

Single-nucleotide polymorphism of GABA (A) receptor gamma 2 submit in familial febrile seizures.

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Abstract: The aim of this study was to evaluate the genetic variations in the GABA-A receptor gamma2 (GABRG2) gene in children with febrile seizures (FSs). The study was carried out on 61 children of ages ranging from 6 months to 6 years. Of which, 41 suffered FSs and 20 age- and sex-matched healthy children served as a control group. Diagnosis of FS and its subclassification to simple and complex FS was done according to the guidelines of the ILAE (2006). Children with FSs were classified into two groups, according to the presence or absence of family history of FSs; Group A included 20 children with family history of FSs and Group B included 21 children without family history of FSs. All children were subjected to history-taking, physical examination and determination of plasma GABA. Detection of DNA was carried out on samples obtained from children suffering FSs. Results showed that the mean plasma GABA was significantly lower in children with FSs than that in control children. The majority (90%) of children with familial FSs are products of consanguineous marriage compared to 52% in children with non-familial FSs, with significant difference. All children with non-familial FSs had simple FSs. Meanwhile, 60% of children with familial FSs had simple FSs and 40% had complex FSs, with significant difference. Both groups of children with FSs showed significant differences as regards the genotype proportions and allele frequencies for GABRG2. It is concluded that the GABRG2- CC genotype is overrepresented in children with familial FSs compared with that in children with non-familial FSs, 2) the GABRG2 C allele frequencies are significantly higher in children with familial FSs than in children with negative family history of FSs.

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1. Introduction

Febrile convulsion, one of the most common seizure disturbances in children with an approximate rate of 3 to 4 percent, is defined as seizure associated with fever as high as 38.5°C in children 6 months to 5 years without any infection within the central nervous system (CNS) or other factors explaining its incident (Talebian et al., 2009). FSs tend to occur in families. In a child with FS, the risk of FS is 10% for the sibling and almost 50% for the sibling if a parent has FS as well (Audenaert et al., 2006).

The pathogenesis of FSs remains obscure. Possible causes include viral infection of the CNS and lowered threshold for seizures in the presence of fever. An alteration of gamma-amino butyric acid (GABA)-ergic neurotransmission has been implicated as an etiologic factor. Neuronal inhibition in the mammalian brain is largely mediated by the binding of GABA to heteromeric GABA receptors (Oslen and Avoli, 1997).

Genetic evidence for a potential role of the GABA-ergic system in epileptogenesis has been obtained initially by the discovery of different GABRG2 mutations identified in two families (Oslen and Avoli, 1997; Wallace et al., 2001). A study in alpha2-gamma 2 GABA(A) receptors

proved that $\gamma 2$ subunit is critical for receptor trafficking, clustering, and synaptic maintenance. Those mutations in the $\gamma 2$ subunits have been monogenically associated with autosomal dominant transmission of the FSs (Kang et al, 2006).

Single-nucleotide polymorphism, the most abundant types of DNA sequence variation in the human genome, is used as a tool to search for genetic markers of FSs (Kwork and GU, 1999; Tsai et al., 2002). It is a single base pair on the DNA that varies from person to person and may provide a new way to identify complex gene-associated diseases as FSs (Winterer et al., 2000). This study aimed to evaluate the mean circulating levels of GABA and the genetic variations in the GABA receptors (GABR), in children who suffer from familial FSs.

2. Subjects and Methods**Subjects:**

This study was a cross-sectional association study conducted on 61 children of ages ranging from 6 months to 6 years recruited from the Emergency Room and Pediatrics Department of Joint Operation Hospital at Al-khafji, Kingdom of Saudi Arabia, during years 2011 through 2012. An informed consent was obtained from the parent(s) of each child

before commencement of the study. Out of these subjects 41 suffered FSs. Twenty age- and sex-matched healthy children, with no history of FSs served as a control group.

Diagnosis of FS and its subclassification to simple and complex FS was done according to the guidelines of the International League Against Epilepsy (Engel, 2006).

Exclusion criteria:

Children with the following criteria were excluded from the study:

- Afebrile seizures.
- Febrile seizures in children older than 6 years.
- Epileptiform EEG traits.
- Evidence of metabolic disorder that might be the etiologic factor underlying seizures.
- Evidence of intracranial infection.
- Any cardiac, pulmonary or renal diseases.

Children with FSs were classified into two groups, according to the presence or absence of family history of FSs:

Group A: children with family history of FSs:

They were 20 children from 8 families, each family has at least 2 members with a history of FSs. Their ages ranged from 6 months to 6 years (mean \pm standard deviation: $X \pm SD = 3.9 \pm 1.5$ years).

