Analysis of the Asn752Thr Polymorphism in Exon16 of the KCNQ2 Gene in Four Families Afflicted with Idiopathic Generalized Epilepsy (IGE)

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Citation

Abstract

To examine the relationship between KCNQ2 polymorphism and idiopathic generalized epilepsy syndromes. Four patients having idiopathic generalized epilepsy according to the ILAE-89 classification and with a positive family history followed-up in our epilepsy outpatient clinic are presented in this study. The phenotype of patients and their affected family members were determined according to the ILAE- 89 classification. The genotype analysis in all four families affected with the idiopathic generalized epilepsy syndrome revealed a possible linkage of the disease to the KCNQ2 gene in only one family (Family I). The pattern of inheritance could either be inherited in recessive mode or a dominant mode with a low penetrance. As a conclusion, in order to assess the relationship between the polymorphism and IGE, a large study aimed at screening both idiopathic generalized epilepsy patients and healthy subjects in a larger number of families.

INTRODUCTION

Idiopathic epilepsies account for 40% of all epilepsies and are predominantly determined by genetic factors that are complex and poorly understood [12]. Most subtypes such as juvenile myoclonic epilepsy, childhood absence epilepsy may display a complex rather than a monogenic pattern of inheritance[3]. The other issue is two or more different IGE phenotypes are frequently found within a single pedigree and obvious clinical and EEG overlap may be observed [1456].

Recently, mutations in genes encoding ion channels have been identified in some types of epilepsy such as benign familial neonatal convulsions [78910 11], autosomal dominant nocturnal familial frontal epilepsy [1213141516171819 20], rolandic epilepsy [21222324] and febrile seizures [25262728293031]. Mutations involved in voltage- gated channels might have widespread effects on threshold excitability of neurons. A search of genes related to ion channels function in epilepsy syndromes may be worthwhile. Two novel voltage-gated potassium channel genes KCNQ2 and KCNQ3 that contribute to the M current, a slowly activating and deactivating potassium conductance were identified in idiopathic generalized epilepsies, BFNC [323334].

In this study, we examined the Asn752Thr polymorphism in exon16 of the KCNQ2 gene located on 20q13.3 in families with idiopathic generalized epilepsy syndromes in Turkish population.

MATERIALS AND METHODS

Subjects: Four nuclear families with 33 members were included in this study. The sample contained 14 idiopathic generalized epilepsy syndromes, six patients with primary generalized tonic-clonic seizures, one patient with childhood absence epilepsy, one patient with juvenile absence epilepsy, one patient with juvenile myoclonic epilepsy and three patients with primary generalized tonic-clonic seizures with myoclonias. The pedigrees of the included families were described in detail in figures: 1,2,3,4. Diagnostic classification was performed according to the probands major epilepsy subtype and followed the proposal of the commission on classification and terminology of the international league against epilepsy [35]. Results of neurological and psychiatric examination were normal in all

patients. EEG recordings obtained from all patients demonstrated normal background activity and most of them with paroxysmal generalized spike-and-wave discharges. Clinical and EEG data for each individual were documented in a standardized, anonymous protocol that was reviewed by Genetic Subcommittee of Turkish Society of Epilepsy. The Ethics Committee of the Medical Faculty, University of Marmara, Istanbul, Turkey approved our study, and written informed consent was obtained from all participants.

DNA analysis: Genomic DNA was extracted from 10 ml aliquots of EDTA- anticoagulated blood samples, using a salting-out method [36].

Polymorphism: The DNA fragment in exon16 of KCNQ2 gene contains two invariable sites which serve as an internal control and one variable site as shown in the figure. (+) allele results in 134bp, 54bp, 45bp and 43bp fragments while (-) allele results in 177bp, 54bp and 45bp fragments when the 276 bp PCR product was digested.

