency in CAE patients. A reporter gene assay was carried out to illustrate the possible effects of this haplotype on transcription. The result showed that this particular haplotype reduced the transcriptional level of *GABRB3* gene by interfering with the binding site of the neuron-specific transcriptional activator N-Oct-3. However, reduced levels of the β3 subunit would have also decreased the level of inhibitory $GABA_A$ receptors. This disease susceptible haplotype when assessed in the German IGE samples did not confirm that this haplotype was common in absence patients of different ethnic groups.[24]

Differences based on ethnicity were further illustrated by mutations found in *GABRB3* in 4 families of 48 CAE Mexican families of American Indian or Spanish European descent. Three mutations resided in the exon1a in signal peptide, while the Gly32Arg mutation in exon 2 affected protein maturation, topology, assembly, and subcellular localization of a GABA receptor, resulting in hyperglycosylation.[25]

GABRB3 deficient mice showed abnormal EEG activity including generalized tonic clonic seizures, clonic and myoclonic seizures, as well as behavioral arrest during the abnormal EEG activity, similar to that found in absence seizures.[26] However, there was no evidence that the mice had experienced pure epileptic seizures;

Table 1
Association and mutation analysis of the candidate genes for idiopathic absence epilepsies.

Gene	Protein	DNA Mutation/association analysis	Protein mutation	Clinical features and origin of Patient(s)/reference	Functional study
GABRG2	γ2 subunit of $GABA_A$ receptor	c.245G > A	R43Q	CAE patients with febrile seizures in a large family[11]	Reduced $GABA_A$ receptor currents[12]
		IVS6 + 2T > G	–	CAE patient with febrile seizures[14]	Probably truncated protein
GABRA1	α1subunit of $GABA_A$ receptor	975delC	Premature stop codon	CAE patient[19]	No $GABA_A$ receptor current
GABRB3	β3 subunit of the $GABA_A$ receptor	Significant association of a common haplotype	–	45 Austrian CAE patients[21]	Reduced transcriptional level of GABRB3 gene
		c.31C > T	P11S	CAE Mexican families with American Indian	Altered protein maturation, topology,
		c.44C > T	S15F	and Spanish European ancestry[25]	assembly and subcellular localization
		c.G962 > A	G32R		of GABA receptors
CACNA1A	α1A subunit of P/Q channels	5733C > T	R1820stop	A patient with severe phenotype of absence seizures and ataxia[39]	Reduced Ca^{2+} current
		439G > A	E147K	In a family with absence seizures and ataxia[40]	Reduced Ca^{2+} channel function
CACNA1G	α1G subunit of LVA channels	c.1709C > T	A570V	A Japanese patient with sporadic case of JME and with a history of early CAE[46]	Increased Ca^{2+} current and faster inactivation decay rates
		c.3295G > A	A1099T	Japanese CAE patients[46]	No alteration in Ca^{+} currents
		c.3728G > A	R1243Q		No alteration in Ca^{+} currents
CACNA1H	α1H subunit of LVA channels	562C > A	F161L	Chinese CAE patients[52]	Increased T-type channel activity[53–55]
		923G > A	E282K		
		1445T > A	C456S		
		1574G > A	G499S		
		2022C > T	P648L		
		2310G > A	R744Q		
		2322C > T	A748V		
		47152C > T	P314S	Chinese CAE patient[56]	Predicted to change potential phosphorylation site
		48684C > T	P492S		Predicted to change the charge status of the channel and phosphorylation site
		47247C > T	N345N		Predicted to alter potential splicing site
		49016C > G	L602L		Predicted to affect transcription factor binding site
		49067A > G	S619S		Predicted to affect transcription factor binding site or splicing
		Significant association of SNP (rs2745150) in intron 11	–	100 Chinese CAE patients[56]	Predicted to change potential splicing site
		Significant association of SNP (rs9934839) in exon 9 and haplotype	–	218 Chinese CAE patients[57]	Predicted to change the transcription binding site
CACNG3	γ3 subunit of neuronal voltage-gated calcium channel	Strong association of 3 common SNPs (rs4787924, rs965830, rs2214437) and a haplotype	–	217 CAE trios and the 65 nuclear pedigrees with Caucasian origin[66]	–
ME2	Malic enzyme 2	Association of a nine-SNP haplotype	–	68 JAE patients with GTCS[67]	–
Glud1	glutamate dehydrogenase	833C > T	R221C	3 children in the same family with photosensitive myoclonic absence epilepsy and HI/HA[68]	Decreased level of GABA in patients compared to the controls
JRK/JH8	JRK jerky homolog (mouse)	c.1367C > T	T456M	CAE patient evolving to JME[70]	–
LGI4	leucine-rich, glioma inactivated 4	Association of a polymorphism (c.1914GC > AT)	–	42 CAE patients[72]	–

