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ABSTRACT
GABRB3 gene is a recently identified gene located in 15q12 chromosome and encodes for gamma-aminobutyric acid (GABA) receptor subunit beta-3 protein, which is linked to the GABAA receptor. The gene is believed to share a role in inhibitory GABAergic synapses, GABA iron-gated channel function, and possible cellular response to histamine. The β3 subunit is expressed in cerebral grey matter, thalami, hippocampi, and cerebellum, among other structures. Faulty *GABRB3* function is linked to several neurological disorders and clinical syndromes. However, the spectrum of such disorders is not yet well known. We present three case reports highlighting the potentially expanding clinical phenotype and variable expression in children with mutated *GABRB3* gene.

The *GABRB3* gene is a recently identified gene in a locus known with various clusters of major genes in the long arm of chromosome 15.[1] This 15q11-q13 locus is a known region of different genomic DNA deletions and duplications related to some neurodegenerative and neurodevelopmental disorders including, for example, Angelman syndrome and autism spectrum disorder (ASD).[2,3] Several gamma-aminobutyric acid (GABA) genes are mapped to the nearby 15q12 locus.[4] It is believed that any changes in the GABAergic signaling pathway are likely to result in several neurological disorders. However, the spectrum and extension of such clinical implications are yet to be unraveled. Our three reported cases do illustrate the likely wide variation in clinical phenotype in heterozygous GABRB3 mutations.

CASE REPORT

Case one

A previously healthy male infant was thought to have a normal neurodevelopmental profile till the age of 20-month-old at presentation. He was born at term after an uneventful pregnancy and uncomplicated normal delivery. His family history was negative for any neurological or developmental disorders in close blood relatives. He was noted to have several worrisome observations around the age of 20 months old, including progressive loss of consistent eye contact, consistently irritable mood with inconsolable high pitch cry, and worsening loss of prior vocabulary and speech abilities. He was described as more clumsy and noted to have more frequent falling than usual as he started walking around the age of one-year-old. Multiple family members noticed overall worsening with decreased interaction with family members and usual social cues. Because of the numerous concerning observations, his primary care physician initiated an urgent referral to the neurology specialist service. Upon evaluation by neurology service, he was found to be well-looking with no signs of acute illness. However, he had multiple abnormal neurological exam findings such as poor eye contact, irritable mood, diffusely decreased axial and appendicular tone, along with brisk deep tendon reflexes throughout all extremities. No nystagmus, cranial nerve palsies, striking abnormal facial features, or neurocutaneous signs were noted. No joint contractures or deformities were found. With this concerning neurological decline and abnormal physical exam, a brain magnetic resonance imaging (MRI) with magnetic resonance spectroscopy was completed and interpreted as an unremarkable study. Basic and targeted metabolic screening was obtained, including lysosomal, thyroid, amino acid, fatty acid, and immune disorders, which were all found to be negative. DNA genomic micro-array sequencing test was then done and showed heterogeneous de

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novo p.L3101 pathogenic variant of *GABRB3*. Although his variant was not previously reported, it was thought to be pathological and likely represents the underlying etiology of his clinical symptoms and signs. The patient was started on an intensive rehabilitation program. Interestingly, his response to physical, occupational, and speech therapy services were positive and remarkable, and he continues to make developmental progress.

Case two

An 18-month-old female infant presented to the emergency department for the first time with likely a brief febrile seizure. She was a product of an uneventful pregnancy, regular reassuring prenatal care, and spontaneous vaginal delivery at 38 weeks estimated gestational age. She attained all her motor and social developmental milestones within the normal range expected timelines with no regression. However, she was observed to have mild expressive speech delay as she was able to only say five to six words at the age of 18 months and could not combine words. Her family history included a cousin with childhood-onset epilepsy and another cousin with febrile seizures. Upon presentation to the emergency room, she had an apparent focal onset for her seizure with lateralized versive eye gazing, focal unilateral facial twitching, and unilateral body shaking. She was febrile with some upper respiratory symptoms but no other symptoms or signs of serious or central nervous system infection. Her exam was reassuring after recovering from the postictal period, and no focal neurological signs were elicited. Extensive infectious workup was negative. During hospitalization, she had two more similar seizures. Because of seizure recurrence and onset focality, she was ultimately diagnosed with a complex febrile seizure. She was temporarily started on levetiracetam because of seizure recurrence with anticipation of the need for only short-term course. Her electroencephalogram (EEG) study was normal. Her brain MRI demonstrated a borderline Chiari 1 malformation but was otherwise unremarkable and showed no evidence of hippocampal sclerosis or focal cortical dysplasia. Given the mild speech delay background, atypical clinical course for febrile seizures, and family history, genetic testing was then considered. Chromosome microarray revealed p.P54L heterozygous autosomal dominant pathogenic variant of *GABRB3*. A diagnosis of generalized epilepsy with febrile seizures plus (GEFS+) was determined. Her seizures were well-controlled with levetiracetam and medication weaning is planned.