Figure 1
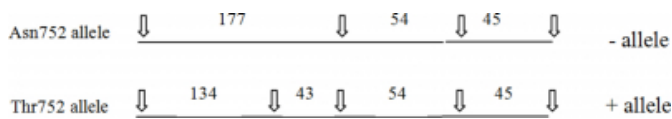
PCR AMPLIFICATION

276 bp DNA fragment which contains the polymorphic site was amplified by using the following primers:

Forward: 5'-TCC TGG CAG CCA CAG AGC CA

Reverse :5'-GGA GAT GCT GAA GCC GCT GA

The amplification was done by the following PCR programme: Initial denaturation at 95°C for 5 min, followed by the cycle: 94°C for1 min;64°C for 1 min; 72°C for 1 min repeated 33 times and final extension at 72°C for 7 min.

Restriction Enzyme Digestion:

276 bp PCR product was digested by using restriction endonuclease Ava II for four hours at 37 °C water bath. Digested products were analyzed 10 per cent polyacrylamide gel.

FAMILY I

Index case was a 12 years-old male whose seizures began at age 6. He was referred to our clinic because of staring periods. His staring periods characterized with sudden onset, movementless periods only associated upward deviation of his eyes. If these periods occurred over 1-2 minutes, twitching around his eyes and mouth are also seen. When poorly controlled, he could have several attacks per day. Interictal EEG showed generalized 3Hz spike and wave discharges characteristic for childhood absence epilepsy (CAE). His medical history was unrevealing.

The proband's 19 year-old brother had his first seizure, which was characterized by throbbing headache followed by light-headedness and fisting of the hands and staring for 1 to 2 minutes. After two years, he had his second seizure and third one, which was ended-up with generalized tonic-clonic convulsions. Interictal EEG showed generalized spike, polyspike and slow wave discharges. His medical history has no characteristic other than febrile seizure that occurred at age one.

The proband's 25 year-old brother had his convulsions between ages 1 and 5 years. His seizure began with tonic contractions and deviation of eyes. He was well controlled on phenobarbital treatment. EEG obtained when he was seizure free and revealed no abnormality.

The proband's 17 year old cousin had generalized tonic-clonic convulsion which was stopped after appropriate antiepileptic treatment. EEG was not obtained while DNA analysis was done for polymorphism analysis.

The proband's 25 year old aunt had generalized tonic-clonic seizure which was controlled after administration of antiepiletic drug. EEG and DNA analysis were not obtained.

This family had five individuals with seizures spanning two generations. Seizure semiology was heterogeneous. Index case had typical CAE with his EEG traits and seizure pattern. Although, the two brothers of the index case and the other two affected people of this family classified as a generalized epilepsy syndrome. All of the affected subjects have well responded to antiepileptic drug treatment.

FAMILY II

The index case was a 15 year-old female, whose seizure began at 10 year of age. Her first seizure began with tonic contractions of the extremities and ended with clonic phase during awakening period. She had no seizures under valproic acid and interictal EEG obtained during the period was normal. Meanwhile, she had aggressive behavior and disinhibition.

The proband's 20 year old sister had absence seizure, when she was at 13 year of age. These absences occurred several times per day and associated with upward deviation of eyes. Interictal EEG showed generalized sharp wave activity. She was well controlled on valproic acid treatment. The proband's $3^{1/2}$ year-old brother had generalized seizures started with absences associated upward deviation of eyes and myoclonias. He also had atonic seizures. He was resistant to treatment in contrast to his sisters. In addition, the onset and the type of his seizures were different from his sisters. The patient did not respond to appropriate dosages of valproic acid, phenobarbital and clonazepam. He still experiences several seizures per day. Interictal EEG showed generalized 2-4 Hz spike and polyspike discharges. This patient was said to be hyperactivite with normal intelligence by his pediatric neuropsychologist.

This family had four individuals with seizure spanning two generations. Seizure semiology was heterogeneous. The seizure types were classified as grand mal on awakening in the index case and juvenile absence epilepsy in the proband's sister. But the brother of the proband with his myoclonic absences and atonic seizures was classified as IGE because of normal intelligence and developmental milestones he had. There was no history of paroxysmal disorder in other members of the family. The last patient is a 3rd degree relative. Epilepsy with tonic-clonic seizures had begun in late adolescence period and well responded to antiepileptic drug.

