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Original Article

The $GABA_A$ Receptor γ2 Subunit (R43Q) Mutation in Febrile Seizures

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ABSTRACT

BACKGROUND: Febrile seizure is the most common form of childhood seizure. Although its exact cause is unclear, many researchers emphasize the importance of its genetic predisposition. Recent genetic studies revealed the importance of the mutations of the gamma-aminobutyric acid A receptor as the etiology of the febrile seizures. R43Q mutation affecting the γ2-subunit N-terminal domain has been related to childhood absence epilepsy and febrile seizure. **METHODS:** We investigated R43Q mutations of the GABRG2 gene, located on the long arm of chromosome 5 encoding the γ2-subunit of the gamma-aminobutyric acid A receptor. We studied 44 patients with febrile seizure and 49 children without any febrile seizure who were admitted to our clinic. **RESULTS:** We found that 36% of our patient group, the children who experienced febrile convulsions, had heterozygous R43Q mutation. Statistical studies revealed that heterozygous R43Q mutation of gamma-aminobutyric acid A receptor γ2 subunit was higher in the study group than in the control group ($P < 0.01$). **CONCLUSIONS:** Heterozygous gamma-aminobutyric acid A receptor γ2 subunit (R43Q) mutation may have an effect in the development of febrile seizures.

Keywords: $GABA_A$ receptor, febrile seizure, γ2 subunit, R43Q mutation

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Background

Febrile seizure is the most common childhood seizure with a prevalence of 2% to 14%.[1,2] The exact cause of febrile convulsion is unclear. Its pathogenesis may have a major genetic component, with dominant inheritance in some families, but multifactorial inheritance is probably responsible in the majority of instances.[3,4]

In recent years, genetic studies revealed the importance of the mutations of voltage-gated ion channels (sodium, calcium, and potassium) and ligand-gated ion channels (nicotinic cholinergic receptor and gamma-aminobutyric acid A [$GABA_A$] receptor) in the etiology of febrile seizure.[5-7] $GABA_A$ receptors, which are members of ligand-gated ion channels, are made up of pentameric assemblies of different subunit subtypes (α1-α6, β1-β3, γ1-γ3, δ, ε, π, θ, and ρ1-ρ3), and form chloride ion selective channels.[7] The majority of $GABA_A$ receptors in the brain have two α1 subunits, two β2 subunits, and one γ2-subunit.[7,8] There is considerable evidence that the γ2-subunit plays an essential role in response to benzodiazepine modulators and receptor targeting.[8] R43Q mutation of the γ2-subunit N-terminal domain has been found to cause childhood absence epilepsy and febrile seizure.[8,9]

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We investigated R43Q mutations of GABRG2 gene on the long arm of chromosome 5 encoding γ2-subunit of $GABA_A$ receptors and their effect on the generation of febrile seizures.

Materials and Methods

This is a prospective case controlled study involving patients who were admitted to the pediatric outpatient clinic and pediatric emergency department of the Haydarpasa Numune Training and Research Hospital between January 2010 and January 2011.

The study group consisted of 44 children with simple or complex febrile seizures diagnosed according to International League Against Epilepsy diagnostic criteria[10] and who were between ages 6 months and 5 years. Children with central nervous system infections or inflammation findings, acute systemic metabolic abnormality, or history of previous afebrile seizures and abnormal neurological development were excluded. Use of antiepileptic or seizure threshold-reducing drugs were among other exclusion criteria, and patients with recent antibiotic use were also excluded from the study because of the possibility of incompletely treated meningitis.

The control group, aged between 6 and 12 years, consisted of 49 children who applied to our outpatient clinic for a febrile disease without febrile or afebrile convulsions and with normal neurological development.

Demographical features, age at first convulsion, the total number of seizures, family history of febrile convulsion or epilepsy, and consanguinity were recorded.

