Introduction

Recent progress has been made in the molecular genetics of childhood absence epilepsy (CAE) regarding the γ-aminobutyric acid (GABA)A and GABAβ receptor genes,\(^1,9\) voltage-dependent Ca\(^{2+}\)-channel genes,\(^5,10-14\) the epilepsy childhood absence susceptibility 1 gene on chromosome 8q,\(^5\) and potassium channel genes (\(KCNK9\) and \(TASK3\)).\(^5,15,16\) Even though some studies have found consistent results for each gene, there also have been opposing results in other studies for the same genes.\(^1-16\) Therefore, these genetic findings need to be reviewed while bearing that 1) genotypic mutations should result in the phenotypic changes (e.g., neuronal hyperexcitability), 2) mutant forms of genetic components should be able to demon-

Background and Purpose

Since the γ-aminobutyric acid type-A receptor subunit γ2 gene (\(GABRG2\)) mutation was discovered in an Australian family with childhood absence epilepsy (CAE) and febrile convulsions, a few screening studies for the \(GABRG2\) mutation have been conducted in sporadic individuals with CAE from other ethnic groups. The aim of this study was to determine whether or not the previously reported genetic mutations and single-nucleotide polymorphisms (SNPs) of \(GABRG2\) can be reproduced in sporadic Korean individuals with CAE, compared to healthy Korean individuals.

Methods

Thirty-five children with CAE in Chonnam National University Hospital and healthy controls (\(n=207\)) were enrolled, and the medical records of patients with CAE were reviewed. CAE was diagnosed according to the Classification and Terminology of the International League Against Epilepsy. All nine exons of \(GABRG2\) were directly sequenced. In addition, the two SNPs found in our CAE patients were analyzed: C315T in exon 3 (E3) and C588T in exon 5 (E5). The frequencies of the two SNPs in the CAE patients were compared with data from healthy controls (for E3 and E5) and from previously reported Korean population data (only for E3).

Results

No mutation of \(GABRG2\) was found in our CAE patients. In addition, the allele and genotype frequencies of the two polymorphisms did not differ significantly between CAE patients, healthy controls, and the Korean general population (\(p>0.05\)).

Conclusions

Our study of sporadic Korean individuals with CAE found no evidence that \(GABRG2\) contributes to the genetic basis of CAE.

Key Words

GABA\(_\alpha\) receptor gamma subunit, absence epilepsy, child.
strate the penetrance not only in specific families but also in sporadic individuals, and 3) genetic mutations or polymorphisms should be reproducible in different racial groups.

Childhood absence epilepsy and genetic epilepsy with febrile seizure plus (GEFS+) are known to be associated with mutations of the GABA_A receptor subunit γ2 gene (GABRG2), causing changes in electrical currents through the channel.1,2,17,18 R43Q in a family with CAE/febrile seizure1,2 and K289M and Q351X in a GEFS+ family.18,19 These genetic mutations, which were discovered in particular families, have been studied subsequently in sporadic patients.3-6 However, the genetic mutation could not be reproduced in these other ethnic sporadic patients.3-4 However, the genetic mutation could not be reproduced in these other ethnic sporadic patients.3-4 The aim of this study was to determine whether or not the previously reported genetic mutations of GABRG21,2 discovered in a large Australian family with CAE and febrile convulsions can be reproduced in Korean sporadic individuals with CAE. In addition, the allele and genotype frequencies at polymorphic sites of GABRG2 were compared between CAE patients and healthy Korean individuals.

Methods

Subjects

Patients with an established clinical diagnosis of CAE (n=35) were recruited from the epilepsy clinic of Chonnam National University Hospital, and their medical records were reviewed retrospectively by two neurologists at the Chonnam National University Hospital epilepsy clinics. Healthy volunteers (n=207) were enrolled for the control groups. The study was approved by the Institutional Review Board of the hospital, and informed consent to participate was obtained from all study subjects or their proxy. The diagnostic criteria for CAE are as follows:20

1) Typical absence seizures appearing as the initial seizure type at 3-12 years of age.
2) Electroencephalography revealing normal background activity and regular paroxysmal bilateral, symmetric generalized, and synchronous 3-Hz spike-and-wave discharges.
3) Normal findings from general physical and neurological examinations.
4) Normal neuroradiographic findings (e.g., brain computed tomography or magnetic resonance imaging).

