Association Analysis of γ 2 Subunit of γ -Aminobutyric Acid Type A Receptor Polymorphisms with Febrile Seizures

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ABSTRACT

An alternation of γ -aminobutyric acid (GABA)-ergic neurotransmission has been implicated as an etiologic factor in epileptogenesis. Missense mutations in the *GABRG2* gene, which encodes the γ 2 subunit of central nervous GABA_A receptors, have recently been described in one family with childhood absence epilepsy and febrile seizures (FSs). FSs represent the majority of childhood seizures and have a genetic predisposition. It is not known, however, whether polymorphisms in those genes involved in familial epilepsies also contribute to the pathogenesis of FSs. By performing an association study, we used single-nucleotide polymorphisms to investigate the distribution of genotypes of *GABRG2* in patients with FSs. A total of 104 children with FSs and 83 normal control subjects were included in the study. PCR was used to identify the C/T and A/G polymorphisms of the *GABRG2* gene on chromosome 5q33. Genotypes and allelic frequencies for the *GABRG2* gene polymorphisms in both groups were compared. The *GABRG2* (nucleotide position 3145 in intron G→A) gene in both groups was not significantly

different. In contrast, the number of individuals with the *GABRG2* (SNP211037)-C/C genotype in patients with FSs was significantly greater compared with that in healthy control subjects ($p = 0.017$), and the *GABRG2* (SNP211037)-C allele frequency in patients with FSs was significantly higher than that in healthy control subjects ($p = 0.009$). The odds ratio for developing FSs in individuals with the *GABRG2* (SNP211037)-C/C genotype was 2.56 compared with individuals with the *GABRG2* (SNP211037)-T/T genotype. These data suggest that the *GABRG2* gene might be one of the susceptibility factors for FSs. (*Pediatr Res* 54: 26–29, 2003)

Abbreviations

FSs, febrile seizures
GABA, γ -aminobutyric acid
GABR, GABA type A receptor
GABRG2, γ 2 subunit of GABR

FSs are the most common seizure subtypes, affecting about 2% to 5% of children before the age of 5 y (1). FSs are characterized by a short duration of seizures during rapidly rising fever. Children with FSs are not predisposed to epilepsy, and the condition is not associated with neurologic abnormalities (2). The pathogenesis of FSs remains obscure. Possible causes include viral infection of the CNS and lowered threshold for seizures in the presence of fever (3). In fact, FSs of children may involve a complex interaction between the immunoinflammatory process, cytokine activation, and genetic factors.

It has long been known that approximately 30% of children with FSs have a family history of FSs (2). The genetic susceptibility to FSs seems to involve multiple genes in most instances (4). Some forms of family epilepsy may initially present as FSs and several of these disorders are caused by channelopathies such as neuronal sodium channels (5, 6) or GABA receptors (7). Thus, because of sharing important clinical features, FSs and these family epilepsies may share a common genetic etiology. It is not known, however, whether polymorphisms in those genes involved in familial epilepsies also contribute to the pathogenesis of FSs, because less than 3% of children with FSs progress to persistent epilepsy (1).

An alternation of GABAergic neurotransmission has been implicated as an etiologic factor in epileptogenesis (8–11). Neuronal inhibition in the mammalian brain is largely mediated by the binding of GABA to heteromeric GABRs (10, 11). GABR, a ligand-gated Cl[−] channel, functions as a tetramer

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consisting of α , β , γ , and π subunits. Each subunit has several subtypes, and the main GABR in the CNS is composed of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits. The genes encoding GABR subunits represent high-ranking candidates for idiopathic generalized epilepsy susceptibility genes because of the widespread distribution of GABRs in the CNS, their ability to produce postsynaptic inhibition, and their modulation by clinically important anticonvulsant drug, including benzodiazepines and barbiturates (10).

