SCN1A, SCN2A, KCNQ2 Gene Mutations in Epilepsy With Generalized Tonic-Clonic Seizures

SCN1A, SCN2A, KCNQ2 Gen Mutasyonları ve Jeneralize Tonik- Klonik Nöbetli Epilepsi

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ÖZET

Amaç: Bu çalışmada, SCN1A (R1648H), SCN2A (GAL879-881QQQ), KCNQ2 (V182M) genlerindeki mutasyonlar ile Jeneralize Tonik-klonik nöbetli (JTKN) epilepsi arasında ilişki olup olmadığının araştırılması amaçlandı.

Gereç ve Yöntem: JTKN tip epilepsili 75 hasta ve 75 sağlıklı kişi çalışmamıza dahil edildi. Hasta ve kontrol grubundaki kişilerin DNA'ları izole edildi ve SCN1A, SCN2A, KCNQ2 genlerinin ilgili bölgeleri polimeraz zincir reaksiyonu (PZR) ile çoğaltıldı. PZR ürünlerine DNA dizi analizi yapıldı. Dizi analiz sonuçları MEGA (Molecular Evolutionary Genetics Analysis) programında analiz edildi. Bulgular istatistiksel olarak değerlendirildi.

Bulgular: R1648H, GAL879-881QQQ, V182M mutasyonları hasta ve kontrol grubunda gözlenmedi. R1648P varyantı, 134272 A>G polimorfizmi ve 134347 G>A polimorfizmi SCN1A geninde bulundu. 105649 G>A polimorfizmi SCN2A geninde belirlendi. 28704 T>G polimorfizmi KCNQ2 geninde bulundu. Hasta ve kontrol grubunda belirlenen variant ve polimorfizmler arasındaki fark istatistiksel olarak anlamlı değildi ($p>0.05$).

Sonuç: Bu mutasyonların JTKN ile ilişki olmadığı sonucuna varıldı.

Anahtar kelimeler: epilepsi;KCNQ2 proteini;SCN1A protein insan; SCN2A protein insan

ABSTRACT

Ojective: In this study, we aimed to investigate if there is a relationship between mutations in SCN1A (R1648H), SCN2A (GAL879-881QQQ), KCNQ2 (V182M) genes and epilepsy with generalized tonic-clonic seizure (GTCS).

Materials and Methods: Seventy five patients with GTCS type epilepsy and 75 healthy subjects were included in our study. DNA's of patients and controls were isolated and the interested regions of SCN1A,

SCN2A, KCNQ2 genes were amplified by polymerase chain reaction (PCR). DNA sequence analysis was applied to PCR products. The sequence analysis results were analyzed in MEGA (Molecular Evolutionary Genetics Analysis) program. The findings were evaluated statistically.

Results: R1648H, GAL879-881QQQ, V182M mutations were not observed in the patient group and the control group. R1648P variant, 134272 A>G polymorphism and 134347 G>A polymorphism were found in SCN1A gene. 105649 G>A polymorphism was determined in SCN2A gene. 28704 T>G polymorphism was found in KCNQ2 gene. The difference between variant and polymorphisms determined in patients and control groups were not statistically significant ($p>0.05$).

Conclusion: SCN1A, SCN2A and KCNQ2 mutations may not be related to GTCS.

Key Words: epilepsy; GTCS; KCNQ2; SCN1A; SCN2A;

INTRODUCTION

Epilepsy is a central neural system damage characterized with repetitive seizures(1).It is one of the most-frequently observed neurological diseases that influence 1% of the general population (2). Nearly 40% of the reasons of the occurrence of epilepticsyndromes is idiopathic(3). The underlying factors of idiopathic epilepsies(IE) are not fully clear yet. However, it is considered that more than one gene has a role in these types of epilepsies, and epilepsy is transferred to next generations with complex inheritance (4). Generalized Tonic Clonic Seizure (GTCS) typed epilepsy is a form of Idiopathic Generalized Epilepsy (IGE) and is observed at a rate of 14% among Idiopathic Generalized Epilepsies (5).

Ion channels have the main duty in the basis of signal transmission between neurons and along neuron. A mutation that may occur in these channels make these ion channels fail to perform their functions in a correct manner. For this reason, the balance between neurons is disrupted, and epileptic seizures may occur due to abnormal neuron discharges. The studies conducted so far have demonstrated that the epileptogenic process is dependent on the channel pathologies. Some of genes that are responsible for ligand-gated channels and voltage-gated channels, which are among ion channels, have been associated with different forms of epilepsy(2,6). Mutations in these genes change the stimulation of neurons in various ways. The majority of these mutations change the sensitivity in synapse areas and the neurotransmitter substance secretion. In addition, they prolong depolarization time which is observed during transmission of action potential in neurons (7).