Table 1 (*Continued*)

Gene	Protein	DNA Mutation/association analysis	Protein mutation	Clinical features and origin of Patient(s)/reference	Functional study
SLC2A1	Glut1 glucose transporter	c.668G > C	R223P	Early onset absence epilepsy[74]	Decreased level of glucose transport
		c.971C > T	S324L		
		c.376C > T	R126C		
		c.680-11G > A	227–228ins PPV		
		c.971C > T	S324L	Aglo-saxon absence families with different syndromes[75]	–
		c.668G > C	R223P		
INHA	inhibin alpha subunit	Significant association of SNP (rs7588807) in intron 1	–	72 Turkish JAE patients[76]	–
		n.370C > T	R124C	Turkish JAE patients	–
		n.525C > G	H175Q		
		n.747G > A	L249L		

instead they showed features of the Angelman syndrome, which is known to have deletions on 15q11–13. Patients with Angelman syndrome, like the *GABRB3* deficient mice, experience different types of epileptic seizures such as, atypical absence, myoclonic, atonic, tonic, and tonic-clonic seizures. The mice were treated with carbamazepine, a well-known antiepileptic drug (AED), which is used for the treatment of focal epilepsies. After treatment, the absence seizures were aggravated, as is the case in humans with absence seizures, indicating an involvement of absence-like pathophysiology in these mice.[27,28]

The α5-subunit of $GABA_A$ receptors (*GABRA5*) of 50 CAE patients were also subjected to mutation analysis; however, no causative variation was identified.[23]

2.1.2. $GABA_B$ receptors

Metabotropic $GABA_B$ receptors mediate their activity via G-coupled proteins by activating K^+ and Ca^{2+} ion channels, second messenger systems, phospholipase C, and adenylate cyclase.[10] These proteins are seven transmembrane receptors, and the functional $GABA_B$ receptor is formed from heterodimers of $GABA_{(B1)}$ and $GABA_{(B2)}$ subunits. These receptors produce both slow and prolonged inhibitory signals, mainly located in the presynaptic terminals, which have an essential role in neurotransmitter release.

The mice models, with knocked down $GABA_{(B1)}$ and $GABA_{(B2)}$ subunits, displayed spontaneous SWDs, which indicated a possible role for $GABA_B$ receptors in absence epilepsy.[29,30] Also, in the neocortex of the Wag/rij rat (one of the established animal models for human absence seizures), researchers found a reduced expression and function of $GABA_B$ receptor.[31] Although the animal models emphasized the possible role of $GABA_B$ receptors in absence seizures, mutation and association analysis performed on Chinese CAE patients again revealed negative results.[32]