Genetic evidence for a potential role of the GABAergic system in epileptogenesis (9–11), however, has been obtained only recently by the discovery of different GABRG2 mutations identified in two families. The phenotype in one of these families was described to be compatible with generalized epilepsy with FS plus, but no further details regarding the seizure types observed in the affected pedigree members were given (12). In the second affected family, individuals predominantly had childhood absence epilepsy and FSs (7). Accordingly, these findings raise the question of whether genetic variation of the *GABRG2* gene confers susceptibility to the epileptogenesis of FSs.

Genetic studies of complex diseases such as FSs are difficult to approach because of the uncertainty of polygenic traits. We previously used single-nucleotide polymorphisms as a tool to search for genetic makers of FSs (13, 14). Single-nucleotide polymorphisms are the most abundant types of DNA sequence variation in the human genome (15, 16). It is a single base pair on the DNA that varies from person to person. Single-nucleotide polymorphisms are markers that may provide a new way to identify complex gene-associated diseases such as FSs. In this study, we tested the hypothesis that genetic variation in the *GABRG2* gene confers susceptibility to FSs in children. Two synonymous polymorphic repeat markers have been identified in single-nucleotide polymorphism (17): the G→A nucleotide exchange at nucleotide position 3145 in the intronic sequence, and SNP211037 (Asn196Asn), at nucleotide position 588, allowing researchers to detect disease-causing gene association.

METHODS

A total of 104 Taiwanese children with FSs and 83 normal control subjects were included. This study was approved by the ethics committee of the China Medical College Hospital, Taichung, Taiwan. All parents signed informed consent before blood tests were performed. There were no significant differences in age, weight, and height between the groups. Diagnosis of FSs followed the criteria established in the 1989 International Classification of Epileptic Syndromes. FSs were defined as seizures associated with a febrile illness as described previously. The EEG was normal for all patients or showed mild nonspecific abnormalities. A patient with 1) afebrile seizures, 2) FSs at older than 6 y (FSs plus), 3) epileptiform EEG traits, or 4) evidence of intracranial infection was not included in the study.

All children underwent peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood using a DNA extractor kit (Genomaker DNA extraction

kit; Blossom, Taipei, Taiwan). A total of 50 ng of genomic DNA was mixed with 20 pmol of each PCR primer in a total volume of 25 μ L containing 10 mM Tris-hydrochloride, pH 8.3; 50 mM potassium chloride; 2.0 mM magnesium chloride; 0.2 mM each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, CA, U.S.A.). Four PCR primers were used to amplify the associated gene. The sequences of these primers were as follows (from 5' to 3' end): GABRG2 (nucleotide position 3145 in intron G→A): upstream, AGAAATTTACCAACTGGTCTAGC-CGG; downstream, AAATCAAATATTGTGTCATGCT-TAGT; and GABRG2 (SNP211037, Asn196Asn): upstream, GAGTGCCAATTACAATTGCAAAA; downstream, AAT-CAGAAAGACTGTAGGTGAGG.

The PCR conditions were as follows: 35 cycles at 94°C for 30 s, 60°C for GABRG2 (nucleotide position 3145 in intron G→A) for 30 s, and 55°C for GABRG2 (SNP211037, Asn196Asn) for 30 s, and 72°C for 45 s, then standing at 72°C for 7 min and holding at 4°C. The polymorphisms were analyzed by PCR amplification followed by restriction analysis: *NciI* for GABRG2 (nucleotide position 3145 in intron G→A) and *ApoI* for GABRG2 (SNP211037, Asn196Asn). The PCR products were directly analyzed on 2% agarose gel by electrophoresis, and each allele was recognized according to its size. Allelic frequencies were expressed as a percentage of the total number of alleles. Genotypes and allelic frequencies for GABRG2 (nucleotide position 3145 in intron G→A) and GABRG2 (SNP211037, Asn196Asn) polymorphisms in both groups were compared.

The SAS system with χ^2 test was used for statistical analyses. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Genotype proportions and allele frequencies for the intronic *GABRG2* gene in both groups were not significantly different (Table 1). The most common genotype for intronic *GABRG2* gene in group 1 was G homozygote, and in group 2 was also G homozygote. Proportions of A homozygote, A/G heterozygote, and G homozygote for GABRG2 were as follows: in group 1, 10.6%, 39.4%, and 50%, respectively; and in group 2, 14.5%, 31.3%, and 54.2%, respectively. The allele A and G frequencies for GABRG2 in group 1 was 30.3% and 69.7%, respectively; in group 2, 30.1% and 69.9%, respectively (Table 1).