Voltage-gated sodium ion channels are important for beginning and progression of the action potential that ensures signal transmission within neurons. An anomaly that is observed in one part of ion channel, cause problems in the progression of action potential. It has been reported that the anomalies related with the subunits with SCN1A, SCN2A and SCN8A are associated with IGE(8). SCN1A gene is located in2q24 area(9). SCN1A gene product ensures that voltage-dependent sodium ion channels are inactivated in a fast manner in suitable conditions (10). In the studies that have been conducted so far, it has been reported that, it is one of the most important genes that has role in epilepsy transferred sporadically and genetically (6). SCN2A gene is located in the 2q24 chromosomal area (9). In the studies conducted on SCN2A gene, many mutations that caused different forms of epilepsy have been found. Different seizure types are observed upon the different effects of each mutation that change the function of the channel. Some of the mutations are wrong-base matching mutations. They cause slide in hyper-polarization range during signal transmission in neurons, and this situation lead to early opening of ion channel gates (6).

The mutations related with voltage-gate potassium ion channels cause the disruption in channel formation and protein folding, slide in the depolarization range, and slow-down in the channel activation and inactivation. The mutations that cause

epilepsy in voltage-gate potassium ion channel gene KCNQ2 have been determined in the studies that have been conducted so far (11,12). KCNQ2 gene is located in 20q13.3 area. It consists of 18 exons and has a length of 50kb (13).

In some *in vivo* studies, it has been reported that the genetic interaction may be related with GTCS. It has been reported that GTCS developed in rats that carried R1648H in SCN1A gene, GAL879-881QQQ mutation in SCN2A gene (14), or V182M mutation in KCNQ2 gene and GAL879-881QQQ in SCN2A gene (2).

In our literature review, we did not come across any studies that were conducted in humans on relationship between GTCS type epilepsy and the mutations in SCN1A, SCN2A, KCNQ2 genes. We aimed to investigate if there was a relationship between mutations in these genes and GTCS type epilepsy.

MATERIALS AND METHODS

Ethical approval was received from Celal Bayar University, Faculty of Medicine, Local Ethical Committee. The subjects approved the volunteering form and were included in the study. R1648H mutation in SCN1A gene; GAL879-881QQQ mutation in SCN2A gene and V182M mutation in KCNQ2 gene were examined in 75 patients who had GTCS type epilepsy and in 75 healthy control.. Venous blood was drawn into EDTA-filled tubes.

Patient and Control Groups

Control Group: *This group consisted of* seventy five healthy individuals without epilepsy who admitted to Manisa Celal Bayar University, Faculty of Medicine Hospital, Neurology Department (male:36, female:39)

Patient Group: *This group consisted of* seventy five patients, who admitted to Manisa Celal Bayar University, Faculty of Medicine, Neurology Department, and were diagnosed with GTCS (male:39, female:36)

Genomic DNA Isolation

GeneMatrix Quick Blood DNA Isolation Kit (Poland)was used to perform DNA genomic isolation from the whole blood. DNA concentration and purity were measured in nano-drop measurement device (Maestro Gen, America) at 260nm and 280 nm wavelengths.

Polymerase Chain Reaction

The 947bp part which included whole of exon26of SCN1A gene; and a part of intron 26;the 989bp area which included the whole of exon19 of SCN2A gene, and a part of intron 19; and the 684 bpwhich included exon9and intron 10 of KCNQ2 gene were amplified from DNAs that were isolated from the blood, and by using the primer pairs given in Table 1. PCRmixture that included buffer, $MgCl_2$, dNTP mixture, forward primer, reverse primer, Taq polymerase, distilled water and template DNA was prepared for each gene. In order to amplify the interested region properly, graded temperature reduction PCR Technique (Touchdown PCR) was used. According to this technique, the annealing temperature was reduced 0,5°C at each cycle. The reaction was performed for each gene in proper PCR cycle conditions (Table 2). Subsequently, obtained PCR products were analyzed in 1,5% agarose gel electrophoresis.