2.2. Calcium channels

Calcium channels are voltage dependent channels whose conductance depends on changes in transmembrane potential. In excitable cells, they conduct Ca^{2+} ions which function in muscle contraction, as well as releasing hormones and neurotransmitters through a diverse calcium involved processes.[33] Calcium channels are composed of one main α1 subunit, i.e. an integral membrane protein and its smaller auxiliary subunits (β, α_2, δ and γ).[34] The biological and physiological properties of calcium channels depend on the α1 subunits, consisting of four repeats of the six transmembrane domains. As in all other voltage gated channels, these domains include one S4 segment which functions as voltage sensor, a selective P-loop, and S6 segment that forms the inner part of the channel. The C-terminus of the α1 protein is also essential for interaction with the auxiliary subunits, Ca^{2+}-calmodulin-mediated inactivation and for G-protein regulation.[35] There are at least two distinct classes of Ca^{2+} channels depending on the voltage requirement for activation. Low-voltage-activated (LVA) channels activate after a small depolarization of the membrane while high-voltage-activated (HVA) channels function in the case of a larger depolarization. Calcium channels are possible targets for induction of SWDs because of their excitatory function in the thalamocortical region. Ethosuximide, which is an essential AED used to treat absence seizures, is also known to suppress T-channel currents.[36] Attempts to locate epilepsy genes in both animal models and absence patients revealed that both the LVA and HVA channels play roles in the pathogenesis of absence seizures (shown in Table 1).

2.2.1. HVA calcium channels

HVA calcium channels include L-, P/Q-, N- and R-subtypes depending on their different electrophysiological and pharmaco-

logical properties, along with amino acid identity.[37] P/Q type calcium channels, mainly found in presynaptic terminal distribution, are known to have an essential role in modulating neurotransmitter release.[38] Therefore, dysfunction of these channels would impair the balance between neuronal inhibition and excitation leading to burst firing.

In a patient with severe phenotype of absence seizures and ataxia, for the first time, a nonsense mutation has been identified in the α1A subunit of P/Q channels (*CACNA1A*).[39] The gene resided on 19p13, with the mutation located in the C-terminus of the protein, which caused a premature stop codon. Functional studies showed that this mutation had a dominant negative effect, leading to a reduced Ca^{2+} current. A second mutation was also identified in the *CACNA1A* of a family with three generations of absence and ataxia in their medical history.[40] The 439G → A nucleotide transition in exon 3 in heterozygous state caused E147K amino acid substitution in the second transmembrane segment of domain I of the channel. Functional studies for this mutation revealed a partial reduction in calcium channel function due to impairment in traffic to the membrane.

In two animal models, the tottering and leaner mouse, which showed both the absence and ataxia phenotypes, mutations were found in *CACNA1A* (Cav2.1). In the tottering mouse the mutation was located in the S4–S5 linker region of the third transmembrane domain near the pore-forming region of the channel.[41] This mutation reduced the whole-cell current density and voltage dependent inactivation during a prolonged depolarization in dissociated Purkinje somas.[42] In the leaner mouse, the mutation in *CACNA1A* had more severe effects, its location in the C-terminus, reduced both current density and open probability of single P/Q type channels.[43]

While Cav2.1 channels appear to be good candidates for absence seizures, both patients and mouse models failed to exhibit not only pure absence seizures, but also other neurological disorders like ataxia and dystonia. Therefore, mutations in *CACNA1A* could have been the cause of the other diseases, with another locus responsible for the epileptic phenotype. Another possibility could be that malfunctions in other proteins, secondary to the mutations in *CACNA1A*, were the actual cause of the absence seizures.[28] Recent studies indicate that this second possibility is more likely: specifically a study on mouse models with *CACNA1A* mutations, where an increase in the current of LVA channels was measured in the thalamocortical region of the brain.[44] In mutant mice with null mutation of *CACNA1A,* LVA current was elevated in the thalamocortical region and the mice were found to be more likely to have spike wave discharges.[45]

2.2.2. LVA calcium channels

There are three genes that code for the α1 subunits, which have low-voltage activation, namely *CACNA1G* ($Ca_v3.1$), *CACNA1H* ($Ca_v3.2$) and *CACNA1I* ($Ca_v3.3$). *CACNA1G* is located on the 17q21.33, and this channel is mainly expressed in the thalamocortical neurons where the spike wave discharges occur. As a potential candidate gene, *CACNA1G* was screened in 73 Japanese and 50 non-Japanese patients. Of this group, 13 variants were identified and 5 of those caused amino acid substitutions.[46] One of these variants was found in a patient with a sporadic case of JME and a history of early childhood absence epilepsy. The mutation caused alanine to valine amino acid exchange at position 570, located in the intracellular portion of the protein within I-II loop. The mutation was not found in 360 control samples and when expressed in HEK cells, the mutated channels caused a larger Ca^{2+} current compared to the wild type, however the result was statistically insignificant. However, other variants may have affected the alternative splicing of the gene: at least five different isoforms with different kinetics and steady-state properties have been identified for the Cav3.1 channel.[47]

