A novel missense mutation (N258S) in the KCNQ2 gene in a Turkish family afflicted with benign familial neonatal convulsions (BFNC)

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SUMMARY: Yalçın Ö, Çağlayan SH, Saltık S, Çakar Ö, Ağan K, Dervent A, Steinlein OK. A novel missense mutation (N258S) in the KCNQ2 gene in a Turkish family afflicted with benign familial neonatal convulsions (BFNC). Turk J Pediatr 2007; 49: 385-389.

Benign familial neonatal convulsions (BFNC) is a rare monogenic subtype of idiopathic epilepsy exhibiting autosomal dominant mode of inheritance. The disease is caused by mutations in the two homologous genes KCNQ2 and KCNQ3 that encode the subunits of the voltage-gated potassium channel. Most KCNQ2 mutations are found in the pore region and the cytoplasmic C domain. These mutations are either deletions/insertions that result in frameshift or truncation of the protein product, splice-site variants or missense mutations. This study reveals a novel missense mutation (N258S) in the KCNQ2 gene between the S5 domain and the pore of the potassium channel in two BFNC patients in a Turkish family. The absence of the mutation both in the healthy members of the family and in a control group, and the lack of any other change in the KCNQ2 gene of the patients indicate that N258S substitution is a pathogenic mutation leading to epileptic seizures in this family.

Key words: potassium channel gene, benign familial neonatal convulsions (BFNC), mutation.

Benign familial neonatal convulsions (BFNC) is a rare subtype of idiopathic epilepsy that exhibits an autosomal dominant mode of inheritance. Frequent brief seizures start within the early days of life and disappear spontaneously after weeks or months. BFNC is associated with the loss of function of a potassium channel; the pathological neuronal hyperexcitability in this epilepsy syndrome is a result of impaired repolarization. Two voltage-gated potassium channel genes, KCNQ2 on chromosome 20q13.3 and KCNQ3 on 8q24, have been shown to be the genes responsible for BFNC[1-3]. Voltage-gated potassium channels constitute a superfamily of proteins that share several common features, such as six conserved hydrophobic regions, including the voltage sensor contained in the S4 helix, an ion channel pore formed partly by the loop between S5 and S6, and a long C-terminal domain[4]. KCNQ2 gene has at least 18 exons spanning 50kb and is expressed in the brain[5]. Co-expression of KCNQ2 and KCNQ3 in most brain tissue raises the possibility that they may assemble together to build a hetero-tetrameric channel in the central nervous system. This hetero-tetrameric channel was found to contribute to the native M-current, one of the most important regulators of neuronal excitability[6,7]. The disease-causing mutations were mostly detected in the KCNQ2 gene, and KCNQ3 gene was identified as a minor locus. Most KCNQ2 mutations are deletions, insertions or splice-site mutations, most of them causing frameshifts and premature stop codons in the predicted protein, and they are mainly clustered in two conserved regions of the channel protein, in the P-loop which forms the pore

domain and C-terminus which is responsible for assembly of heteromeric channels and for surface expression[8,9]. The mutations in the P-loop affect ionic conductance of the channel and reduce K^+ current, while the mutations in the C-terminal abolish the formation of heteromeric channels or the heteromeric channels can not be transported to the membrane surface. Thus, the reduced current is the result of reduced number of channels on the surface[10]. To date, only two mutations were identified in the S4 voltage-sensor segment, which is responsible for sensing the electric field over the membrane. These mutations are either missense or result in a truncated protein which leads to a dramatic slowing of activation of the channel upon depolarization[11,12]. In a more recent study, a missense mutation was identified in the S5 domain. The mutation was found to reduce the channel current by more than 80%[13]. This study reports the first missense mutation between the S5 domain and the pore region leading to BFNC in a Turkish family with single patients in each of the three successive generations.

Material and Methods

The BFNC family and controls

Clinical diagnosis and blood sampling of the BFNC family (Fig. 1) was performed in the Department of Neurology at İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey, upon informed consent of the family. For controls, DNA samples from 60 German and 77 Turkish individuals without a history of epilepsy were used.

