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ÖZGÜN MAKALE / ORIGINAL ARTICLE

A Novel Mutation of SCL26A4 Gene in Turkish Family with Pendred Syndrome

Pendred Sendromlu Türk Ailede SCL26A4 Geninde Yeni Mutasyon

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ABSTRACT

Aim: This study aimed to investigate the molecular testing of congenital hearing loss by using next generation sequencing technology. Pendred syndrome (PS) is described by severe bilateral sensorineural hearing loss with goiter. The mutations of SCL26A4 gene can cause PS.

Material and Method: We evaluated the feasibility of target-enrichment and massive parallel sequencing technologies to interrogate all mutations of genes (GJB2, GJB3, GJB6, SLC26A4 and for the mitochondrial mutation A1555G) implicated in NSHL, we performed molecular analyses of 14 NSHL families and patients by using Miseq system (Illumina Inc.). Next-Generation sequencing (NGS) technologies provide specificity, sensitivity and reproducibility at levels sufficient to perform genetic diagnosis of hearing loss.

Results: We found two different mutations in SCL26A4 gene such as F354S and I588T in both consanguineous families as diagnosed with Pendred syndrome and we reported a novel mutation in SCL26A4 gene. We found no mutation in GJB2, GJB3, GJB6 gene and A1555G mtDNA in this study.

Conclusion: These results highlight the benefits using targeted gene panels with NGS technologies in the molecular analysis of nonsyndromic, congenital hearing loss patients. This study assessed the frequency of deafness genes in Turkish children with congenital hearing loss who had been treated with cochlear implantation, and we found a novel mutation (I588T) in SLC26A4 gene.

Key Words: Pendred syndrome, Congenital Hearing Loss, Next-Generation sequencing

ÖZET

Amaç: Bu çalışmada konjenital işitme kaybının moleküler testini yeni nesil dizileme teknolojisi kullanılarak araştırmayı amaçladık. Pendred sendrome (PS) yaygın çift taraflı işitme kaybıyla ve guatrla tanımlanmıştır. SCL26A4 gen mutasyonu Pendred sendromuna sebep olur.

Gereç ve Yöntem: Tüm ilgilenilen genlere ait mutasyonların (GJB2, GJB3, GJB6, SLC26A4 genlerinin mutasyonlar ve mitokondrial A155G mutasyonu) incelenmesi masif paralel dizileme teknolojisi ile değerlendirilecektir. 14 NSHL ailenin moleküler genetik analizleri Miseq sistemi kullanılarak gerçekleştirildi. Yeni nesil dizileme teknolojileri, işitme kayıplarının genetik tanısını yapmamızı yeterli düzeyleri spesifite, sensitivite ve üretkenlik sağlar.

Bulgular: Her iki akrabalık olan Pendred sendromu tanılı ailede SCL26A4 geninde F354S ve I588T mutasyonları bulduk ve SCL26A4 geninde yeni mutasyon bildiriyoruz. GJB2, GJB3, GJB6 ve mitokondrial mutasyon saptanmamıştır.

Sonuç: Bu sonuçlar, nonsendromik işitme kaybında moleküler analizlerinde NGS teknolojileri ile hedeflenmiş gen panellerinin avantajlarını vurgulamaktadır. Bu çalışma, kongenital işitme kayıplı kohlear implantlı Türk çocuklarında sağırlık genlerindeki mutasyon sıklığın değerlendirildi ve SCL26A4 geninde I588T mutasyonu saptandı.

Anahtar Kelimeler: Pendred Sendromu, Kongenital İşitme Kaybı, Yeni Nesil Sekanslama

