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Original Article

Mutational screening of GABRG2 gene in Pakistani population of Punjab with generalized tonic clonic seizures and children with childhood absence epilepsy

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Abstract

Background: Epilepsy is a multifaceted and multistep disorder that disrupts the proper functioning of neurons. It is becoming increasingly clear that the responsiveness of neurons depends on the appropriate trafficking of ions across the channels in the membrane of neurons. In line with this notion, impairment among these ion channels due to mutations has gain increasing attention in molecular neuroscience.
Methods: Mutation analysis of the coding exons (exon 3, 5 and 9) was performed by sequencing GABRG2 to identify any complex biological entities among two different types of epilepsies.
Results: Sequencing of the candidate gene "GABRG2" revealed a single polymorphic site in exon 3 in the children with absence epilepsy and generalized tonic clonic seizures. However, this single nucleotide alteration was more common in the patients with childhood absence epilepsy patients compared to the generalized cases.
Conclusion: A silent mutation was identified at locus 27,909 C > T in 30.66% of the total screened or analyzed cases. However, no single nucleotide polymorphism was identified in exon 5 of GABRG2 in a Pakistani population, in contrast to a study of Chinese patients with childhood absence epilepsy.

Keywords: Childhood absence epilepsy (CAE); GABA receptor; GABRG2 gene; Generalized tonic clonic seizures (GTCS)

1. Introduction

Epilepsy is multifactorial and one of the most common types of brain disorder, characterized by recurrent seizures and reported to occur in 0.5−1.0% of the world's population. In Pakistan, epilepsy is also a prevalent paroxysmal neurological disorder that accounts for 1% of the population.[1] Seizures are the core symptom of epilepsy, and are defined as aberrant discharges of electrical signals from a variety of neurons in the brain. Various brain insults can cause epilepsy, including birth injuries, birth defects, head injuries, bleeding in the brain, brain tumors, brain infections or stroke, however few epileptic cases have been reported to be inherited. More than 20 abnormal genes have been reported to be key biological culprits of epilepsy, and their abnormal molecular cross-talk has been linked with the onset of different types of epilepsies.[2]

Conflict of interest: The authors declare that they have no conflict of interest related to the subject matter or materials discussed in this article.

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Absence seizures tend to be more frequent in children, however they can also occur in adults. Some children can have hundreds of absence seizures per day, which can cause substantial disruption to their lives. Absence seizures can be identical to and confused with daydreaming. Some absence seizures last longer than a few seconds and involve minor movements of the body, such as jerking of the shoulders. These seizures are defined as atypical absences, and these patients can also have learning disabilities, and may also suffer from other types of seizures.[3,4] Epileptic seizures occur more frequently in women than in men, and some women have seizures as a result of fluctuations in estrogen and progesterone during their menstrual cycle referred to as catamenial epilepsy.[5]

Epileptic seizures can be divided into a variety of categories. They can cause behavioral problems such as staring (absence epilepsy), involuntary movements (generalized tonic clonic seizures, GTCS), or seizures restricted to selective regions of the brain (focal seizures) in one hemisphere.[6] According to the International League Against Epilepsy (ILAE) classification 2017, epilepsies are categorized under three broad levels. Seizure type, epilepsy type and epilepsy syndrome. Non-epileptic and epileptic events are distinguished according to the seizure type on the basis of focal, generalized and unknown onset. A new subtype, "combined generalized and focal epilepsy" has been introduced at the epilepsy type level along with focal, generalized and unknown epilepsies. Generalized epilepsy encompasses absence, tonic, atonic, tonic-clonic and myoclonic. Seizures originating from one hemisphere are categorized as focal epilepsy that may be multifocal or focal disorders. The unknown type has a heterocomplex nature and has to be evaluated at the molecular level. The final level of the recent classification is "Epilepsy Syndrome Diagnosis," which is made on the basis of clinical findings from Electroencephalography (EEG), imaging studies, etiology and prognostics and is also associated with treatment.[7] Absence epilepsy involves loss and regain of consciousness within a brief period of time and is characterized by recurrent blank spells. Some important types of epilepsy are illustrated in Fig. 1.

