Studies of Epileptic Encephalopathies with GABRB3 variant

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Abstract. Epilepsy has been accounting for a significant proportion of human population. Estimated 50 millions people or 4 out of 1000 population have been affected by the epilepsy. As one of the categories of epilepsy, Epileptic Encephalopathy has been affecting a certain portion of people, especially among children, from infant to the age of 16. It contributes to severe cognitive and behavioral impairments. In recent studies on the genetic cause of the epileptic encephalopathy, scientists have found the association with GABRB3 gene. This review article is going to introduce an overview of the properties and function of the GABRB3 gene, including the receptor it is located in. Then this article will introduce different types of epileptic encephalopathy, including dravet syndrome, west syndrome, Lennox-gastaut syndrome, and myoclonic astatic epilepsy. And then summarize recent research and studies of patients with different types of epileptic encephalopathy, including the conditions of seizure onset, types of seizure appeared, position of mutation, and the type of mutation.

Keywords: epilepsy, Epileptic Encephalopathy, GABA, GABRB3.

1. Introduction

Epilepsy has been accounting for a significant proportion of human population. Estimated 50 millions people or 4 out of 1000 population have been affected by the epilepsy. The history of epilepsy can be traced back to the ancient civilization of the middle east. Throughout the history, from people first discovered the symptom to present time, the suspected treatments change from prescribed diets or living conditions, and surgery such as bloodletting or skull trephination and medical herbs [1], to the application of anti-epilepsy drugs in recent years.

As one of the categories of epilepsy, Epileptic Encephalopathy has been affecting a certain portion of people, especially among children, from infant to the age of 16. It contributes to severe cognitive and behavioral impairments usually above what might be expected from the underlying pathology. While the detailed cause of epileptic encephalopathy is unknown. Many studies have revealed an underlying association of epileptic encephalopathy with mutation of the GABRB3 gene.

Research found that gamma-Amionobutyric acid (GABA), the major inhibitory neurotransmitter in the cerebral cortex, plays a significant role in the mechanism of epilepsy. Abnormalities of GABA receptors are found to be associated with a large group of people diagnosed with epilepsy. GABA receptor is composed by 6 receptor subunits, GABAA1, GABAA6, GABAB2, GABAB3, GABAG2 and GABAD. Among these subunits, GABAB receptors appear to hyperpolize neuron, decrease calcium entry, and diminish the release of other neurotransmitter from presynaptic neuron [2].

This review article is going to introduce an overview of the properties and function of the GABRB3 gene, including the receptor it is located in. Then this article will introduce different types of epileptic encephalopathy, including dravet syndrome, west syndrome, Lennox-gastaut syndrome, and myoclonic astatic epilepsy. And then summarize recent research and studies of patients with different types of epileptic encephalopathy, including the conditions of seizure onset, types of seizure appeared, position of mutation, and the type of mutation.
2. **GABRB3**

2.1. **Gamma-aminobutyric acid (GABA)**

GABA is an amino acid that functions as the primary inhibitory neurotransmitter in the Central Nervous System (CNS). GABA is synthesized from the conversion of glutamic acid through glutamic acid decarboxylase [3], in GABAergic neurons axon terminal. GABA functions by binding to GABA receptors in the postsynaptic neurons of GABAergic neurons in the CNS.

2.2. **GABA receptor**

GABA receptor is considered the major inhibitory receptor in the central nervous system (CNS), it is subdivided into 2 subtypes, GABRA, GABRB [4].

GABAA subtypes are ligand-gated transmembrane ion channels on the postsynaptic neurons. A GABAA receptor consists of 5 receptor subunits [5], a pentamer in transmembrane receptor. However, there are numerous subunit isoforms for GABAA, including GABRA1, GABRA2, GABRB1, GABRB3, and, GABRG1. At the center of the receptor is the chloride ion pore [6]. Once specific ligands like GABA neurotransmitter bind to the GABA site, the chloride ion pore is opened, allowing chloride ion to go through the membrane [7].

GABAB subtype, unlike GABAB receptor, are G protein-coupled receptors [8]. When agonists like GABA neurotransmitter is bound to GABAB, Binding of GABAB receptor with agonists like GABA neurotransmitter release the G beta-gamma complex that is activated from inactive heterotrimeric G protein complexes (G alpha-beta-gamma complexes) [9]. Finally, the G gamma-beta protein activates a specific type of potassium channel, G protein-coupled inwardly-rectifying potassium channel (GIRKs), which results in the inward of potassium ion and hyperpolarize the membrane potential.

![Figure 1](https://via.placeholder.com/200)

Figure 1. Structural location of GABAA receptor variants [10].

2.3. **GABRB3**

GABRB3 is a protein subunit of GABAA receptor. According to National Library of Medicine, the GABRB3 gene is located on the long arm of chromosome 15, the q12 region in the human genome. It spans 250kb, including 18 exons in the region. Studies have revealed that this gene is not only associated with epilepsy, but also autism, Angelman syndrome, Prader-Will syndrome, and no syndromic orofacial clefts [11].