Group B: children without family history of FSs:

This group included 21 age- and sex-matched children with FSs but did not have family history of FSs.

Methods:

All children were subjected to the following:

1. Complete history- taking with special emphasis on:
 - a. The character of seizures (type- duration- number of previous attacks – number of seizures during the same illness- conscious level).
 - b. Clinical features suggesting CNS infection, e.g. meningeal irritation, projectile vomiting and disturbed conscious level.
 - c. Past history of other neurological illness.
 - d. Perinatal histories.
 - e. The age of achievement of developmental milestones.
 - f. Any significant illness, e.g. jaundice, cerebral palsy, metabolic disease or cardiac disease.
 - g. Consanguinity between parents.
2. Thorough physical examination including neurological examination.
3. EEG was done to exclude any subject with epileptiform EEG trait.
4. Determination of GABA

Plasma GABA levels were measured by a modification of the high-performance liquid chromatography-electrochemical method, using ESA Coulmetric Electrode Array system (ESA Inc. Chelmsford, MI), (Donazanti and Yamamoto, 1988).

5. Polynucleotide chain reaction.

Two ml of venous blood were extracted by aseptic technique, from children suffering FSs in sterile EDTA-vacutainer tubes.

Procedure:

1. Genomic DNA extraction:

DNA extraction was performed by Mag NA Pure Compact Nucleic Acid Isolation kit I (Roche Diagnostics, Mannheim, Germany) on whole blood samples, using the MagNa Pure Compact Instrument to purify high-quality, undegraded genomic DNA from mammalian. The nucleic acid isolation procedure is based on the proven Mag Na Pure Magnetic Glass Particle Technology.

After the purification run has ended the Elution Tubes were closed with the supplied tube caps and removed immediately and stored at 20°C till further analysis.

2. Detection of DNA samples:

Light Cycler® 480 High Resolution Melting Master (Roche Diagnostics, Mannheim, Germany) is a ready-to-use reaction mix developed for the detection of DNA samples that differ in sequence from others.

Kit components:

1. Master Mix, 2x conc, contains Fast Start Tag DNA polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP) and high resolution melting dye.
2. Mg Cl₂, 25 mM.
3. H₂O, PCR-grade.

Procedure:

- The solutions were briefly spun in a microcentrifuge before opening.
- Vial contents were carefully mixed by pipetting up and down and stored on ice.
- Twenty ml conc. solution of the PCR primer were prepared. The primer sequence were as follow:
 - GABRG2 (SNP211037), Asn 196
Asn): upstream primer
(GACTGCCAATTACAATTGCAAAA) and down
stream primer
(AATCAGAAAGACTGTAGGTGAGG).
 - PCR mix was prepared in a 1.5 ml reaction tube on ice, for each reaction tube by adding the following components in the order listed below.
 - Master mix, 2x Conc
10.0µl.

- Primer mix, 1.0 ml (0.2 μ M of each primer)
 - Mg Cl₂ 1.6 ml (of 2.0 mM).
 - Water, PCR-grade 2.4 μ L.
- The 15 μ l total PCR mix was pipetted into each well of the Light Cycler ® 480 Multiwell plate.
- 5 μ l of concentration- adjusted DNA template were added to the previous mixture and mixed well.
 - The Multi-well plate was sealed with Light Cycler® 480 sealing foil.

- The Multi-well plate was centrifuged for 2 min at 1500xg in a standard swing-bucket centrifuge, containing a rotor for multi-well plates with suitable adaptors.
- The palte was loaded into the Light Cycler ® 480 instrument.
- The PCR program was programmed as follows, Table 1.

Table 1: The PCR program .

Target temperature	Aqisition mode	Cycle hold
Preincubation 95°C	Non	10 min
Amplification (repeated for 40 cycles) 95°C 53°C 72°C	None None Single	10 sec 15 sec 10 sec
High resolution melting 95°C 40°C 60°C 95°C	None None None Continuous	1 min 1 min 1 sec

After PCR amplification, the amplicons were analyzed by high resolution melting curve analyses and data were evaluated using the Light Cycler ® 480 Gene Scanning Software, **Figure 1**.