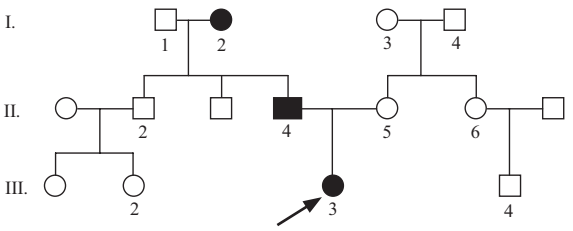

Fig. 1. Pedigree of the Turkish family. Arrow indicates the index patient. Individuals I-2, II-4 and III-3 were clinically diagnosed with BFNC. Individuals II-4 and III-3 were heterozygous for N256S mutation while all healthy members carried only the wild type allele. DNA sample of I2 was not sufficient for DNA sequence analysis. The other members of the family were not available for testing.

Mutational Analysis

Genomic DNA was extracted from white blood cells by using NACI method[14]. The primer sequences for the amplification of all 16 exons of the KCNQ2 gene were as described in Biervert and Steinlein, 1999[5]. Each polymerase chain reaction (PCR) was prepared in 25 μl volume containing 1 X Mg^{2+} free reaction buffer, 1 or 1.5 mM of $MgCI_2$, 0.2 mM of each dNTP, 1.25 units of Taq DNA polymerase, 5% DMSO or 5% glycerol, 5 pmol of each primer and 50 ng of genomic DNA. The PCR products were purified by using the Qiagen (USA) purification kit and sent to MWGAG Biotech, Germany for automated sequencing.

For Single Strand Conformational Analysis (SSCP), PCR products were run on 10% polyacrylamide gel at both room temperature and at 4°C.

For restriction enzyme analysis degenerate primers (F-5' CATGATGGTGGCGCCGTCTGCC, R-5' TCCGCGTAGGTG TCAAAGTGGGCG) were designed to create two restriction sites (1 variable and 1 invariable) for CfoI enzyme (Fig. 2). The invariable site in the 135bp PCR product is digested in all samples with CfoI enzyme and the variable site is digested only when there is A→G substitution (N258S mutation).

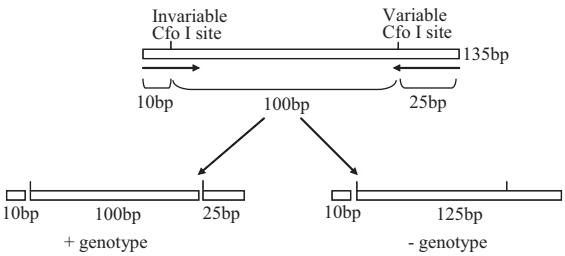

Fig. 2. Schematic diagram of CfoI digestion of exon 4 of KCNQ2 gene. The arrows indicate the primers.

Results

Clinical history

The index patient (III-3) (female, born in July 1998) had 6-7 seizures - about 30 seconds in duration repeated at 15-20 minute intervals - on the third day of life. The attacks were characterized by staring, facial cyanosis, fisting and elevation of both arms in a tonic posturing. She was afebrile during the seizures. All routine laboratory tests in the emergency

unit revealed normal results. During our first examination at age one month, her neuro-developmental status, neuro-metabolic tests and cranial magnetic resonance imaging (MRI) were normal. She received oral phenobarbitone up to nine months of age. At age 10 months she had a febrile seizure of a few minutes duration with clinical features similar to the previous ones. Presently, she is in perfect health, seizure-free and without medication. The patient had serial EEGs up to present. The earliest one at age one month and all others after age six months revealed normal results. In two EEGs at two and four months of age, irregular slowing on both fronto-centro-parietal regions and rare spikes with left fronto-central (F3-Cz-C3) predominance were present. Background activity in all EEGs was normal and physiological elements of sleep were appropriate for developmental stages. Patient's father (II-4) had two, and paternal grandmother (I-2) had single seizures, each on the third post-natal days. No descriptive information was available regarding their seizures. They had received no medication and had no health problems related to epilepsy later in their lives.