GABRG2 encodes a gamma-aminobutyric acid (GABA) receptor. GABA is the major inhibitory neurotransmitter in the mammalian brain, where it acts on GABAA receptors, which are ligand-gated chloride channels. This ligand-receptor binding results in the influx of chloride ions through ion channels. It has been suggested for many decades that disruption of GABAergic neurotransmission mediated by GABA may be involved in epilepsy.[8] Enhanced activation of GABAA receptors of neurons limit the pacemaking capacity of these cells, and therefore play a prominent role in decreasing the tendency of generating absence seizures.

Exonic mutation in the gene encoding the gamma-2 subunit of the type-A gamma-aminobutyric acid receptor (GABRG2)

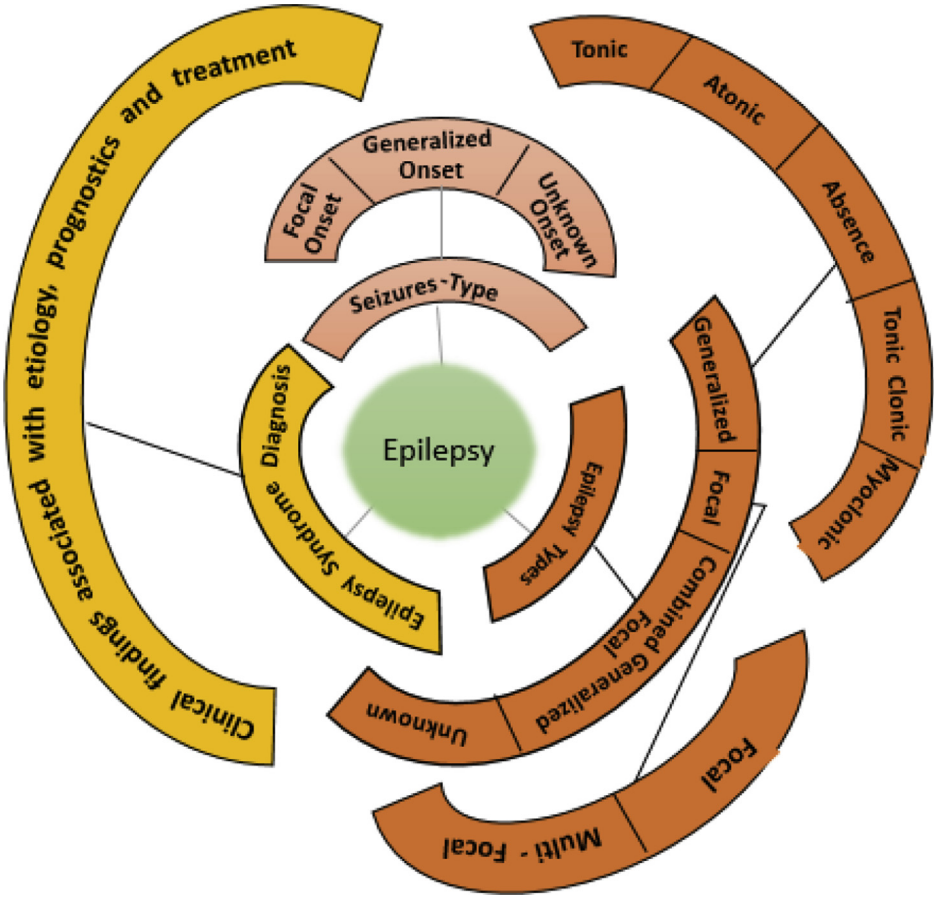

Fig. 1. Classification of epilepsy according to the International League Against Epilepsy (ILAE) (2017 report of ILAE Classification core group).