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INTRODUCTION

GJB2, GJB3, GJB6 and mt DNA mutations in congenital hearing loss sensorineural hearing loss is the most common congenital deafness disease, occur in approximately 1 in 1000 births and 50% of these patients are hereditary (1). Genetic deafness is divided into syndromic and nonsyndromic forms. Over 20 genes for non-syndromic autosomal recessive deafness have been well-characterized. In nonsyndromic genetic deafness of prelingual or congenital onset, autosomal recessive inheritance predominates; autosomal dominant and mitochondrial forms have also been described. Mutations in the Connexin 26 (GJB2/Cx26) gene are responsible for more than half of all cases of prelingual non-syndromic autosomal recessive deafness in different populations. Autosomal recessive genes are responsible for about 80% of the cases of hereditary non-syndromic deafness of pre-lingual onset with 23 different genes identified to date. The wide range of functions of these DFNB genes reflects the heterogeneity of the genes involved in hearing and hearing loss. Mutations in the **GJB2** gene encoding connexin 26 are responsible for as much as 50% of pre-lingual or congenital deafness (2). Mutations in the connexin 26 gene (GJB2) account for probably 20% of the nonsyndromic congenital deafness (2-4). In some populations this figure may be higher (especially, 80% in Jewish Ashkenazi children). The most common mutations are the 35delG and the 162delT mutations. Mutations in the connexin 26 gene are presumed to change cochlear potassium recirculation via an effect on the gap junctions in cochlear cells, leading to the accumulation of potassium ions in the cochlear endolymph, and resulting hair cell dysfunction and deafness. The c.35delG carrier frequency, however, differs significantly between European populations, highest frequency being in the Southeastern Europe (4). The other frequent mutations in specific populations are c.167delT in Ashkenazi Jews (5), c.235delC in Japanese (6). Biallelic mutations in 42 different genes have been reported for autosomal recessive nonsyndromic hearing loss (ARNSHL), which explains more than 50% of families with this type of deafness in different populations. Mutations in GJB2, encoding 26, are most commonly identified cause of sensorineural deafness in many populations. The 35delG mutation in the connexin 26 gene (GJB2), at the DFNB1 locus, is the most common mutation in patients with autosomal recessive sensorineural deafness. The GJB2 gene is located on chromosome 13q12 at the DFNB1 locus that encodes the connexin 26 protein (7)**.** Mutations in the connexin 30 (GJB6) gene have been reported to cause autosomal recessive and autosomal dominant, nonsyndromic hearing loss. The connexin 30 protein functions as a component of gap junctions channel of cochlea cells. Biallelic GJB3 mutations have been reported once in two ARNSHL families in which patients were compound heterozygote mutations (7). Mitochondrial hearing loss is believed to be responsible for less than 1 per cent of non-syndromic HL. Nevertheless, the A1555G mutation in the 12S ribosomal RNA gene of the mitochondrial genome related to aminoglycoside ototoxicity has been reported to be important in the etiology of NSHL in some populations (9). Denoyelle et al. found that the mutation in Cx26 gene underlies the dominant form of deafness DFNA3. GJB2 mutations can be

responsible for both a dominant and a recessive form of deafness (10).

Pendred Syndrome and SCL26A4 mutations

Everett et al. used a positional cloning strategy to identify the PDS/SLC26A4 gene at 7q31, and demonstrated that PDS mutations underlie most, if not all, cases of Pendred Syndrome. The SLC26A4 gene mutations could therefore cause sensorineural hearing loss through impairment of endolymph ionic and osmotic homeostasis, pH regulation, thyroid hormone biosynthesis, or any combination of these mechanisms. The SLC26A4 gene encodes pendrin, which is a transmembrane anion exchanger that belongs to the solute carrier 26 family and exchanges chloride, iodide, bicarbonate and formate. It is expressed in different tissues, including thyroid, kidney, and inner ear. SLC26A4 mutations may account for as much as 10% of hereditary deafness in diverse populations. Each ethnic population has a different mutation spectrum, with one or few prevalent founder mutations (7,18). A large consanguineous family from India with congenital severe NSHL described SLC26A4 gene where Pendred syndrome gene (SLC26A4) was located in the chromosome 7q31 **(**15**).** PS is characterized by hearing loss, goiter and hypothyroidism, with/without EVA or other inner ear malformations. Three known genes account for nearly half of PDS/DFNB4 cases were SLC26A4, FOXI1 (<1%), and KCNJ10 (<1%), suggesting further genetic heterogeneity. SLC26A4 mutations can cause both Pendred syndrome and recessive NSHL (DFNB4), two autosomal recessive disorders that share hearing loss associated with EVA as the common feature (17, 20).

Targeted Sequencing With Next Generation Sequencing in NSHL

Sequencing of all many genes by Sanger DNA sequencing is labor intensive and not cost effective. Targeted DNA sequencing with Next-Generation sequencing provide sensitivity, specificity and reproducibility at levels sufficient to perform genetic diagnosis of congenital hearing loss. We have developed a deafness gene panel to improve the molecular diagnosis of autosomal recessive nonsyndromic hearing loss (ARNSHL) by simultaneous sequencing of the exons of 4 deafness genes (GJB2, GJB3, GJB6, SCL26A4) and A1555G mutation of mtDNA. Most of nonsyndromic hearing loss (ARNSHL) genes consist of long and/or many exons making classic genetic methods to screen for mutations very expensive and time-consuming. Next generation technologies provides a transformational approach for identifying causative mutations in Mendelian disorders such as hearing loss. Different targeted genomic capture methods and next-generation sequencing have been successfully applied to screen mutations in hereditary hearing loss in relatively small sets of families (3, 4, 6, 8).

Herein, we report the evaluation of a PCR based enrichment strategy followed by a Miseq system (Illumina Inc.) in 14 Turkish families using NGS implicated in ARNSHL.

MATERIAL AND METHOD

The study informs consents were approved by the Ethics Committee of the Mustafa Kemal University School of Medicine. A signed informed consent was obtained from each participant or parent.

Clinical and Audiological Data

We collected peripheric blood samples of twenty-four patients after examined patients in Mustafa Kemal University, School of Medicine, Department of Medical Genetics outpatient clinic. We examined the patients with facial features and other features for excluding other syndromic hearing loss, and we evaluated the pedigree of these families.We evaluated all the patients who are suffering with hearing loss. Most of the families have consanguinity.