The diagnosis of CAE followed the criteria established in the 1989 International Classification of Epileptic Syndrome.20

GABRG2 mutation analysis in CAE patients

Blood samples were drawn after obtaining informed consent. Genomic DNA was extracted from peripheral blood lymphocytes using a standard protocol. All samples were analyzed by direct sequencing after amplification by PCR as described below. Appropriate forward and reverse primer sets for each string of exons and exon-intron boundaries of the GABRG2 cDNA sequence were prepared (Table 1) based on GenBank sequences (accession number: NM_000816.3). In addition, PCR was carried out under the following conditions. Genomic DNA (100-300 ng) was amplified in a total volume of 50 µL: 5.0 µL of 10× h-Taq storage buffer (SolGent, Daejeon, Korea), 1.0 µL of 10 mM deoxynucleotide triphosphates, 2.0 µL of each primer (at 10 pmol/µL), 0.5 µL of h-Taq DNA polymerase (2.5 U/µL; SolGent, Daejeon, Korea), and distilled water. The amplification conditions were as follows: an initial denaturation cycle at 95°C for 15 minutes; followed by 40 amplification cycles of denaturation at 95°C for 20 seconds, annealing at 56-58°C for 40 seconds, and extension at 72°C for 1 minute; and a final extension at 72°C for 5 minutes. The PCR products were electrophoresed on a 2.0% agarose gel, and the amplified genomic DNA fragments were extracted from the gel and purified using a GeneAll Expin gel-extraction kit according to the manufacturer’s instructions. Direct sequencing of both strands was performed using the BigDye terminator kit (PE Biosystems, Foster City, CA, USA). DNA sequences were obtained using an ABI 3100 Genetic Analyzer. Electropherograms were analyzed visually using Chromas version 2.13 software (Technelysium, Queensland, Australia).

Table 1. Sequences of forward and reverse primers and annealing temperatures

| Exon | Forward and reverse primer sets | AT (ºC) |
|------|---------------------------------|---------|
| 1    | F TACTCCCCCCCAGACTTGGAA 56     | R GCCAAAAAGGGCACAATCTTA 56 |
| 2    | F TCTTTTCCACTGGTGGTCTG 58     | R TCTTCCTGTCCTGGACTAATC 58 |
| 3    | F CAAATGTGGTGAATTAGTAACTGG 56 | R TCAACATTTCTCCTCAAACATGC 56 |
| 4    | F TTGCGCAAACGTGGTATG 56       | R AGCATGCCACACCTGGATG 56  |
| 5    | F TGTGTTTTCAATCAGAATGTGAAG 57 | R GGCAATCAGAAGACTGTAAGG 57 |
| 6    | F CATGTCATAGAGATGCTGGTC 56    | R TCTGATTCATATCATTGGAGGG 56 |
| 7    | F AATTTAAATGTGIGTGGTGCATAC 56 | R GGCCTAAATTGAAAGCGATCAAC 56 |
| 8    | F TCAGTTACCCACCTGCTTATG 57   | R AGCCCTGACATAGCTGTAATG 57 |
| 9    | F GACATTGTCGAAAGACAGGCC 56   | R AACAGATCCAGACATCATGAAAC 56 |

AT: annealing temperatures, F: forward primer, R: reverse primer.
Analyses of two candidate SNPs in healthy controls: rs11135176 (C315T in exon 3) and rs211037 (C588T in exon 5)

The two SNPs discovered in CAE patients [C315T in exon 3 (E3) and C588T in exon 5 (E5)] were analyzed in healthy and unrelated controls; this was conducted in the same manner as for the CAE patients. In addition, the previous Korean population data were collected on the Internet homepage of the National Center for Biotechnologic Information (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=11135176), and were available only for E3.