There are two translation start sites in the GABRB3 exons: exon 1a and exon 1, where exon 1 is located downstream from 1a. The two exons are followed by the same exon 2-4, which respectively result in the alternative transcriptional isoform 2 and 1 [12].
3. Epileptic encephalopathy

3.1. Epileptic encephalopathy

Epileptic encephalopathies (EE) are characterized by a group of seizures in which cognitive, sensory, and motor functions are dysfunctional. It is usually diagnosed within the younger age group [13]. EE can be categorized into two types according to the pathology. One type of EE is typically produced by frequent severe seizures, for example, frequent onset of febrile seizures is likely to progress into dravet syndrome. This type of EE includes Migrating Partial Seizures, Dravet Syndrome, and Ring Chromosome 20. Another type of EE is typically observed to have continuous or nearly continuous spike and slow wave activity [14], which is examined through EEG. This type of EE includes West Syndrome, Lennox-gastaut syndrome, and Myoclonic Astatic Epilepsy.

3.2. Dravet syndrome

Dravet syndrome is the type of epileptic encephalopathy produced by frequent onset of severe seizures. The sign of dravet syndrome usually begins in the middle of the first year of life (between 2 and 10 months), with febrile seizure, in which patients experience a seizure triggered by fever. Progressively, convulsions will occur without any fever, and additionally will develop partial seizures, generalized seizures, myoclonus, and absences [13]. Most the Dravet syndrome is because of the mutation of SCN1A gene. In recent studies, Mutations of GABRB3 gene, have been identified in patients with dravet syndrome [15].

There is an observation of a case where a mutation of GABRB gene corresponds to dravet syndrome. In the observation, a female infant had experienced 3 episodes of febrile seizures by her eighth month. The electroencephalography (EEG) showed bilateral slow waves mainly evident in occipital regions. At the age of 2.5 years, the EEG pattern showed a slow background activity with poly spikes and wave discharges. These examinations led to the diagnosis of DS. At the age of 7, Next generation sequencing (NGS) was carried out and two likely pathogen Eric mutations were found, one was a heterozygous missense variant of GABRB3(geneNC_000015.9:g.26806317C>T) (p.Thr281Ile; c.842 C>T) at chromosome 15q.12, and another was a heterozygous nonsense variant of the BBS4 gene (NC_000015.9:g.73023914C>T) (c.883 C>T; p.Arg295Ter) at chromosome 15q24.1 [4].
There is another observation on 2017 that shows the correspondence of DS and the mutation of GABRB gene. In the study, exome sequencing and variant validation is conducted in 6 patients who were diagnosed with the DS. The study came out that they identified 22 de novo variants, of which 5 were missense variants. One of the 5 missense variant was a heterozygous variant in GABRB3(c.695G>A, p. [Arg232Gln]) [12]. Notice that unlike the case reported above, patients in this study do not find abnormality in EEG examination. In addition to another study, two families whose family members have all experienced the febrile seizure are studied. In this study, all patients haven't been detected to have abnormalities in EEG. Plus, the protein position detected to have GABRB3 mutation is identical within a family, while it is different between two families. For one family, the protein position detected is c110T>G, p. V37G, paternal, another is c.470C>T. P. T157M. Maternal [16].

In conclusion to the three studies above, although most of the cases diagnosed with Dravet Syndrome do not find abnormality in EEG examination, one case is detected with slow spike waves in EEG. Also, within a family, members who are diagnosed with Dravet Syndrome can find GABRB3 variant in the same protein position.

3.3. West syndrome

The West Syndrome is a well-defined epileptic encephalopathy. Patients with the combination of clusters of spasms, psychomotor deterioration, and hips arrhythmia are considered as west syndrome. The condition of hypsarrhythmia consists of generalized high-amplitude activity of spike and delta theta slow waves that are continuous when awake and fragmented in sleep, with absence of normal physiologic activity. The most common group of people with West Syndrome onset is infant age between 3 and 12 months. Onset of Lennox-gastaut syndrome can be followed after the West Syndrome.

A study focuses on 3 male infants who are identified with the West Syndrome associated with GABRB3 variant. The seizures onset appeared between the first day of birth and the 8th month after birth, experiencing generalized tonic-clinic seizure, tonic seizure, infantile spasm, febrile seizures, and fever. Two types of features are found in EEG examinations. The EEG on one patient reveals a hypsarrhythmia and generalized synchronized spike wave. While the EEG on the other two patients reveals a multi focal low spike wave. Through next-generation sequencing panel diagnostics, the group detected 3 areas of mutation respectively, including first, the position of mutation 767T>A, de novo mutation, and the position of protein encoded, L256Q, second, the duplication of exon 1-9 of the GABRB3 gene, de novo mutation, and third, the position of mutation 905A>G, de novo mutation, and the position of protein encoded, Y302C [17].