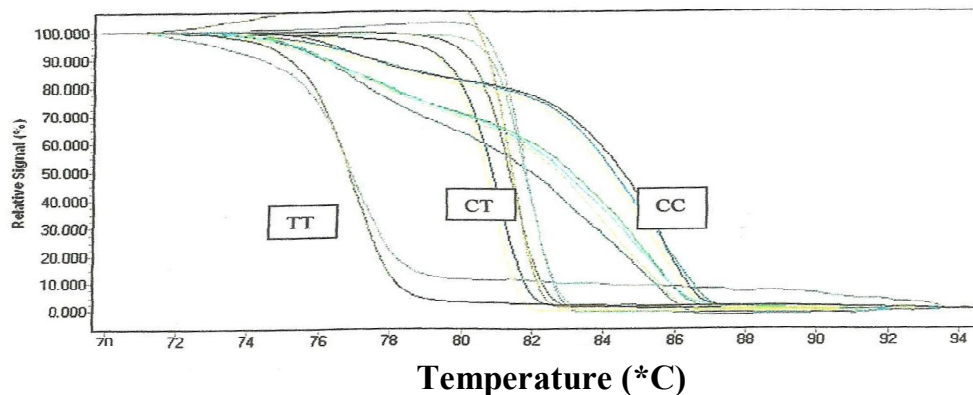


Fig 1: Normalized, temperarue-shifted melting curves from GABRG2 (SNP211037) amplicons carrying sequence variation. Sequence variants can be distinguished by different shape of melting curve.

Statistical analysis (Daniel, 1995)

Standard computer program SPSS for windows, release 10.0 (SPSS Inc , USA) was used for data entry and analysis. All numeric variables were expressed as mean \pm standard deviation ($X \pm SD$). Chi-sqaure (χ^2) test was used to compare frequency of the qualitative variables among the different

groups. For all tests a probability (p) less than 0.05 was considered significant.

3. Results

Table 2 summarizes the demographic characteristics of 41 children with FSs versus 20 healthy control children. There were non significant differences between cases and controls regarding

their age and sex. This means that both cases and controls are matched. The mean plasma GABA was significantly lower in children with FSs than that in control children, $p = 0.003$.

Table (3) presents the characteristics of 20 children with familial FSs (Group A) versus that in 21 children with non familial FSs (Group B). There were non-significant differences in age of onset of FSs or sex distribution between the two groups, $p > 0.05$. The majority (90%) of children with familial FSs are products of consanguineous marriage compared with 52% in children without family history of FSs, with significant difference. All children with no family history of FSs had simple FSs, and no complex FSs. Meanwhile, 60% of children with familial FSs had simple FSs and 40%

had complex FSs, with significant difference. Both groups of children with FSs showed statistically significant difference as regards the genotype proportions and allele frequencies for GABRG2. The most common genotype for GABRG2 gene was C homozygote in both groups (70% in familial FSs), versus 43% in non familial FSs, with significant difference. Proportions of C/T heterozygote were 30% and 33%, with non significant difference. Non of children with familial FSs were T homozygote for GABRG2 versus 24% in non-familial FSs, with significant difference. The allele C and T frequencies for GABRG2 in patients with family history of FSs were 83% and 18%, respectively, with significant difference.

Table 2: Demographic characteristics of 41 children with febrile convulsions versus 20 healthy control children.

Characteristics (s)	Cases n = 41	Control n = 20	p-value
Age (months), $\bar{X} \pm SD$	41.44 \pm 15.62	38.45 \pm 16.21	0.63 (NS)
Gender, n & (%)			
Males	24 (58.5)	12 (60)	0.75 (NS)
Females	17 (41.5)	8 (40)	
Plasma GABA (ng/ml)			
$\bar{X} \pm SD$	76.4 \pm 25.0	104.7 \pm 30.6	0.003 (S)
Range	34.0 – 113.0	80 – 157.0	

$\bar{X} \pm SD$: mean \pm standard deviation, NS: non significant, n: number.

GABA: gamma aminobutyric acid, ng: nanogram, S: significant

Table 3: Characteristics of 20 children with familial febrile convulsions (Group A) versus 21 children with non familial febrile convulsions (group B).

Characteristics	Group (A) n = 20	Group (B) n = 21	P-value
Age at onset (months), $\bar{X} \pm SD$	14.9 \pm 4.62	16.2 \pm 5.21	> 0.05 (NS)
Consanguineous marriage, n(%)	18 (90)	11 (52.4)	< 0.05 (S)
Gender, n(%)			
Males	13 (65)	14 (66.7)	> 0.05 (NS)
Females	7 (35)	7 (33.3)	
Type of febrile seizures, n(%)			
Simple	12 (60)	21 (100)	< 0.05 (S)
Complex	8 (40)	0 (-)	
Allele frequency, n(%)			
C allele	33 (82.5)	19 (45.2)	< 0.05 (S)
T allele	7 (17.5)	23 (54.8)	
Genotype distribution, n(%)			
CC	14 (70)	9 (42.9)	< 0.05 (S)
CT	6 (30)	7 (33.3)	> 0.05 (NS)
TT	0 (-)	5 (23.8)	< 0.05 (S)

n: number, %: percent, S: significant NS: non significant.