Mutation analysis was also performed on 48 Chinese patients who were shown to have a similar genomic structure to the Japanese in the HapMap project. Unfortunately, the research did not reveal any pathological change, but six new variants were detected. An association analysis was carried out to determine if there was a significant difference between controls, but it exhibited negative association.[48]

To assess the possible role of the $Ca_v3.1$ channels in inducing spike wave discharges, *CACNA1G* was deleted in mice models. Interestingly, animals became resistant to spike wave discharges, due to the LVA T-type channel being abolished.[49] In another study, *CACNA1G* was overexpressed in a mouse with low and high transgene copy numbers. This led to an elevation in α1G expression, and consequently, in the functional T-type currents in thalamic neurons.[50] Both transgenic lines showed SWD but did not indicate much of a difference in the frequency of seizures. Further, the mouse did not exhibit any other neurological disorders; providing direct evidence that an increase in $Ca_v3.1$ did lead to pure absence seizures.

The *CACNA1H* gene ($Ca_v3.2$) is located on chromosome 16p13.3 and expressed in the thalamic reticular nucleus.[49] It is extensively alternatively spliced and generates a family of variant transcripts.[51] The different variants shift voltage-domain for gating, the kinetics of activation, inactivation and recovery from inactivation, and the magnitudes and voltage midpoints for functional window currents. Thus, changes that affect the ESE regulatory sites in exons or splicing in intronic regions could predispose seizures. The first mutation analysis in *CACNA1H* was carried out with 118 Chinese CAE patients and revealed 12 missense mutations in 14 patients who were in a heterozygous state.[52] These mutations were introduced into human $Ca_v3.2a$ cDNA and transfected into HEK-293 cells for whole-cell patch-clamp recordings.[53–55] T-type channel activity was found to have increased in all mutant types causing SWD in absence seizures due to a shift in activation potentials. The channels were activated in response to a smaller voltage change, or a change in the rate of recovery of channels from the inactivated state (deinactivation), or an increase in the surface expression of the channels.

The possible role of *CACNA1H* in absence epilepsy was further confirmed by a study performed on another Chinese population where five exonic variations (P314S, N345N, P492S, L602L and S619S) and nine rare intronic variations were identified. While it was predicted that exonic variations would change either the transcription binding, or splicing sites, or the secondary structure of the channel, a common variation (rs2745150) in intron 11 was found to be highly associated with CAE, and was suggested to alter the potential splicing site.[56] Later on Liang et al. carried out an association analysis on 218 Chinese CAE patients and both a common variation (rs9934839) in exon 9 of *CACNA1H* and a common haplotype covering the gene were found to be significantly associated by both a case-control study and a transmission disequilibrium test.[57]

The *CACNA1H* gene was also screened in 192 Chinese IGE patients while the researchers looked for a common susceptibility allele.[58] The four variants found in patients were also found in some of the patient's unaffected family members, indicating a polygenic inheritance of IGEs. These variants were also assessed for functional studies and shown to have increased T-type currents with one exception; the A480T variant which did not lead to different current when compared to the wild type.[59] Considering the alternative splice variants, this variant may affect the regulation of transcription, causing the expression of a channel which is more prone to excitation. However, evaluation of *CACNA1H* in populations of Caucasian origin revealed no linkage or mutation in this gene.[60] These contradictory results emphasize the presence of population specific susceptibility alleles in complex disorders.

In a polygenic rat model of absence epilepsy (GAERS), a mutation was found in exon 24 causing Arg158Pro.[61] Further studies showed that *CACNA1H* had two splice variants in the thalamus, one with exon 25 and one without. The mutation caused significantly quicker recovery from channel inactivation and larger Ca^{2+} influxes during high-frequency bursts, but only when it was on the variant with exon 25. Therefore, the mutations and the spliced variant should be considered together when studying the function of the channel. These splice variants could provide an explanation of the mechanism of epileptic seizures in relation to the following questions: why are they temporal, have cell type specific effects, and why are seizures present in certain age groups, but not before or after that period?