Table 1. Genotypes and allele frequencies of the intronic *GABRG2*. Polymorphisms in children with febrile seizures and normal control subjects

	Febrile seizures No. (%) (n = 104)	Controls subjects No. (%) (n = 83)	<i>p</i> value*
Genotype			
A/A	11 (10.6)	12 (14.5)	0.455
A/G	41 (39.4)	26 (31.3)	
G/G	52 (50)	45 (54.2)	
Allelic frequency			
A	63 (30.3)	50 (30.1)	0.972
G	145 (69.7)	116 (69.9)	

* *p*-values were calculated by χ^2 test.

In contrast, the genotype proportions and allele frequencies for GABRG2 (SNP211037) in both groups were significantly different (Table 2). The most common genotype for GABRG2 (SNP211037) gene in group 1 was C/T heterozygote, and in group 2 was T homozygote. Proportions of C homozygote, C/T heterozygote, and T homozygote for GABRG2 (SNP211037) were as follows: in group 1, 16.5%, 53.4%, and 30.1%, respectively; and in group 2, 10.8%, 38.6%, and 50.6%, respectively. The allele C and T frequencies for GABRG2 (SNP211037) in group 1 was 43.2% and 56.8%, respectively; and in group 2, 30.1% and 69.9%, respectively (Table 2).

The GABRG2 (SNP211037)-CC genotype was over-represented in patients with FSs compared with healthy control subjects (16.5% versus 10.8%). Thus, the GABRG2 (SNP211037)-C allele was significantly higher in patients with FSs than in healthy control subjects ($p = 0.009$). The odds ratio for developing FSs in individuals with GABRG2 (SNP211037)-CC genotype was 2.56 compared with the GABRG2 (SNP211037)-TT genotype, and it was significantly different ($p < 0.001$). The odds ratio for developing FSs in individuals with the GABRG2 (SNP211037)-CC and GABRG2 (SNP211037)-CT genotype was 2.38 compared with the GABRG2 (SNP211037)-TT genotype, and it was significantly different ($p < 0.001$).

DISCUSSION

The present study investigated the influence of genetic variations at the intronic GABRG2 and GABRG2 (SNP211037) gene cluster on 5q33 chromosome in children with FSs. We found that FSs were not associated with intronic GABRG2 gene polymorphism. In contrast, children with the GABRG2 (SNP211037)-C allele had a higher incidence of febrile seizures. The relative risk of FSs in individuals with the GABRG2 (SNP211037)-CC genotype was 2.56 times greater compared with those with the GABRG2 (SNP211037)-TT genotype. The relative risk of FSs in children with the GABRG2 (SNP211037)-CC and GABRG2 (SNP211037)-CT genotype was 2.38 times higher than in individuals with GABRG2 (SNP211037)-TT genotype. This evidence indicates that the GABRG2 (SNP211037)-C allele is a candidate genetic marker for FSs.

FSs are an age-specific disease, and remit spontaneously without treatment (2). Developmental changes in GABR *per se*

have been well characterized (18, 19). In the adult CNS, GABA is the primary inhibitory neurotransmitter. Early in development, however, GABAergic synaptic transmission is excitatory and can exert widespread trophic effects. During the postnatal period, GABAergic responses undergo a switch from being excitatory to inhibitory. The decreased seizure susceptibility of the mature brain may be related to postnatal segregation of GABA-sensitive networks (20).