4. Discussion

Febrile convulsions is a common cause of convulsion in children, about 4% of children in the

age group of one to six years have at least one episode of FS. The pathogenesis of FSs remains obscure. Possible causes include viral infection of the

CNS (**Millichap and Millichap, 2006**), lowered threshold for seizures in the presence of fever (**Schuchmann et al., 2006**), and changes of neurotransmitter and trace elements in the biological fluids (**Amiri et al., 2010**). In fact, FSs of children may involve a complex interaction between the immune-inflammatory process, cytokine activation, and genetic factors (**Tsai et al., 2007**).

Gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter, is produced by decarboxylation of L-glutamate. Zinc modulates the activity of glutamic acid decarboxylase, the rate limiting enzyme in the synthesis of GABA. Fever and / or infections cause a reduction in serum zinc concentrations so affects the level of GABA (**Garty et al., 1995**). It appear that at higher body temperature, serum GABA levels decrease, which in turn can change the brain GABA levels and may lead to preception of seizures during febrile episode (**Mishra et al., 2007**).

In this study the mean plasma level of GABA was significantly lower in children with FSs than that in control children, $p = 0.03$. Nearly similar results were obtained by **Mishra et al (2007)** who reported a significantly lower cerebrospinal fluid and serum GABA both in adults with seizure disorders and in children with FSs compared with seizure- free subjects.

Neuronal inhibition in the mammalian brain is largely mediated by the binding of GABA to heteromeric GABA receptors (**Oslen and Aboli, 1997**). The GABR is the predominant ligand-gated Cl ion channel conferring fast inhibitory synaptic transmission in the CNS and therefore, is a prime candidate for involvement in epileptogenesis. GABR functions as a tetramer consisting of α , β , γ subunits. Each unit has several subtypes. The genes encoding GABR subunits represent high-ranking candidates for idiopathic generalized epilepsy susceptibility (**Bowser et al., 2002**).

Single nucleotide polymorphisms (SNPs), the most abundant types of DNA sequence variation in the human genome, were previously used as a tool to search for genetic markers of FSs. According to the theoretical models, if the genotype of a group of individuals with a common disease and that of a group without the disease are studied, certain genotypes will be consistently associated with those individuals who have the disease (**Rish and Merikangas, 1996**).

Chromosomal mapping indicates that GABR subunit genes are often clustered in the genome. The GABA $\alpha 6$ (GABRA6), $\beta 2$ (GABRB2) and $\gamma 2$ (GABRG2) subunit genes have been assigned to the chromosomal segment 5q33. Two synonymous polymorphic repeat markers have been identified in

single-nucleotide polymorphism; one of which was the SNP122073 (Asn196 Asn), at nucleotide position 588 allowing researchers to detect disease- causing gene association (**Chou et al., 2007**).

In this study, we hypothesize that the genetic variation in the GABRG2 gene confers susceptibility to FSs in children. PCR was used to identify the C/T polymorphism of the GABRG2 gene on chromosome 5q33. Genotyping and allelic frequencies for gene polymorphism was carried out for 2 groups, one group with family history of FSs and the other group without family history of FSs. The age of onset of FSs was non-significantly earlier in children with familial FSs than that in children without family history of FSs. Consanguineous marriage was significantly more common in children with familial FSs than in those without family history. FSs was non-significantly more common in male children than in female ones. In contrast, **Gaumann (2008)** reported a significantly higher incidence of FSs in males than that in females.

In this study, all children with non-familial FSs present as simple FSs. Meanwhile, 40% of children with familial FSs present as complex FSs, with significant difference. Several studies have reported that some forms of family epilepsy may initially present as FSs. Six genetic loci for FSs have been mapped thus far. In addition, mutations of GABR are reported to be associated with autosomal dominant epilepsy with FSs. Thus, because of sharing important clinical features, FSs and family epilepsies may share a common genetic etiology (**Nakayama et al., 2004**).

Although there is clear evidence for genetic basis of FSs, the mode of inheritance is still unclear. Polygenic, autosomal dominant and autosomal recessive models have received support (**Johnson et al., 1996**).