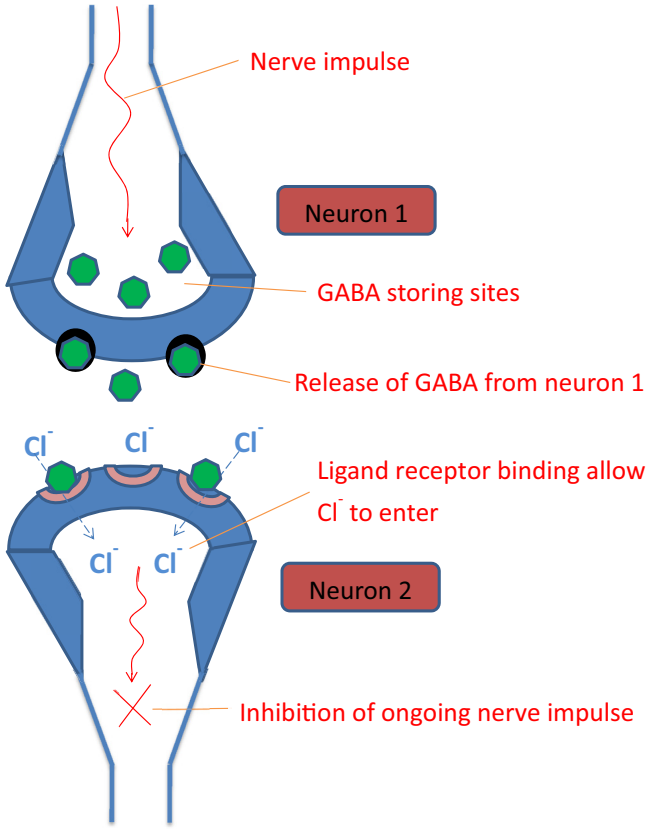

Fig. 2. Showing inhibitory effect of GABA.

have been reported to cause CAE, febrile seizures (FS) and generalized epilepsy with febrile seizures (GEFS+).[8] As previously discussed, $GABA_A$ receptors are ligand gated ion channels and its ligand GABA is an inhibitory neurotransmitter in the central nervous system (CNS). Upon receptor-ligand coupling, the $GABA_A$ receptor successfully conducts Cl^- ions across the boundary through its pores, resulting in hyperpolarization of neurons and imposing an inhibitory effect on neurotransmission (Fig. 2).

The active site in the $GABA_A$ receptor serves as a target site for GABA and a variety of drugs including gaboxadol, bicuculline and muscimol. Along with these target sites, several allosteric sites also serve as targets for ethanol, benzodiazepines, nonbenzodiazepines and other agents to halt seizures.[9,10]

2. Methods

The present study focused on two important types of epilepsy, the most complicated but rare epileptic type in children "CAE," and the general type of epilepsy in both children and adults, "GTCS" by focusing on three exonic sections of GABRG2. The study was conducted at the Molecular Biology and Genomic Laboratory, Institute of Biochemistry and Biotechnology (IBBT), University of Veterinary and Animal Sciences (UVAS), Lahore.

Forty blood samples from patients with CAE and 55 from patients with GTCS were collected. All of the patients had been previously diagnosed by neuro-physicians on clinical grounds supported by EEG findings from Ganga Ram Hospital, Lahore and Children Hospital, Lahore. CAE is characterized by the absence of body jerks during fits (no turning of hands), staring spells, brief seizure duration (4–10 s), and other factors. Ethical approval from the institute and hospitals to conduct the study was obtained. Data on the patient's' age, sex, and location were recorded. An Organic method of DNA extraction was followed in order to obtain

Table 1
Primers, annealing temperature and product size of exons.

Exons	Forward	Reverse	SNP	Temp.	Size (bp)
GABRG2 (NNCBI Gene ID: 2566)					
3	AAACTCTACTATGCGTGCTT	GCTATTTCCATGACATCAC	AAC>AAT	56.0	364 bp
5	CACTCTGTGTTTTCAATCAG	CTTCTTTTGCAGGAATCAC	–	55.0	396 bp
9	GAACCAGGCAATAGAAAAC	CATCTCACAGGGATTATAGC	–	56.0	549 bp

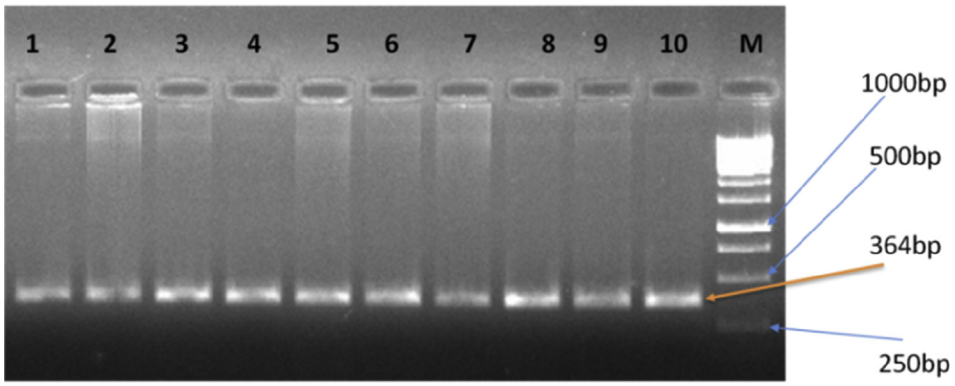

Fig. 3. PCR amplified bands of exon 3 of absence epilepsy patients. The amplicons coincided at 364bp ladder band.