The *CACNA1I* gene is located on the 22q13.1 and mainly expressed in the thalamic reticular nucleus.[49] However, when a mutational analysis was done with Chinese CAE patients, no mutations were detected.[62]

Non-pore forming modulatory subunits of Ca^{2+} channels is also a possible candidate for burst firing as it can regulate channel function, assembly, and localization. The β4 subunit can interact with both α1A (P/Q type) and α1B (N-type) subunits. A mutation identified in the β4 subunit gene (*CACNAB4*) of an epilepsy animal model (the lethargic mouse) displayed epileptic seizures and ataxia.[63] This mutation caused a truncated cytoplasmic protein and possibly caused other β subunits to coassemble with α subunits compensating for the mutation at the hippocampal synapses.[44] However, the thalamus β4 subunit is expressed highly, whereas the β1–β3 subunits are not; suggesting that there may not be a compensatory mechanism in the thalamus for the inhibitory function and thus, and the mutation may lead to SWD.

Mutations were also identified in the γ2 subunit gene (*CACNAG2*) of another epilepsy animal model (stargazer mouse).[64] This subunit has the potential to interact with both HVA and LVA channel types. The γ2 subunit plays a role in elevating the inactivation of calcium channels.[65] In both mutant mice there was an elevation of LVA current, as there was in the case of the tottering and the leaner mutant mice which have mutations in *CACNA1A*.[44]

In human studies, the γ3 subunit gene (*CACNG3*) located on chromosome 16p13.1-p12, was found to be associated with CAE in the European population: confirming the distinct roles of regulatory subunits in channel function, and consequently, in epileptic seizures.[66]

2.3. Non-ion channel genes

Epilepsy has for the most part been considered a channelopathy. However, mutations and susceptibility variations in novel non-ion channel genes have also been identified as the possible causes of the disease (see Table 1). The Malic enzyme 2 (*ME2*), located on chromosome 18, codes for the mitochondrial enzyme that converts malate to pyruvate and is also involved in neuronal synthesis of the neurotransmitter GABA. In a patient group of 88 JME and 68 JAE with EGTCS, *ME2* was found to be associated with all subtypes in a recessive model.[67] 35% of cases were homozygous for the nine SNPs that cover the *ME2* gene and its promoter, while only 8% of controls were homozygous.

In a family with hyperinsulinism/hyperammonemia (HI/HA), three children had both photosensitive myoclonic absence epilepsy and mutations in the glutamate dehydrogenase (*Glud1*) gene (10q23.3). The patients also had low levels of GABA compared to the control group.[68]

In a mouse model of absence epilepsy, named "jerky", mutations were identified in the JRK gene. The homolog of this gene (*JRK/JH8*) resides in humans on 8q24, which is a candidate region based on linkage studies.[69] Further, a rare mutation was identified in the case of a CAE patient who evolved to JME.[70] The protein of the gene has similarities to several nuclear regulatory proteins, suggesting that it might function as a DNA-binding protein.[71]

A polymorphism in the leucine-rich, glioma inactivated 4 (LGI4) gene was found to be associated with childhood absence epilepsy; however, the pathological effect of this variant was not clear.[72] This gene resides on 19q13.11 and has a recessive model of inheritance in absence seizures.

In recent years, a novel gene, *SLC2A1*, has come to be viewed as one of the most prevalent causative genes in early-onset absence seizures (those occurring before 4 years of age). The disease is not common and patients may have additional medical conditions such as movement disorders and intellectual impairment.[73] The gene resides on 1p34.2, coding for the GLUT-1 glucose transporter, which carries glucose across the blood-brain barrier. Mutations have been identified in 10% of examined patients with early onset absence seizures in the Suls et al. study, and in 12% of patients in the Mullen et al. study.[74,75] Functional studies on these mutations pointed out the reduced transport capacity of mutant proteins.