3.4. Lennox-gastaut syndrome (LGS)

LGS is a well-known epileptic encephalopathy. Patients with LGS often have the clinical feature of atypical absences, tonic seizures, cognitive deterioration, and slow spike wave activity in the EEG. Focal, multi focal, or diffuse brain damage are considered as the reason for LGS, also, LGS can be resulted by West syndrome. In addition, LGS is closely related to a syndrome severe epilepsy with multiple independent spike foci (SE-MISF), where patients experienced refractive epileptic seizures, psychomotor retardation, and their EEG shows epileptiform discharges that raised from at least on focus in each hemisphere [18]. The age of LGS onset is usually from 1 to 10 years of age.

A large-scale study of the de novo mutation in epilepsy encephalopathies identified the mutations of GABRB3 in LGS. The study focuses on 115 infants incentivized with LGS. They sequenced the exome of the 115 probands as well as their parents, and confirmed a series of de novo mutations. Among the mutations, GABRB3 variants are found in four patients. Among the four patients, 3 of them are identified with LGS that had evolved from infantile spasms. The variant genes reported in these three patients include D25N, E109G, and Y302C [19].

There is another study focused on a female child who is identified with the LGS associated with GABRB3 variant. The child had her first seizure onset on her 17th month, experiencing atonic seizure,
dyscognitive, generalized tonic-clinic seizure, and fever. The intellectual ability is severely dysfunctional, along with strabismus, hyperactivity, and aggression. The EEG examination shows multi focal low spike waves. By next-generation sequencing panel diagnostic, the study identifies the position of mutation, 905A>G, de novo mutation, and the position protein encoded, T157M [20].

3.5. Myoclonic astatic epilepsy

Myoclonic astatic epilepsy, also known as Doose syndrome, is the type of EE that typically with continuous spike and low wave activity. Normally affects children between 2 and 5 years old, mainly boys. In the first month of disease, generalized tonic-clinic seizures are the only type of seizures onset, where patients lose consciousness and have stiffening and jerking of muscles, affected by the abnormality brain activity on both sides of the brain. Then the seizure occurs more frequently, normally causing several times a day. Bursts of generalized slow spikes and waves are detected with EEG.

A study has revealed the association of GABRB3 variant with the Myoclonic astatic epilepsy. The study focuses on 4 boys and 1 girl who have the myoclonic astatic epilepsy, and find the GABRB3 variant using next generation sequencing panel. The patients typically experienced frequent myoclonic atomic seizure, generalized tonic-clinic seizure, febrile seizure, and fever. In addition, the observations on EEG vary from generalized spike wave to poly spike wave [21]. 5 different mutation sites were found, including first, the position of mutation, 8delG, unknown type of mutation, and the position of protein encoded, Gly3fs*26, second, the position of mutation, 227C>G, de novo mutation, and the position of protein encoded, p.S76C, third, the position of mutation 331C>T, maternal mutation, and the position of protein encoded, R111* fourth, the position of mutation, 425G>T, maternal mutation [22], and the position of protein encoded, R142L, (mosaic: 10%–20%), and fifth, the position of mutation 550T>C, de novo mutation and the position of protein encoded, Y184H, de novo mutation. [23]

3.6. Other seizures and epilepsies associated with GABRB3 variant

In some cases, patients may have onset of only one seizure, which is not able to be diagnosed as epilepsy. However, the seizure may be associated with an epilepsy, either having the similar cause as an epilepsy, or being developed into an epilepsy later. For example, the three LGS patients studied by Allen et al. 2017 are developed from the seizure infantile spasm. As a result, it is important to notify the seizures associated with EE. In the same study done by Allen et al. 2017, among the four patients identified to have GABRB3 variant, three of them are found LGS developed from infantile spasm, while the remaining one patient is identified with infantile spasm and is not present with any other seizure, in addition, hypsarrhythmia is found through EEG examination. In this patient, the de novo missense mutation is identified, which is N25D.

Variant of GABRB3 is also found to be associated with Childhood Absence Epilepsy (CAE). A study of GABRB3 variant in CAE reveals a physiological change as a result of a mutation. In analysis of four unrelated families where typical CAE phenotype are shown, three mutations of GABRB3 are identified, P11S, S15F, and G32R. The P11S and S15 locate in GABRB3 exon 1a, and the G32R locates in exon 2 and is located at the N-terminus of the GABRB3 subunit. The group compared a wild-type GABAA receptor with a GABAA receptor consisting of a mutated GABRB3 subunit, and it shows a different electro physiological property. The GABAA receptor with variant GABRB3 shows an attenuated chloride ion current. This dysfunction of chloride ion current is a cause of the hyperglycosylation in CAE.

4. Conclusions

A certain amount of studies and observations have indicated the association of GABRB3 gene variant with the epileptic encephalopathy, leading a wide range of diseases. The studies show that cases of EE with GABRB3 mutation have variety of symptoms (seizure onset, age of onset, etc.), and
the variety of mutation position. Although many cases have been recorded, study on the pathology is lack. It will be important to refer to the study on pathology of GABRB3 variant on other similar diseases, or the EE diseases with other gene mutation such as the SCNIA mutation.

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