Statistical comparison of two SNP allele and genotype frequencies between patients and healthy individuals

Allele frequencies are expressed as a ratio of the total number of alleles. Allele and genotype frequencies for each GABRG2 polymorphism in both patient and control groups (for E3 and E5) or for the Korean population (only for E3) were compared with Pearson chi-squared test analysis and Fisher’s exact test. The genotype frequencies at each SNP were assessed for deviations from the Hardy-Weinberg equilibrium. Statistical significance was accepted at $p<0.05$. SPSS version 18.0 (SPSS, Chicago, IL, USA) and MedCalc software (MedCalc Software, Mariakerke, Belgium) were used for these statistical analyses.

Results

Patient characteristics

The age of the patients with CAE ($n=35$) was $9.51\pm3.37$ years (mean $\pm$ SD; range: 5-20 years) and the gender ratio (males : females) was 0.6 : 1 (Table 2). The age at the onset of absence seizures was $7.03\pm2.30$ years. Most patients had simple CAE (31 out of 35), with the other 4 having a history of febrile convolution. Among the entire cohort of patients, there were three related CAE members in a family: a pair of monozygotic twins and one of their siblings. Four patients had a family history of epilepsy (not CAE) and five patients had family members with febrile convolution.

Mutation analysis of GABRG2

The nine exons and the exon-intron boundaries were analyzed thoroughly in patients with CAE, but no mutation was found. Only two SNPs were discovered: rs11135176 (E3) and rs211037 (E5).

The major (C) allele frequencies of the two SNPs (E3 and E5) discovered in CAE patients were 0.81 and 0.36. In healthy controls ($n=207$), the major C-allele frequencies in exons 3 and 5 were 0.74 and 0.57. There was no significant difference between the two groups ($p>0.05$). Genotype frequencies for E3 in the patient group were 0.63 for CC and 0.37 for CT. The TT genotype was not found in the patient group. In healthy controls, the CC, CT and TT genotype frequencies were 0.54, 0.41, and 0.05, respectively. There was no significant difference between the two groups ($p>0.05$). Genotype frequencies for E5 in patient groups vs. healthy controls were 0.11 vs. 0.21 for the CC genotype, 0.49 vs. 0.44 for the CT genotype, and 0.40 vs. 0.35 for the TT genotype. No significant difference was observed between the two groups ($p>0.05$) (Table 2).

The Korean population SNP data for rs11135176 (E3) on National Center for Biotechnologic Information showed that the major allele frequency ($C$) was 0.75 and that the genotype frequencies for CC, CT, and TT were 0.567, 0.367, and 0.067, respectively. We found no significant difference between these data ($n=90$) and our patient data ($n=35$; $p>0.05$).

Discussion

γ-aminobutyric acid, the main inhibitory neurotransmitter in the brain, mediates its rapid inhibition through GABAA receptors. GABAA receptors have a pentameric structure with five subunits: 1) two of α1, α2, α3, or α5 subunits; 2) two β2 or β3 subunits (or one each); and 3) one γ2 subunit. These are ar-

| Table 2. Comparison of allele and genotype frequencies at polymorphic sites of GABRG2 between patients with childhood absence epilepsy ($n=35$) and healthy controls ($n=207$) |
|---|---|---|---|
| SNP| Allele frequency| Genotype frequency| p value |
| | Pt | Con | p value |
| | Pt [n] | Con [n] |
| E3*| C | 0.81 | 0.74 | 0.19 | CC | 0.63 (22) | 0.54 (111) | 0.41 |
| | T | 0.19 | 0.26 | 0.37 (13) | CT | 0.41 (85) | 0.80 |
| | | | | 0.00 (0) | TT | 0.05 (11) | 0.34 |
| E5†| C | 0.36 | 0.57 | 0.25 | CC | 0.11 (4) | 0.21 (43) | 0.29 |
| | T | 0.64 | 0.43 | 0.49 (17) | CT | 0.44 (92) | 0.79 |
| | | | | 0.40 (14) | TT | 0.35 (72) | 0.69 |

*E3 is rs11135176 in exon3 of GABRG2. The Hardy-Weinberg equilibrium (HWE) is 1.82 in patient group ($p=0.57$, exact) and 1.05 in control group ($p=0.31$).
†E5 is rs211037 in exon 5 of GABRG2. The HWE is 0.11 in patient group ($p=1.00$, exact) and 1.6 in control group ($p=0.18$).