FAMILY III

The index case was 23 year-old female who had generalized tonic-clonic seizures after 16 years of age during sleep. Interictal EEG obtained during hyperventilation period showed generalized sharp waves.

The proband's 20year-old sister had her first seizure at 15 years of age. Her seizures were provoked by visual stimuli and characterized by shaking of the upper extremities and loss of contact. She also had generalized tonic-clonic seizures during sleep. Interictal EEG was normal. Her medical history revealed a febrile convulsion occurred at 10th day of her life.

The proband's grand-grandmother had also epilepsy which was reported by history. The patient died before the study was undertaken.

The proband's 85 year-old grandmother had her first seizure

at age twenty. Her seizure was best described as myoclonias occurring in upper extremities, which was culminated in generalized tonic-clonic seizure upon withdrawal from antiepileptic drug. Interictal EEG demonstrated right centrotemporal sharp waves.

The proband's 55 year-old aunt had generalized tonic-clonic epilepsy, which had begun, in twenties of her life and which was reported by history alone.

The proband's cousin had generalized tonic-clonic seizure in the late adolescence period.

This family had six individuals with seizures spanning four generations.

Seizure semiology was heterogenous. One individual had GTCS, one individual had myclonias alone and one individual had both myoclonias and GTCS. All seizure types are well controlled under valproic acid treatment. The proband's mother and 26 year-old sisters had also migraine.

FAMILY IV

Index case was a18 year-old male. His seizure began with bilateral myoclonic jerks in shoulders and arms without loss of consciousness, occurring after awakening at age 16.

First GTCS on awakening occurred after a sleepless period at age 17. Interictal EEG showed generalized 4 Hz spike and wave complexes.

The proband's 15 year-old cousin had her first GTCS on awakening at 13 years of age.

She also had JTCS on daytime. Interictal EEG showed generalized spike and/or wave discharges.

This family had 2 individuals with seizure spanning three generations. The phenotypes of this family composed of grand mal on awakening and JME. The affected peoples of this family are well responded to valproic acid treatment. In addition, the proband's cousins had right centrotemporal epileptiform discharges on EEG, although he had no clinical history of seizure.

RESULTS

All of the families included in this study have heterogenous IGE syndromes in their families. One patient also has febrile convulsion history. In only one patient, the seizures were uncontrolled. Subsequent results were found after the analysis of exon 16 polymorphism in four families with

idiopathic generalized epilepsy syndrome. Genotyping of family members showing exon 16 polymorphism as (+) threonine allele and (-) asparagines allele were demonstrated in family pedigrees (Figure 1, 2, 3, 4). Polyacrylamide gel electrophoresis results were also shown.
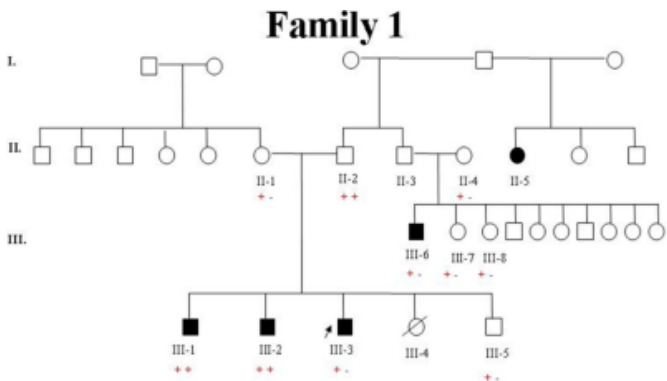
Figure 2

Figure 1: Pedigree of the first family
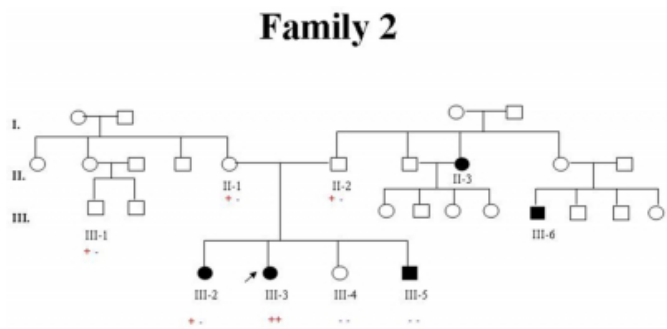

Family 1

Figure 3

Figure 2: Pedigree of the second family.