An association study with 205 Turkish IAE patients revealed a strong association of a common SNP (rs7588807) in the inhibin alpha precursor gene (*INHA*) in 2q36 to JAE and/or to IAE with GTCS.[76] The *INHA* gene has also been screened through DNA analysis and three potentially damaging mutations have been identified. Inhibin protein, commonly known as a gonadal glycoprotein, inhibits the secretion of follicle-stimulating hormone which in turn induces the production of progesterone and estradiol. This has also been shown to have expression in different parts of the brain with unknown function. The mutations and the associated SNP in inhibin protein are predicted to have either an indirect effect on absence seizures, as progesterone was shown to have enhanced SWD through allopregnanolone, a positive modulator of $GABA_A$ receptors,[77] or to have a direct effect by increasing the excitability of the brain.

3. Conclusion

These types of mutations in genes that code for the subunits of GABA and Ca^{2+} channels, identified in both patients and animal models, explain the cellular mechanism of absence seizures and the generation of SWD. Loss of function mutations found in the subunits of $GABA_A$ and $GABA_B$ receptors are the reason for the loss of inhibition in the thalamocortical network, while the gain of function mutations in the subunits of LVA Ca^{2+} channels cause excitatory activity, leading to a susceptibility to seizures. Mutations in *CACNA1A*, *CACNAB4*, and *CACNAG2* also reduce the function of the HVA channels, but this reduction results in an increase in the LVA current in the thalamocortical region of the brain. Unfortunately, the mutations and susceptibility alleles in ion channels are rare and restricted to a few patients or populations (see Table 1). However, this does support the possibility that the onset of SWDs may vary among individuals with similar or different absence syndromes, showing the complexity of this disease. Thus, negative results in one gene, in one population, should not mark the gene as "not a candidate" for absence seizures, as has been the case with many of the causative mutations identified in *CACNA1H* in the Chinese population which appeared to contradict the negative results of studies involving Caucasian patients.

A recent study examining the exonic variations of 237 ion channel subunit genes in both healthy individuals and patients with idiopathic epilepsies (IE) confirms the high genetic heterogeneity and complex pathogeneity in IE.[78] The results of this study suggest that a single variant, even if its effect was shown to be pathogenic by functional studies, may not be responsible for the disease, because missense mutations in ion channels have been identified in both study groups. Instead, each individual may have

a unique channotype (ion channel sequence variation profile) variant pattern which determines the network's excitability and the possibility of possessing the epileptic phenotype. Therefore, mutation analysis in complex diseases like epilepsy should not be restricted to single ion channel studies; instead, using next generation sequencing methods, such as exome sequencing, would be a more efficient way to reveal an individual's channotype and the possibility of risk for the disease.

In large families of epilepsy where there is a major gene, the genotype-phenotype relations are clearer as the mutations in the disease-related gene in other independent large families of epilepsy confirm the involvement of the gene in the pathogenesis of the disease. However, in the complex subtypes of epilepsy, like absence epilepsies, it is difficult to establish the link between genotype and phenotype, because the susceptibility variant identified by genetic studies may have little effect in the pathogenesis of the disorder. Thus, functional studies are essential to validate the mutations in the study of complex disorders.[79] For example, in the DNA analysis of *CACNA1G* in the Singh et al. study, a possible pathological mutation (A570V) was identified. The location of the mutation in the channel and the absence of the mutation in 360 control samples strongly support the argument for the pathological role of the gene. However, functional analysis of the mutated protein revealed an insignificant difference in the channel function when compared to the wild-type protein.

Besides the known function of the ion channel genes in membrane excitability, in recent years novel non-ion channel mutations have begun to emerge in absence epilepsies. Some could be explained through the mechanism of SWD by low levels of GABA in patients; however, some of them are still in need of further functional studies in order to verify their roles in absence seizures.

In other subtypes of IGEs, novel approaches such as deletion/duplication analysis and next generation sequencing methods have already revealed novel causative variations.[80] In the following years, in order to clarify the complex pathogenesis of the absence seizures, the search for the mutations and susceptibility alleles will be reinforced by studies in CNV, CGH analysis, exome sequencing, and epigenetic modification studies.

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