Con: control, Pt: patient, SNP: single nucleotide polymorphism.
ranged like the spokes of a wheel with a central chloride pore. Modifications of the GABA_A receptor subunit genes (e.g., GABRA1, GABBR3, and GABRG2) are thought to alter receptor function and/or impair receptor biogenesis via multiple mechanisms, which may predispose affected patients to seizures.

Some types of GABA_A receptor subunit gene mutations have been associated with epilepsy, CAE, GEFS+, febrile seizures, juvenile myoclonic epilepsy, and Dravet syndrome.

The γ-aminobutyric acid type-A receptor subunit γ2 gene is one of the GABA_A receptor subunit genes initially discovered to have mutations in patients with epilepsy. Mutations involving the γ2 subunit (GABRG2) are known to be present in absence epilepsy with or without febrile convulsions (R34Q, IVS6+2T → G) and GEFS+ (K289M, Q351X). R34Q mutations were found in over 4 generations of a large Australian family with 35 epilepsy patients in the early 2000s. Typical CAE was even observed in eight of them. Other seizure phenotypes were also observed: GEFS+, febrile seizure, myoclonic atatic epilepsy, generalized epilepsy with tonic/clonic seizures, and partial epilepsy. Subsequent animal studies have shown that a good animal model of familial CAE can be created with this heterozygotic mutation. Nevertheless, this mutation has not been reproduced in either unrelated sporadic individuals or in other ethnic groups.

Screening for the GABRG2 mutation in sporadic individuals with CAE was performed in two ethnic groups: German (46 CAE and 59 juvenile absence epilepsy: 154 controls) and Chinese (68 CAE, Han ethnicity; trio). However, neither of these studies showed the previous missense mutation in GABRG2. Only Kanamura et al. (German) found a point and splice donor mutation (IVS6+2T → G) leading to a nonfunctional protein.

The three aforementioned studies, SNPs of GABRG2 were also reported for exons 3 and 5. In the Chinese patients, Lu et al. identified SNPs in exon 3 with allele frequencies of 0.75 for G and 0.25 for A, and in exon 5 with allele frequencies of 0.47 for C and 0.53 for T. However, it appears that both of these SNPs lead to synonymous substitutions in the translated protein and probably do not affect protein function. In addition, transmission disequilibrium tests in 68 trios with CAE revealed no significant discrepancies in allele frequencies of the two SNPs between the CAE patients and the ‘internal controls’. The SNP in exon 5 is identical to the ES SNP sequence that we found in our Korean group. In a German-population-based association study, a common exon 5 polymorphism (C588T) was also evaluated. Genotype frequencies of C588T in the German CAE patient group vs. controls were 0.615 vs. 0.675 for CC, 0.348 vs. 0.273 for CT, and 0.037 vs. 0.052 for TT. Even though these proportions of genotypes differ from those for our Korean groups, Kanamura et al. did not find any significant differences in the allele and genotype frequencies for C588T between patients with idiopathic absence epilepsy and controls (p=0.35); this concurs with what we found.

As in the previous studies, we were unable to find a mutation at R34Q in GABRG2. Furthermore, there was no newly discovered mutation in GABRG2, and the SNPs did not differ between the CAE group and the healthy Korean controls. A limitation of this study is that we included only a small number of Korean CAE patients; more patients should be recruited in future studies in order to check our suggestion that GABRG2 contributes to the epileptogenesis of CAE. Our negative results, unlike the findings for the previous family study, need to be reviewed, especially regarding the following two points: 1) a racial discrepancy can exist, and there is still a shortage of data for large ethnic groups, including Koreans; and 2) the previous mutation was discovered in a family with diverse epileptic phenotypes except for the two main phenotypes of CAE and febrile seizure, while most of the individual studies for GABRG2 have focused on CAE patients.

Conflicts of Interest
The authors have no financial conflicts of interest.

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