To detect R43Q mutations of the GABRG2 gene encoding the γ2-subunit of $GABA_A$ receptors, 2 mL of venous peripheral blood was collected into ethylenediaminetetraacetic acid tubes from both groups. Genomic DNA isolation was performed with a High Pure polymerase chain reaction (PCR) template purification kit (Roche, Mannheim, Germany); after isolation, it was analyzed with agarose gel electrophoresis. $GABA_A$ receptor γ2 subunit codon 43 was amplified with the Amplification Refractory Mutation System PCR. Allele-specific PCR was optimized with mutation-specific and wild-type–specific primers used for setting up two different reactions. PCR reactants were 15.5 μL double-distilled water, 2.5 μL buffer, 1.25 μL F primer, 1.25 μL R primer, 0,5 μL deoxynucleoside 5'-triphosphate, 2 μL MgCl2, and 1 unit Taq polimerase within 23 μL of final PCR volume. PCR conditions occurred for 30 cycles: 94°C for 4 minutes, 94°C for 30 seconds (denaturation), 64°C/57°C (normal/mutant) for 25 seconds (annealing), 72°C for 30 seconds (elongation), and 72°C for 8 minutes for last elongation. Primer sets were forward F1-R43Q 5′-CACAGAAAATGACGGTGTGGATTCTGC-3′, reverse-1 R1-R43Q 5′- ACGTTGGCTTCACTCCTATATCAGG**C**-3′, and reverse-2 R2-R43Q 5′-ACGTTGGCTTCACTCCTATATCAGG**T**-3′ (IDT). The primer set for detection of the polymorphism was designed specifically for the 3′OH region. Although the reactions were set up and run at the same time for two different primer sets at two different test tubes, because of specific hybridization of the 3′OH region, the allele-specific PCR selectively detects the polymorphic area from the same genomic DNA template.

GABRG2 amplicons were examined on 2% agarose gel. Amplification at both primer set tubes were considered as heterozygote and if the amplicon was detected in only one tube, it was considered as a homozygote for that primer set–specific region.

Informed consent was received from all parents. The study was approved by the ethical committee of the hospital.

The data were evaluated with NCSS 2007 and PASS 2008 Statistical Software (NCSS, Kayesville, UT). Besides the descriptive statistical methods as mean and standard deviation, the Kruskall Wallis test was used to comparison parameters without normal distribution between the groups. For comparison of the groups with normal distribution, quantitative data were analyzed using Student *t* test. The chi square test was also used compare quantitative data. The results were considered significant if the *P* value was lower than 0.05 and within 95% safety interval.

Results

The study group contained 44 children (24 males) with a mean age of 2.78 ± 1.37 years; the control group consisted of 49 children (21 males) with mean age of 8.34 ± 1.69 years. There was no significant gender difference between the study and control groups, but two groups had a statistical difference in age ($P < 0.01$). Febrile convulsion and epilepsy history of the families was significantly higher at the study group than those of control group ($P < 0.01$) (Table 1).

The first seizure occurred between 5 and 48 months (14.27 ± 7.25). Twenty-eight (63.6%) patients had simple seizures and 16 patients (36.4%) had complex febrile seizures. Fourteen patients (31.8%) had only one seizure, 10 (22.7%) patients had two seizures, four (9.1%) patients had three seizures, and 16 (36.4%) patients had four or more seizures.

We determined that 36.4% of the children who experienced a febrile convulsion had a heterozygous mutation of R43Q (Table 2). Statistical analysis revealed that the R43Q heterozygous mutation of the $GABA_A$ receptor γ2 subunit was significantly higher in the study group than in the control group ($P < 0.01$). There was no difference in homozygous R43Q mutation frequency between these groups. The ratio of carrying the wild-type genotype in the control group was higher than in the study group, with a significance of $P < 0.01$.

The study group exhibited no statistical relevance for mutation carrier status, age at first febrile convulsion, family and/or epilepsy history, and the number of febrile convulsions compared with controls ($P > 0.05$) (Table 3).

Discussion and Conclusions

Clustering of febrile seizures in some families suggests genetic predisposition in the etiology of convulsions.[6] Genetically defined eight-gene loci have been found responsible for febrile seizures so far. These are: 8q13-21 (FFB1), 19p13.3 (FEB2), 2q23-24 (FEB3), 5q14-15 (FEB4), 6q22-24 (FEB5), 18p11.2 (FEB6), 21q22 (FEB7), and 3q26.2-q26.33.[4,6]

GABA is the major inhibitory neurotransmitter in the central nervous system and binds to three different receptors named $GABA_A$, $GABA_B$, and $GABA_C$. $GABA_A$ receptor is the predominant ligand-gated ion channel mediating fast inhibitory transmission in the central nervous system

TABLE 1.
Demographic features of the groups

	Study Group (n = 44) n (%)	Control Group (n = 49) n (%)	*P*
Sex (female/male)	20 (45.5)/24 (54.5)	28 (57.1)/21 (42.9)	0.260
Family history of febrile seizure			0.002†
Absent	29 (65.9)	45 (91.8)	
Present	15 (34.1)	4 (8.2)	
Family history of epilepsy			0.033*
Absent	32 (72.7)	44 (89.8)	
Present	12 (27.3)	5 (10.2%)	
Consanguinity			0.181
Absent	34 (77.3)	43 (87.8)	
Present	10 (22.7)	6 (12.2)	

* $P < 0.05$.
† $P < 0.01$.