Table 1. ThePrimer pairs for SCN1A, SCN2A and KCNQ2 genes

Genes	Primer Type	Primer Sequences
SCN1A	Forward Primer	TGTTGCATACCTCTGGAAGAAG
SCN1A	Reverse Primer	AAGCAATTTTCGTGAGTACC
SCN2A	Forward Primer	ACCTGTACATTTGCCCTGTTAAT
SCN2A	Reverse Primer	CTAGGCACCGAAAACCCCAT
KCNQ2	Forward Primer	TGGGCATACACCTTCCCG
KCNQ2	Reverse Primer	CCGCAGCTTAAACAACCA

Table 2. The PCR cycle protocol for SCN1A, SCN2A and KCNQ2 genes

GENES	PCR CYCLE			
	STAGES	TEMP (ºC)	TIME	NUMBERER
SCN1A	Pre-Incubation	95	15 minutes	1
	Denaturation	94	45 seconds	35
	Annealing	64	45 seconds	
	Extension	72	60 seconds	
	Last Extension	72	5 minutes	1
SCN2A	Pre-Incubation	95	15 minutes	1
	Denaturation	95	45 seconds	35
	Annealing	66	45 seconds	
	Extension	72	60 seconds	
	Last Extension	72	5 minutes	1
KCNQ2	Pre-Incubation	95	15 minutes	1
	Denaturation	94	45 seconds	35
	Annealing	59	45 seconds	
	Extension	72	60 seconds	
	Last Extension	72	5 minutes	1

DNA SequenceAnalysis

DNA sequence analysis was applied to PCR products that were obtained from SCN1A, SCN2A and KCNQ2 genes. Results were examined by using MEGA (Molecular Evolutionary Genetics Analysis) program. The number of people who had nucleotide change in the amplified areas of the genes in both study groups were determined.

Statistical Analysis

The findings obtained in our study were evaluated with SHEsis program. The issue of these genes being whether homozygous or heterozygous was investigated, and the genotype distributions were determined with Chi-Square test. The data were compared between the study groups and statistical evaluations were made. The significance value was accepted as $p< 0.05$.

RESULTS

The data are given in Table 3. In control and patient groups, the mean age was 35,25 years. When the two groups were compared in terms of age and gender distribution, the difference was not significant ($p>0.05$).

***SCN1A*:** R1648H mutation in exon 26 of gene was not found either in the patient or in the control group. Different polymorphisms were determined in exon 26 in the sequence analysis. 134436 G>C variant(R1648P) was determined in 1 patient and in 3 people in the control group as heterozygote. 134272A>G polymorphism (Q1593R, rs765485870) and 134347G>A polymorphism (S1618N, rs200263247) were found in different people in the control group (Table 3). When each determined polymorphism was compared, the difference was not significant ($p>0.05$).

***SCN2A*:** It was determined that GAL879-881QQQ mutation did not exist in neither control nor in the patient group (Table 3). It was shown that 105649 A>G polymorphism (rs188078595) in intron 19 of the gene was carried as heterozygote in the control group.The difference between the groups was not found to be significant ($p>0.05$) (Table 3).

***KCNQ2*:** It was determined that none of the subjects carried V182M (rs201130603) mutation (Table 3). According to the sequence analysis it was determined that 28704T>G polymorphism (rs374252672) in intron 10 of the gene was carried in a heterozygous in 1 person in the control group.When this polymorphism was compared , the difference between them was not found to be significant ($p>0.05$) (Table 3).

Table 3. The data of the patient and control group.

	GENOTYPE	CONTROL	PATIENT
n		75 (%100)	75 (%100)
GENDER			
MALE		36 (%48)	39 (%52)
FEMALE		39 (%52)	36 (%48)
AVERAGE AGE		35,25±9.5	35,25±9.5
SCN1A	R1648H*	-	-
	R1648P (G/G)	72 (%96)	74 (98,7)
	R1648P (G/C)	3 (%4)	1 (%1,3)
	Q1593R (A/A)	74 (%98,7)	75 (%100)
	Q1593R (A/G)	1 (%1,3)	-
	S1618N (G/G)	74 (%98,7)	75 (%100)
	S1618N (G/A)	1 (%1,3)	-
SCN2A	GAL879-881QQQ *	-	-
	Intron 19. 105649. nt (AA)	74 (%98,7)	75 (%100)
	Intron 19. 105649. nt (AG)	1 (%1,3)	-
KCNQ2	V182M *	-	-
	Intron 10. 28704. nt (T/T)	74 (%98,7)	75 (%100)
	Intron10. 28704. nt (T/G)	1 (%1,3)	-

* The polymorphisms that are targeted to be examined in the study.

DISCUSSION

In our study, a possible relationship between R1648H mutation in SCN1A gene; GAL879-881QQQ mutation in SCN2A gene; V182M mutation in KCNQ2 gene and GTCS has been investigated. We concluded that none of the subjects carried the defined mutations in both study groups.

In our study, it was determined that R1648H mutation in exon 26 of SCN1A gene did not exist in the patient and control group. It was shown that the R1648P variant in the same area was carried by 3 people in the control group and by 1 person in the patient group in heterozygous. When the data obtained from both groups were evaluated statistically, the difference was not significant ($p>0,05$). There was no relation between this mutation and GTCS.