Case three

This male patient was born full-term (40 weeks estimated gestational age) after a largely uncomplicated pregnancy and delivery with no neonatal resuscitation or critical care needs. He had normal behavioral and developmental progress up to near puberty age, and no major health comorbidities were noted. With a rather acute history, he presented at the age of 12 years old with an acute mental status change in the form of sudden onset of repeating certain phrases inappropriately outside the situational context. Other interesting behaviors were also observed, such as continuous scribbling and walking around in circles. Mental state examination revealed deficits in short-term memory and abstract thinking. However, physical and neurological exams were otherwise unremarkable. He was diagnosed preliminarily with acute encephalopathy. Complete blood count, chemistry profile, drug screening, and basic metabolic profile were all within normal limits. Head computed tomography scan was normal. As a part of medical evaluation, EEG was requested and showed near continuous spike and slow-wave complexes consistent with non-convulsive status epilepticus. Anti-seizure medications were initiated, and he showed a good initial response to the benzodiazepine trial with lorazepam and midazolam. However, his seizures were difficult to control over time, so detailed epilepsy evaluation, including genetic studies, was pursued. Epilepsy expanded panel was then obtained, which showed a p.R217C heterozygous autosomal dominant pathogenic variant of the *GABRB3* gene. His seizures remain difficult to control with standard anti-seizure drugs. Although his neuroimaging studies did not suggest any additional epilepsy risk, he is now undergoing further epilepsy surgery evaluations.

DISCUSSION

Chromosome 15q11-13 is a complex genetic locus that encloses three GABAA receptor subunit (GABR) genes, *GABRB3*, *GABRA5*, and *GABRG3*. *GABRB3* gene encodes for a member of the ligand-gated ionic channel protein, which is one of the

subunits of a multi-subunit chloride channel that serves as the receptor for GABA, a major inhibitory neurotransmitter of the mammalian central nervous system.[5] Usually, both the wild-type and mutant β3 subunit-containing α1β3γ2 GABAA receptors are attributed to impaired intracellular β3 subunit processing. It is believed that the β3 subunit is expressed in various neuromodulatory circuits in the cerebral cortex, hippocampus, and thalamic reticular nucleus, where they mediate phasic and tonic inhibition.[6] *GABRB3* gene mutation disorders continue to constitute a diagnostic challenge given the wide spectrum of linked clinical features and associated syndromes.[7–10]

The relationship between GABRB3 mutations and traits of ASD has been studied in several systemic reviews, but results seem to be conflicting. Some studies have demonstrated that single-nucleotide polymorphisms (SNPs) in *GABRB3* have a statistically significant predictor value for development of ASD.[11–13] However, expression of *GABRB3* in a cohort of patients with ASD seems to be aberrant. A recent meta-analysis by Noroozi et al,[14] suggested that some GABRB3 variants are not associated with increased risk of ASD. In fact, there is some evidence that different SNPs of GABA receptor B3, A5, and G3 subunit genes located on chromosome 15q11-q13 are not associated with the development of ASD in different ethnic populations.[15] Our first case testing showed a de novo pathological p.L3101 variant in the *GABRB3* gene. To our knowledge, this variant was never reported before in a cohort of patients with ASD and GABRB3 mutations.

The contribution of *GABRB3* gene alterations to increased risk for febrile seizures in children is still not well understood. Across the spectrum of GEFS+, GABRB3 mutations were found in a small group of patients. Mutations in SCN1A or GABRG2 are better studied in GEFS+ cohort of patients.[16] In a recent review by Møller et al,[17] 416 patients with febrile seizures and early life epileptic encephalopathy were screened, identifying 22 patients with heterozygous mutations in *GABRB3*, including three probands from multiplex families. At the severe end of the clinical spectrum, GABRB3 mutations are thought to be closely associated with early-onset epileptic encephalopathy, although SCN8A and CDKL5 mutations are more prevalent.[18] Some recent reports suggest *GABRB3* is a potential etiological substrate for severe myoclonic epilepsy of infancy, also known as Dravet syndrome.[19] Our reported second patient is thought to fit the diagnosis of GEFS+ as she continues to progress developmentally so far. Her overall clinical progress and good control of seizures with low-dose levetiracetam make the possibility of other epileptic encephalopathies less likely.

Whether there is a substantial correlation between alterations in the *GABRB3* gene and lower seizure threshold is an actively debated topic in the era of epilepsy epigenetics. The spectrum of possibly linked epilepsy syndromes is expanding, from more benign syndromes like childhood absence epilepsy to epileptic spasms and Lennox Gastaut syndrome. Recent studies suggested that impaired receptor localization to synapses is a common pathophysiological mechanism for GABRB3 mutations, although the extent of impairment may be different among mutant subunits, leading to variable epilepsy-related phenotypes.[20,21] GABRB3 mutations were also noted in children with unclassified epilepsy along with other genetic alterations after more utilization of epilepsy genetic panel testing. No specific seizure classification has been described, thus emphasizing the role of genetic heterogeneity. Whether GABRB3 mutations can result in more severe phenotype and refractory epilepsy is not yet known. Our third reported patient is running a super refractory epilepsy clinical course and is currently under evaluation for possible epilepsy surgery consideration. Our patient had non-convulsive status epilepticus upon presentation, which has not been reported in patients with GABRB3 alterations. A recent review of the genetics of pediatric intractable epilepsy did not consider GABRB3 among the genetic mutations which are known to be associated with difficult-to-treat epilepsy.[22]

CONCLUSION

GABAA receptor β3 subunits are widely expressed in the developing brain. Mutations in *GABRB3* gene appear to have a wide spectrum of clinical implications. We believe our three reported cases will add to the available knowledge about variations across both genotype and phenotype spectrum. More reports of this type will ultimately improve our understanding of how these genetic alterations are being translated into clinical manifestations for a wide range of neurological disorders, particularly in

the era of improved access and evolution of available genetic testing techniques and the whole future of precision-based medical care.

Disclosure

The authors declared no conflicts of interest. The authors confirm that patient identifiers have been omitted from the study and legal guardians have given a no objection verbal consent to the publication of general scientific data.

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