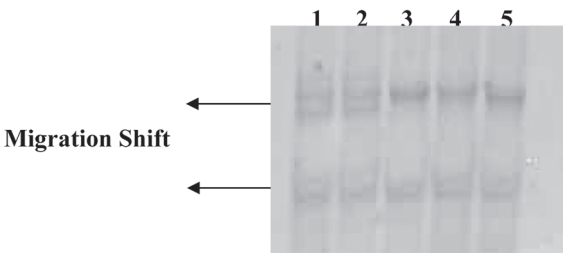

Fig. 3. SSCP gel pattern of exon 4. Lane 1 and 2: BFNC patients II-4 and III-3, lane 3: healthy individual in the family (II-5), lanes 4 and 5: normal controls.

Detection of the N258S Missense Mutation

Twenty-one regions covering all 16 exons of the KCNQ2 gene of patients II-4 and III-3 and healthy individuals - one from the family (II-4) and two from the control sample - were amplified as described in the methods section. The PCR products were run on SSCP gels both at room temperature and 4°C. All exon patterns of the patients were normal when compared to the controls except exon 4 (Fig. 3).

The exon 4 of patient II-4 was sequenced and compared with that of normal KCNQ2 sequence in the database. The analysis revealed an AàG transversion in heterozygote form at nucleotide position 773 (Fig. 4). The normal A allele codes for asparagine while the G allele codes for serine at codon 258 (AAC→AGC). Amino acid 258 is located in a region between S5 and P-loop of the potassium channel and evolutionary conservation of asparagine at this site of the KCNQ family genes is shown in Table I. This

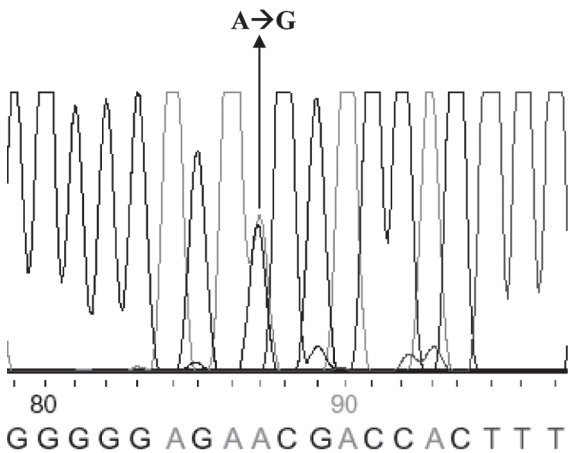

Fig. 4. A section of the DNA sequence of exon 4 sequence of the KCNQ2 gene in patient II-4. A→G transversion in heterozygote form is indicated by the arrow.

Table I. Evolutionary Conservation of Asparagine at Codon 258 in the Voltage-Gated Potassium Channel Subunits

SEQUENCE	GENE	SPECIES
SFLVYLAEKGENDHFDTYADALWWGLITLT	KCNQ2	Homo sapiens
SFLVYLAEKDANSDFSSYADSLWW	KCNQ4	Homo sapiens
SFLVYLAEKGENDHFDTYADALWWGLITLT	KvEBN1	Homo sapiens
SFLVYLVEKDANKEFSTYADALWWGTITLT	KCNQ5	Homo sapiens
SFLVYLAEKGENDHFDTYADAL	KCNQ2	Rattus norvegicus
SFLVYLAEKGENDHFDTYADALWWGLITLT	KCNQ2	Mus musculus
SFLVYLVEKDANKEFSTYADALWWGTITLT	KCNQ5	Mus musculus
SFLVYLCEKNTNDKYQTFADALWWGVITL	kqt-1	C. elegans

novel A→G change in exon 4 of the KCNQ2 gene was confirmed by CfoI restriction enzyme digestion. Digestion results revealed that the two patients were heterozygous for this change but the normal individuals in the family did not carry it (Fig. 5).