We determined that there was no R1648P variant in SCN1A gene in gene database (15). R1648P variant is reported firstly with this study. The fact that arginine (R), which is a polar basic amino acid, being converted into proline (P), which is an apolar amino acid in the relevant area in both groups, may be evaluated in terms of the relation with the post-translational modifications of the proteins that constituted the voltage-gate sodium ion channels. In our study, Q1593R polymorphism in SCN1A gene was detected in 1 person in the control group. Since the difference between the patient and control groups was not found to be significant, it was concluded that the polymorphism was not related to GTCS.

Owing to SCN1A gene, 134347G>A polymorphism (S1618N) was found in 1 person (1,3%) in the control group. Although the relation with GTCS was not determined statistically, the prevalence of polymorphism (Table 3) provides us the opportunity to compare it with the other societies. According to 1000 Genome Project, the prevalence of G allele in African, American, Eastern Asian, Southern Asian and European societies was given as 100%(16). The findings of our study and the results of the 1000 Genome Project are consistent with each other.

In our study, GAL879-881QQQ mutation was not detected in any people in the patient and

control groups. In addition, no changes were found in the area where sequence analysis was performed in SCN2A gene. This area corresponds to the cytoplasmic area which connects the 4th and 5th segment of the second membrane structure of the voltage-gate sodium ion channel. It poses an important area in the formation of the pore structure of the channel. According to our study, it is possible to claim that there are no mutations related with GTCS type epilepsy in exon 19o f SCN2A gene in our study group. However, it may be recommended that this result is supported with a study with more patients.

The105649 A>G polymorphism was detected in intron 19 of SCN2A gene. This polymorphism was found in 1 person (1,3%) in the control group. Since the difference between the data of the patient and control groups was not found to be significant in terms of this polymorphism. It was concluded that polymorphism was not related with GTCS.

In our study, V182M mutation in exon 9of KCNQ2 gene was not detected in the patient and control group. The 28704 T>G polymorphism in the intron 10 of KCNQ2 gene was detected in 1 person (1,3%) in the control group. The statistical relation of this polymorphism with GTCS has not been determined.

Hawkins et al. conducted an *in vivo* study on rats and reported that the genetic interaction between R1648H mutationin SCN1A gene; GAL879-881QQQ mutation in SCN2A gene; and V182M mutation in KCNQ2 gene was related withGTCSseizure type. They also reported that it had a relation with GTCS type epilepsy in the rats that carried together GAL879-881QQQ mutation in SCN2A gene, and R1648H in SCN1A gene(14). We determined that R1648H in SCN1A gene;GAL879-881QQQ in SCN2A gene, and V182M mutation in KCNQ2 genedid not exist in the patient and in the control group. According to the findings of our study, which is the first one conducted on humans in this mutations, we can suggest that there is no relation between GTCS and these mutations.

Kearney et al. conducted an *in vivo* study on rats and reported that GTCS type epilepsy was observed in the rats which carried together GAL879-881QQQ mutation in SCN2A gene and V182M mutation in KCNQ2 gene (2). In our study, the defined mutations were not detected in SCN2A and KCNQ2 genes in the patient group. For this reason, the results obtained in our study do not support the results of Kearney et al.The findings obtained in our study is important in that it is the first study conducted on humans in terms of these mutations in the group that had GTCS type epilepsy.

Complex inheritance plays an important role in idiopathic epilepsies (17,18).The interaction between the environmental and the geneticfactors in complex inheritanceis relevant. In Addition, genetic heterogeneityis observed in all of the epilepsy syndromes that form the sub-groups of the idiopathic epilepsy (18). For this reason, it is difficult to enlighten the genetic basis of an idiopathic epilepsy phenotype. In addition, more than one gene causing an epilepsy syndrome or a gene causing different epileptic syndromes make it difficult to enlighten the genetic bases of the epilepsy phenotypes(19,20). Genetic heterogeneity also exists in GTCS type epilepsy, which is a sub-type of idiopathic generalized epilepsy. The underlying genetic factor being not determined yet may be due to this reason.

In conclusion, R1648H in SCN1A gene; GAL879-881QQQ in SCN2A gene; and V182M mutation in KCNQ2 gene were not detected in GTCS patient group and in the control group. We concluded that these mutations in humans were not related to GTCS. In addition, the relation of the mutations in SCN1A, SCN2A and KCNQ2 genes with GTCS epilepsy type was not determined. R1648P variant in SCN1A gene was found in 1 patient in the patient group, and in 3 people in the control group; however, it was concluded that this was not related with GTCS. This variant was determined in this study for the first time.We consider that our findings,which is the first ones in Turkish society, will add a scientific contribution to the literature.

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