Family 2

Figure 4

Figure 3: Pedigree of the third family
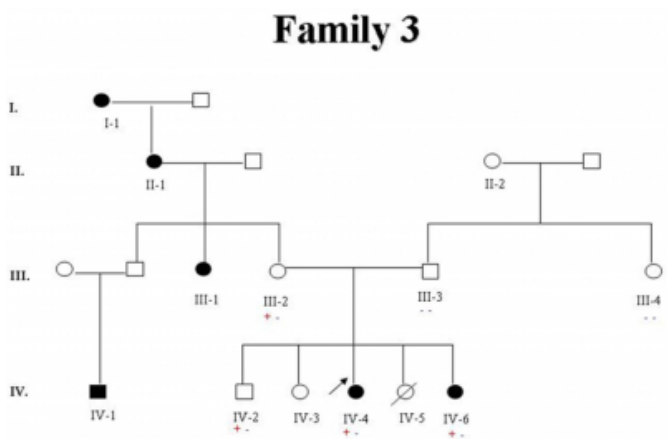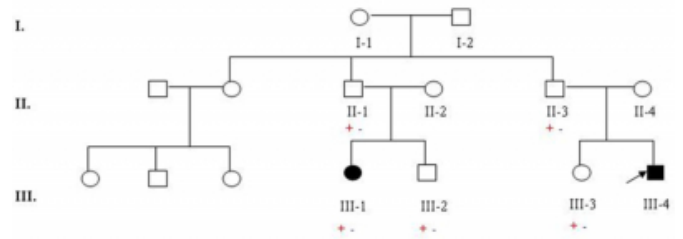

Family 3

Figure 5

Figure 4: Pedigree of the fourth family.

Family 4

FAMILY I

IGE syndrome was seen in two consecutive generation of the index case (II-2). Although, II-2 was not epileptic, his little sister (II-5) and his three sons (III-1, III-2, and III-3) and his cousin (III-6) have IGE syndrome. Although II-2 is not informative concerning the polymorphic site; one of his + alleles associated with his children's IGE inheritance. III-3, III-5 got the one (-) allele from their mother, while III-1 and III-2 got the one (+) allele. The unaffected child (III-5) took the (+) allele, which was unrelated to the syndrome from his father. The effected heterozygote male cousin (III-6) took the (+) allele that related to the syndrome. But the two sister (III-7, III-8) of II-6, also carried (+) allele (Figure 5). Due to lack of genotype of II-3, a possible linkage to the + allele could not be followed.

Figure 6

Figure 5: Polyacrylamide gel electrophoresis of the first family.
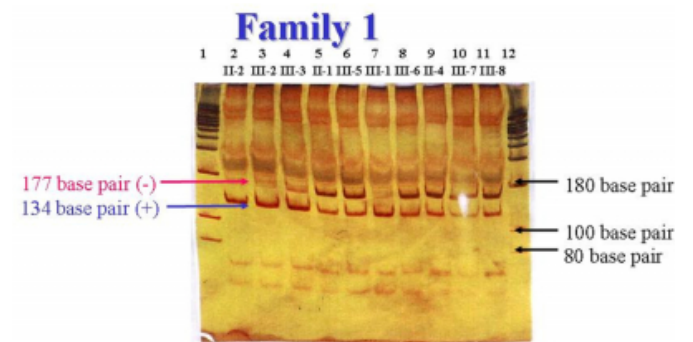
FAMILY II

In this family, genotype analysis of exon 16 polymorphism on three affected children expresses all possible genotypes (Figure 6). Consequently, no evidence of linkage between KCNQ2 and IGE was found in this family. The absence of linkage may be due to the lack of phenotypic homogeneity

or the possibility that the causative mutations reside in other gene(s).