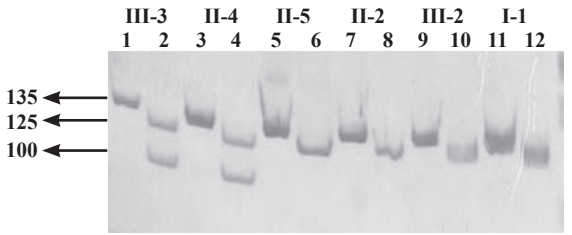

Fig. 5. CfoI restriction enzyme analysis for AàG mutation in exon 4 of the BFNC family. Lanes 2, 4, 6, 8, 10, 12 are digested products of family members III-3 (patient), II-4 (patient), II-5, II-2, III-2, I1 and lanes 1, 3, 5, 7, 9, 11 are uncut PCR products of the respective samples.

In order to confirm the association of the mutation with the clinical phenotype, 137 apparently healthy individuals were analyzed using CfoI restriction enzyme digestion. None of the individuals carried the A→G transversion.

Discussion

The structural analysis of the KCNQ2 gene in members of the Turkish family afflicted with BFNC revealed an A→G transversion at nucleotide position 773 in exon 4 of the KCNQ2 gene resulting in N258S substitution in patients II-4 and III-3. The DNA sample of patient I-2 was not sufficient to complete the analysis; however, the mutation was absent in the unaffected members of the family as revealed by CfoI digestion analysis. It is probable that II-4 inherited this mutant allele from I-2 or it is a new mutation originated in II-4.

The following findings suggested that A→G transversion in exon 4 of the KCNQ2 gene is the pathogenic mutation in the BFNC family. First, the mutation is absent in healthy members of the family and a population-wide analysis of this mutation in 60 German and 77 Turkish individuals showed that they all carried the wild type sequence, excluding its possibility of being a polymorphism. This mutation is also not listed in the SNP database (http://www.ncbi.nlm.nih.gov/SNP/). Secondly, asparagine was found to be highly conserved among homologous proteins as shown in Table I. Conserved asparagine (N) is indicated in bold. Thirdly, the location of the substitution site seems to be in a functionally important region in the protein. Amino acid 258 is located between the S5 transmembrane domain and P-loop, near to the S5 domain, as shown in Figure 6. The amino acids between S5 and S6 form the pore through which K^+ ions pass and asparagine at 258 may locate to the end of the pore or through the extracellular part of the pore. This region may have a role in the opening of the channel upon depolarization. The amino acids lining the pore have conformational change during the depolarization, and asparagine to serine exchange may affect pore conductance, gating properties and probable opening of the channel.

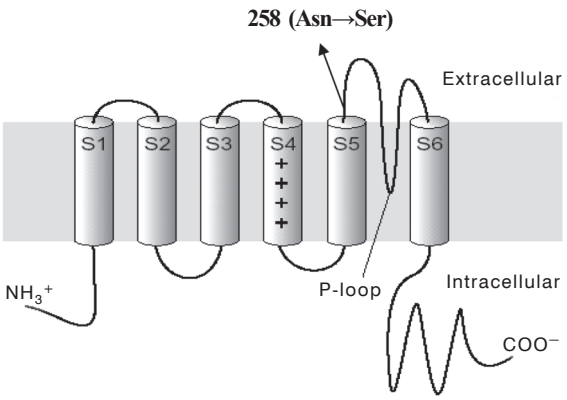

Fig. 6. The location of the N258S missense mutation in the channel[15].

Idiopathic epilepsies, which account for up to 40% of all epilepsies, are mainly caused by genetic factors. However, most idiopathic epilepsies have an oligogenic or multifactorial inheritance rather than a monogenic inheritance. In recent years, studies with large families with rare monogenetic forms of the disease revealed the mutations in different ion channels. Ion channels provide the basis for the regulation of excitability in the central nervous system, so the mutations in ion channels result in a variety of diseases associated with hyper- or hypoexcitability of the affected tissue, as in the case of epilepsy, and hence such diseases are named channelopathies. The studies with rare monogenetic epilepsies or channelopathies are expected to help in clarifying the pathogenesis of more common idiopathic epilepsies that have complex inheritance.

Acknowledgement

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