the required amount of genomic DNA, and conventional PCR amplification was performed. The amplified PCR products were subjected to sequencing. We focused on the three exonic portions of the GABRG2 gene including exon 3, 5 and 9 (Table 1). The set of primers was designed using Primer 3 software.

3. Results

Conventional PCR analysis was performed to amplify exons 3, 5 and 9 of the GABRG2 gene. All of the samples were subjected to PCR amplification, and positive amplification was achieved when the primers were used (Fig. 3).

To compare the sequencing results of mutated sample with healthy controls (wild type) of the same region, we identified a polymorphism (silent mutation) at position 27,909 of the GABRG2 gene, where the nucleotide C had been replaced by T. The molecular analysis strongly suggested that this heterozygosity may play an additional role towards the onset of seizures along with other genetic and epigenetic events. This single nucleotide polymorphism was identified in exon 3 of the patients with CAE. Fig. 4, clearly shows the banding pattern difference between the wild type (healthy controls) and mutated version of absence epilepsy.

However, when the sequencing pattern of the same position was compared with GTCS, a silent mutation was identified instead of a heterozygous pattern of sequencing peaks. Fig. 5, illustrates a comparison of the patients with GTCS and the wild type (healthy controls). Similar silent mutations were also observed in the nine patients with CAE.

In brief, we did not find any mutations in exon 5 or exon 9 of the GABRG2 gene among both types of epilepsy (CAE and GTCS), in contrast to a study of Chinese patients with CAE.[11]

4. Discussion

GABA receptors are G protein-coupled receptors which have been shown to be essential inhibitory neurotransmitters in the CNS. Considerable advances have been made in the understanding of the molecular composition of these

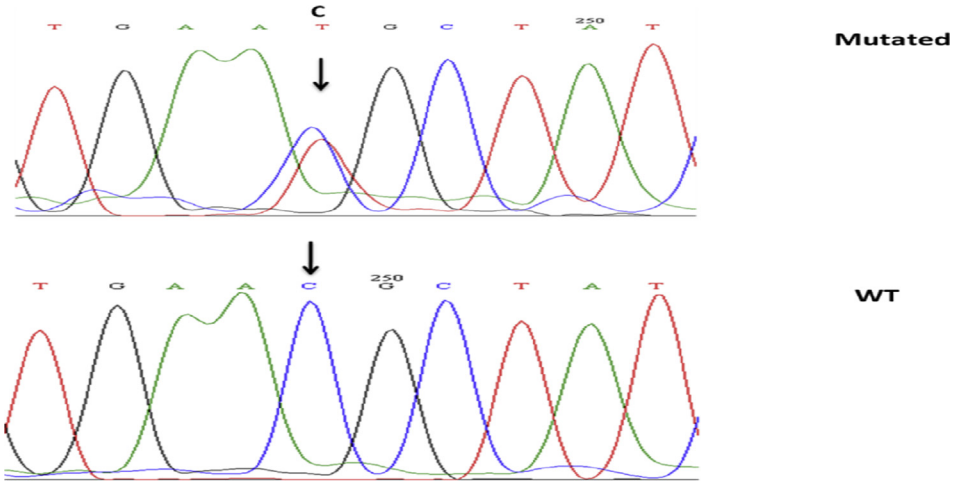

Fig. 4. Sequencing chromatogram showing the wild type (WT) and mutated (silent mutation) sequence at the position 27,909 of GABRG2.