In this study, both groups of children with FSs showed statistically significant differences as regards the genotype proportions and allele frequencies for GABRG2 . The most common genotype for GABRG2 gene was C homozygote in both groups; 70% in the group with family history of FSs and 43% in the group without family history of FSs. Proportions of CT heterozygote were 30% and 33%, respectively. Non of children with familial FSs were T homozygote for GABRG2 versus 24% in non-familial FSs. The allele C and T frequencies for GABRG2 in patients with family history of FSs were 83% and 18%, respectively, while in patients with negative family history of FSs they were 45% and 55%, respectively. Thus, the GABRG2- C allele frequencies were significantly higher in children with positive family history of FSs than in children with negative family history of FSs ($P < 0.05$). These

findings are consistent with a previous study demonstrating an allele association between the GABRG2 polymorphism and idiopathic epilepsy. It is concluded that GABR exerts an inhibitory function on the CNS, thus dysfunction of GABR can lead to seizure activity (Risch and Merikamagas, 1996).

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References

1. Amiri M, Farzin L, Moassesi ME, Sajadi F. Serum trace element levels in febrile convulsion. *Biol Trace Elem Res* 2010; 135: 38 – 44.
2. Audenaert D, van Broeckhoven C, De Jonghe P. Genes and loci involved in febrile seizures and related epilepsy syndromes. *Hum Mutat* 2006: 391-401.
3. Baumann RJ. Technical report: treatment of the child with simple febrile seizures. *Pediatrics* 2008;103: 86.
4. Bowser D, Wagner DA, Czaikowski C, et al. Altered kinetics and benzodiazepine sensitivity of a GABAA receptor subunit mutation [γ 2 (R43Q)] found in human epilepsy. *Proc Natl Acad Sci, USA*, 2002; 99 (23): 15170 – 75.
5. Chou IC, Lee CC, Huang CC, et al. Association of the neuronal nicotinic acetylcholine receptor subunit alpha-4 polymorphism with febrile convulsions. *Epilepsia* 2007;44: 1089 – 93.
6. Daniel WW (1995): *Biostatistics: A foundation for analysis in the health sciences*, 6th edition. Johnwiley and Sons Inc, NewYork.
7. Donazanti BA, Yamamoto BK. An improved and rapid HPLC – EC method for the isocratic separation of amino neurotransmitters from brain tissue and microdialysis perfusates. *Life Sci*, 1988; 43: 913 – 22.
8. Engel J. Report of the ILAE classification core group. *Epilepsia* 2006; 47 (9): 1558 - 68.
9. Garty BZ, Olmucki R, Lerman –Sagie T, Nitzan M. Cerebrospinal fluid zinc concentration in febrile convulsion. *Arch Dis Child* 1995; 73: 338 – 41.
10. Johnson WG, Kugler SL, Stenros ES, et al. Pedigree analysis in families with febrile seizures. *Am J Med Genet* 1996;345 – 52.
11. Kang JQ, Shen W, Macdonald RL. Why does fever trigger febrile seizures? GABAA receptor γ 2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies. *J Neuro Sci* 2006;26: 2590 – 97.
12. Kwork PY, Gu Z. Single nucleotide polymorphism libraries- why and how are we building them? *Mol Med Today* 1999; 5: 538 – 43.
13. Millichap JG, Millichap JJ. Role of viral infections in the etiology of febrile seizures. *Pediatr Neurol* 2006; 35(3): 165 – 72.
14. Mishra OP, Singhala D, Upadhyayb RS, et al. Cerebrospinal fluid zinc magnesium, copper and gamma, aminobutyric acid levels in febrile seizures. *Pediatr Neurol* 2007; 5: 39 – 44.
15. Nakayama J, Yamamoto N, Hamano K, et al. Linkage and association of febrile seizures to the IMPA2 gene on human chromosome 18. *Neurology* 2004; 63:1803-7.
16. Oslen RW, Avoli M. GABA and epileptogenesis. *Epilepsia* 1997;38:399-407.
17. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996; 273; 1516 – 7.
18. Schuchmann S, Schmitz D, Rivera C, et al. Experimental febrile seizures are precipitated by a hyperthermia – induced respiratory alkalosis. *Nat Med* 2006; 12 (7): 817 – 23.
19. Talebian A, Vakili Z, Talar SA, et al. Assessment of the relation between serum zinc and magnesium levels in children with febrile convulsion. *Iranian J Pathol* 2009; 4 (4): 157 – 60.
20. Tsai FJ, Hsieh YY, Chang CC, Lin CC, Tsai CH. Polymorphism for interleukin 1-beta exon 5 and interleukin 1 receptor in Taiwanese children with febrile convulsions. *Arch Pediatric Adolesc Med* 2002;156:545-8.
21. Wallace RH, Marini C, Petrou S, et al : Mutant GABA (A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet* 2001;28:49-52.
22. Winterer G, Smolka M, Samchowec J, et al. Association analysis of GABAA γ 2- unit with event-related prefrontal activity. *Hum Genet* 2000; 107:513 – 8.

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