Targeted DNA Sequencing With Next-Generation Sequencing

Primer design was carried out for the coding regions of the genes of interest (GJB2, GJB3, GJB6 and SLC26A4) and for the mitochondrial mutation A1555G. There were 34 primer for the amplification of 17 amplicons. The sizes of the amplicons varies between 484 bp and 2316 bp. DNA samples were obtained with the isolation from 200 µl blood samples from each individual, by using QIAamp DNA Blood Mini Kit (Qiagen Inc.). PCRs were carried out on isolated DNA samples, by using the designed primers and the reactions were checked by using 2% agarose gel electrophoresis. PCRs belong to each individual were mixed to obtain PCR pools, which have all the amplicons of each individual in one tube. While mixing, the amplification efficiency and length of the amplicons were taken into consideration; the volume for each PCR is directly proportional to the length of the amplicon and inversely proportional to the efficiency of the reaction, which were estimated with the help of gel electrophoresis. The PCR pools for each individual were purified by using NucleoFast® 96 PCR kit (Macherey-Nagel GmbH). The purified pools were quantified and standardized to 0,2 ng/ul, which was needed for sample preparation step. The samples were got ready for Next-Generation sequencing by using NexteraXT sample preparation kit (Illumina Inc.). Next-Generation sequencing of the samples were carried out by using Miseq system (Illumina Inc.). The data were analyzed on IGV 2.3 software (Broad Institute).

In silico Analysis

We detected two mutations in SCL26A4 gene in both consanguineous families; such as F354S and I588T. We used online data filtering by MutationTaster, it shows us those mutation as probably disease causing mutation. We use for data filtering online databases such as PolyPhen2/SIFT and MutationTaster.

RESULTS

Following a detailed history and examination, we underwent genetic testing, including searching for GJB2, GJB3, GJB6 and mitochondrial-DNA A1555G mutation. Genetic investigation has been completed by searching for SLC26A4 mutations and we detected a novel mutation in Family 1 and other heterozygous mutation was in Family 2 (Table 1). Thyroid hormones levels dosage (FT4:1.08 ng/dl, TSH: 1.35 uIU/ml) were in the norm in Family 1. The survey was completed by ultrasonography of the thyroid, kidney and urinary tract. Exams were all within normal ranges.

In physical examination, thyroid gland was palpable. Otoscopy and tympanometry of those patients were normal. Family 1 of the patients molecular analysis results were F354S heterozygote mutation in SCL26A4 gene. Family 2 of the patient was a novel (I588T) heterozygote mutation in SCL26A4. We analyses the molecular testing of other two genes of Pendred Syndrome (KCNJ10, FOXI1), but there is no mutation on these genes.

Table 1. Summary of all deafness gene mutation panel.

	GJB2	GJB3	GJB6	SCL26A4	Mt DNA A1555G
Family 1	-	-	-	One mutation (F354S)	-
Family 2	-	-	-	Novel mutation (I588T)	-
Other 12 Families	two mutation	-	-	-	-

DISCUSSION

All of the studies of non-syndromic sensorineural hearing loss patients from Japan, India, South Korea, no homozygous 35delG mutation were reported (6, 8, 14). Battelino et al. (2012) reported six patient with 35delG mutation and GJB6 mutation was observed in a patient with mild progressive hearing loss (9). Tarkan et al. (2012) showed that 12 (12.7 per cent) patient have been detected in the 35delG mutation of 94 patients with hearing loss in Turkish patients. According to the 35delG mutation detection ratio, the present study results are nearly similar with Tarkan et al. study results (11). This study evaluated Turkish children with congenital, non-syndromic hearing loss with cochlear implantation. We found a high incidence of 35delG mutation in NSHL patients and detected no mutation in GJB2, GJB3 and GJB6 genes and in A1555G mtDNA. Tekin et al. (2003) found that the c.35delG mutation revealed 37 homozygotes and 21 heterozygotes making the allele frequency of this mutation 18.5%. One of the most significant results of their study is to show that although the c.35delG mutation is quite high in certain regions, its distribution is heterogeneous throughout Turkey (13). The frequency of c.35delG mutations are similar in the present study and Tekin et al. study. Coyle et al. (1998) reported four mutations (p.L236P, p.T416P, p.E384G and IVS8 + 1G > A) in SCL26A4 gene in northern Europe (16). Busi et al. (2012) reported two novel mutation (IVS2+1delG and K590X) in SCL26A4 gene and there is no other mutation in GJB2, GJB3, GJB6 and mtDNA (17). The results of study showed that there are two heterozygous mutations (F344S, I588T) in SCL26A4 gene with Pendred syndrome phenotype. Yang et al (2009) described the mutations of KCNJ10 together with mutations of SLC26A4 cause digenic non-syndromic hearing loss associated with EVA syndrome (18, 19). Yazdanpanahi et al. reported two novel mutations (c.863-864insT and c.881-882delAC) in exon 7 of SLC26A4 gene (20).

In conclusion, we detected novel mutations (I588T) in SLC26A4 gene with PS phenotype. There is a great genetic heterogeneity between mutations of all deafness genes in congenital hearing loss patients. So deafness gene panels are facilitate to detect a novel mutations and they are suitable, cost-effective in hearing loss patients.

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