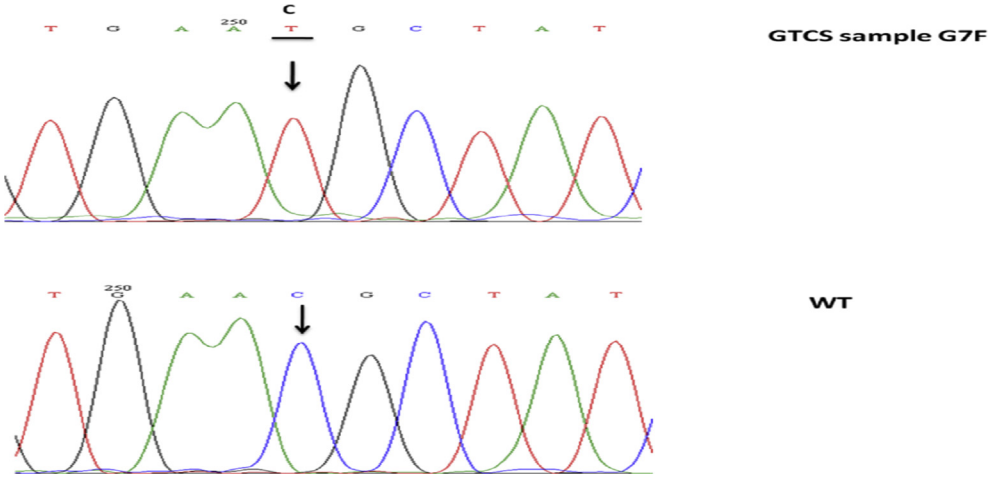

Fig. 5. Sequencing chromatogram showing the wild type (WT) and heterozygous mutant sequence at the position 27,909 of GABRG2.

receptors. GABA receptors are now known to be comprised of principal and auxiliary subunits that trigger receptor properties in a distinctive manner.

Advances in the field of neuroproteomics have helped to clarify the expression, interaction and function of proteins in the nervous system. Accordingly, neuroproteomics can also help to clarify the organization of dynamic, functional protein networks and macromolecular structures that underlie the physiological, anatomical and behavioral processes in epilepsy.

It is unquestionable that therapeutic approaches for the management of epilepsy are limited due to the multifactorial nature of the disease. In this study, we focused on two major types of epilepsy, CAE and GTCS by targeting the GABRG2 gene. Mutation analysis of the coding exons (exon 3, 5 and 9) was performed by sequencing of GABRG2 to identify the complex biological entities in both types of epilepsies. The exons were amplified and the amplicon size was comparable to reported in previous studies.[11,12] The primers used in this study were designed by slightly modifying previously reported sets of primers.[12] This is the first study performed in a Pakistani population focusing on GABRG2.

GABRG2 has recently been identified to be a cause of the onset of epileptic seizures. Mutations in this gene have been associated with both epilepsy (CAE and GTCS) and febrile seizures.[13] GABRG2 encodes GABA receptors that are fundamental inhibitory neurotransmitters in the mammalian brain, and also ligand-gated chloride channels. This ligand-receptor interaction facilitates the inward movement of chloride ions through the channels, and this hyperpolarizes neurons, which then induces the inhibitory effect of neurotransmitters.

Several studies conducted in the Western population of China and Japanese patients analyzed 20 healthy controls and 95 clinically diagnosed patients, and the amplified regions were sequenced in order to identify any missense, silent mutations or single nucleotide polymorphism.[11,12] Sequencing of the candidate gene "GABRG2" revealed a single polymorphic site in exon 3 of the patients with CAE and in those with GTCS. However, this single nucleotide alteration was more commonly identified in the patients with CAE compared to those with GTCS. The Silent mutation was identified at locus 27,909 C > T of 46.66% of the total screened or analyzed cases. These silent mutations at the same location of this exon have already been reported in a Japanese population.[10] However, we did not identify any single nucleotide polymorphism in exon 5 of the GABRG2 gene of our Pakistani population, in contrast to a study on Chinese patients with CAE.[11] This could be useful to elucidate the effect of alteration in nucleotides on disease severity. This population genetics-based information is an initial step, and additional studies are necessary to identify molecular and practical obstacles that stand in the way of realizing genome-driven medicine. Tools such as genome resequencing and genome-wide association studies have been increasingly used to uncover a number of variants that affect drug toxicity and efficacy, as well as potential drug targets. However, how much closer we are to incorporating pharmacogenomics into routine clinical practice is still a question that needs due attention.

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