Figure 7

Figure 6: Polyacrylamide gel electrophoresis of the second family
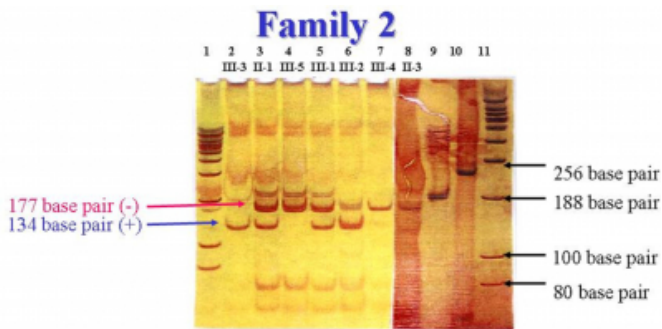
FAMILY III

IV-4 and IV-6 got (+) allele from the mother. Due to IGE syndrome in the mother (III-2), it is possible to say that there is a relation between IGE and (+) allele (Figure 7). However, despite the presence of (+) allele in IV-2, clinical impression was not shown. Consequently, a relationship between the IGE syndrome and KCNQ2 gene could not be shown.

Figure 8

Figure 7: Polyacrylamide gel electrophoresis of the third family
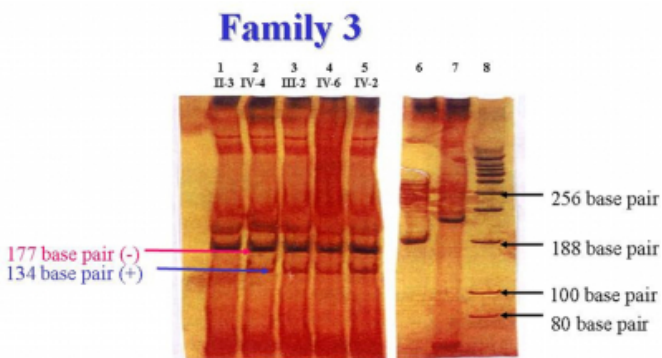
FAMILY IV

In this family, only one affected member was analyzed, and this respect, to reach a relation with the KCQN2 gene is not possible. However, the mode of inheritance displays a similarity (Figure 8). The reason is that in both families, unaffected parents have affected children. Figure 8: Polyacrylamide gel electrophoresis of the fourth family.

DISCUSSION

Most of the epilepsy syndromes do not demonstrate classical Mendelian inheritance pattern. More than one genetic and environmental factors cause an epilepsy type to be expressed [4]. Other than these factors, diagnostic criteria, genetic variety, incomplete penetrance, polygenic inheritance and the effect of the onset age of epilepsy also build epilepsy complex.

Two or more different phenotypes are frequently found within a single pedigree and obvious clinical and EEG overlap may be observed.