Some studies previously demonstrated that the GABA concentration in the cerebrospinal fluid of children with recurrent FSs was lower than that in control subjects and suggested that an immature GABAergic system underlies FSs (21–24). The threshold for FSs is considered to depend on the activity of the GABAergic system; low activity of the GABAergic system allows FSs to occur easily. Hyperthermia-induced seizures in experimental animals have been used to study the mechanism of FSs (25, 26), and glutamate is known to play an important role in the induction of hyperthermia-induced seizures (27). Arias *et al.* (26) reported that glutamate decarboxylase activity is suppressed by hyperthermia in newborn rats. The susceptibility to hyperthermia-induced seizures is higher in developing than in adult animals, similar to the case of human FSs (27). The GABAergic system in developing animals is immature in comparison to the excitatory system (28).

The genetic susceptibility to FSs seems to involve multiple genes in most instances. Our review of the literature found that three loci, FEB1 on 8q (29), FEB2 on 19p (30), and FEB4 on 5q (31), were reported to be related to FSs with an autosomal dominant pattern of inheritance. Additionally, a mutation in the GABRG2 gene has been identified in individuals with FSs either with or without childhood absence epilepsy (7). The gene for generalized epilepsy with FS plus, which is an epileptic syndrome characterized by FSs persisting beyond age 6 y and non-FSs, was identified as SCN1B, the gene coding for the accessory subunit $\beta 1$ of the voltage-gated sodium channel (5, 6). Digenic inheritance was suggested for pedigree segregating FSs and temporal lobe epilepsy and loci mapped to chromosome 1q and 18qter (32).

In the polygenic inheritance of the FSs, therefore, a large number of genes might be involved, and a given single gene might have only a very small impact on the disease. In our previous studies, we found an association between a common polymorphism of the gene encoding the IL-1 receptor antagonist and FSs in children (13). In contrast, we noted that KCNQ2 polymorphism was not a useful marker to predict FSs (14). The present study suggests that GABRG2 gene might be one of the susceptibility factors for FSs. Given that the single-nucleotide polymorphism involved in the association does not change an amino acid, the disease-associated allele must be in linkage disequilibrium with the DNA change, as yet unidentified.

The existence of a second gene in the vicinity of GABRG2, therefore, cannot be excluded at this time. One mutation identified in GABRG2, a missense mutation c.983A→T; K328M, located in the linkage between transmembrane domains 3 and 4, was found in a French family in which the phenotype of affected individuals was GEFS+ (33). GABRs harboring K328M showed reduced Cl^- current in response to

Table 2. Genotypes and allele frequencies of GABRG2 (SNP211037). Polymorphisms in children with febrile seizures and normal control subjects

	Febrile seizures No. (%) (n = 104)	Controls subjects No. (%) (n = 83)	<i>p</i> value*
Genotype			
CC	17 (16.5)	9 (10.8)	0.017
CT	55 (53.4)	32 (38.6)	
TT	31 (30.1)	42 (50.6)	
Allelic frequency			
C	89 (43.2)	50 (30.1)	0.009
T	117 (56.8)	116 (69.9)	

* *p*-value were calculated by χ^2 test.

a physiologic ligand, GABA. Because GABR exerts an inhibitory function, dysfunction of GABR can lead to seizure activities. Another mutation of GABRG2 was a missense mutation (c.245G → A; R82Q) identified in a family in which the phenotype of affected individuals was FSs followed by absences (7). The R82Q mutation resides within the first of two high-affinity benzodiazepine-binding domains of GABRs. Interestingly, R82Q did not alter Cl⁻ current in response to GABA but abolished Cl⁻ current augmented by diazepam (7). GABRs may respond to endozepines, putative endogenous benzodiazepine-like substances, and prevent both FSs and absences. Furthermore, the response of GABR to benzodiazepine is temperature-sensitive (34). Thus, a defect in GABAergic transmission, specifically a mutation of GABRG2 that confers benzodiazepine sensitivity, is a plausible mechanism for FSs, as benzodiazepine is effective in the prophylaxis of FSs (35) and pharmacologic inhibition of GABRs causes seizures (11).

CONCLUSION

In conclusion, our study suggests that genes associated with GABAergic function may be a candidate for FSs. Further studies could be focused on the analysis of GABRG2 RNA and protein in children with FSs. This study may provide the basis for further survey of GABRG2 polymorphism.

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