Recently, studies concentrated on three genes in IGE. One of these genes was $GABA_B$ receptor gene. This is an HLA-linked susceptibility gene on chromosome 6 [373839]. This gene was isolated in members of many JME families. Also, in mouse models, this gene was investigated [40]. In chromosome 6p21 and 8q24, the other epilepsy locus exists whose phenotypes consist of classic JME with convulsions and/or EEG rapid multi-spike wave complexes [373841] .The third gene group, which is responsible for QT syndrome, is KCNQ potassium channel gene family. Voltage-dependent ion channels that control the propagation of action potentials generate the excitable properties of neurons. In epileptic seizures, voltage-dependent channels produce characteristic, highly synchronized discharges of action potentials in coupled neurons. During the period of action potential, efflux of K^+ restores the membrane potential to its resting, hyperpolarized value. Because of their role in repolarizing the membrane, K^+ channels are particularly important in controlling the excitability of neurons [42]. Potassium channels are membrane-bound macromolecules performing regulatory functions in nearly all cell types. Potassium channels are members of voltage-dependent ion channels share a characteristic structural organization, including six putative transmembrane segments and a P-loop, which constitutes the outer part of the pore [43] .More than 80 potassium channel genes in human are known, but mutations in about 10 K+ channel genes only are recognized to cause human disease [44] .KCNQ2 and KCNQ3 are neuron specific isoforms that are co-expressed in broad regions of the nervous system. They assemble to form heteromeric channels that have properties of the M-type K^+ current [34]. M-current is active near the threshold of action potential firing and is an important determinant of neuronal excitability [44]. The M-current slowly activates when excitatory stimulus depolarizes the neuron toward spike threshold, repolarizing the membrane back toward resting potential and suppressing firing[45]. In recent times, it has

been demonstrated that the KCNQ2 and KCNQ3 subunits constitute an M-current in native neurons [32]. KCNQ channels play key roles in heart, brain and other tissues [46]. Mutations in KCNQ1 cause hereditary long-QT syndrome, cardiovascular disorder associated with syncope and sudden death [4346]. Although KCNQ1 has not been found in brain, recently investigators identified four homologous neuronal KCNQ genes (KCNQ2-5)[46]. KCNQ2 and KCNQ3 mutations cause benign neonatal familial convulsions [747]. KCNQ4 mutations result in a form of dominantly inherited deafness [4346]. KCNQ5 is widely expressed in brain, heart, and skeletal muscle [48]. Both KCNQ4 and KCNQ5 may contribute to M-current [444849].

The KCNQ gene family is not specific for only one organ. A person with autosomal dominant deafness also has seizure [33]. All members of the KCNQ gene family can produce M current and therefore there will be a large field of interaction to be examined between KCNQ gene family and seizure production [33]. Benign neonatal familial convulsion is mapped to 8q24, which is the location of KCNQ gene family [750]. For that reason KCNQ gene family will be the responsible gene group. BFNC is a rarely seen type of IGE syndrome. The role of the KCNQ2 and KCNQ3 genes in this syndrome is known. Also, the relationship between M current, which organize the stimulation and KCNQ2 and KCNQ3 genes are also known [101147]. Fong et al mapped CAE to 8q24 [50]. This location is near to the locus of KCNQ3 gene. This close relationship between CAE and this chromosomal locus had led them to consider a relation between CAE and K^+ channel gene [50]. The genotype analysis in all four families affected by the idiopathic generalized epilepsy syndrome revealed a possible linkage of the disease to the KCNQ2 gene in only one family (Family I). But it can not be taken as a conclusive evidence. The reason for this is that we could not be able to examine all the affected family members and also other intragenic polymorphic markers were not included. Another explanation for this might be the complexity of IGE phenotypes. Homogeneous phenotypes were not present in the other three families. K^+ channels have heterotetrameric protein structure and this structure is coded with more than one gene. This may be the reason for insufficient linkage of all the families with KCNQ2 gene. Mutations in the other K^+ channel gene KCNQ3, coding for the other subunit of K^+ channel, might also be responsible for the IGE subtypes. In one study, which was conducted on 38 JME and 33 CAE patients, KCNQ3 polymorphism analysis revealed no

relation between KCNQ3 polymorphism and a phenotype of this family [51]. Epilepsy syndromes do not appear as a single phenotype in all family members. They appear as more than one phenotype. Absence, myoclonic and generalized tonic-clonic seizures in various combinations were also found in our families.

More than one polymorphism locus must be analyzed for the examination of the K^+ channel role on the pathogenesis of IGE syndromes.

As a conclusion, in order to assess the KCNQ2 mutation profile of the Turkish population, a large study aimed at screening both idiopathic generalized epilepsy patients and healthy subjects in larger number of families seems to be necessary.

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