

Heterozygous Deletion of Exon 8 in WFS1 Gene in Two Wolfram Syndrome Probands with Hearing Loss: Case Report

İşitme Kaybı Olan Wolfram Sendromlu İki Olguda WFS1 Geni Ekson 8'de Heterozigot Delesyon

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ABSTRACT Point mutations in the Wolfram syndrome 1 gene (WFS1) are attributed the autosomal dominant and/or recessive mild type sensorineural hearing loss in first degree relatives. Total genomic DNA was isolated from peripheral blood of affected probands and controls. Multiplex polymerase chain reaction was performed and followed by multiplex ligated probe amplification analysis. Sensorineural hearing loss was moderate in a 48-year-old male patient (case 1) and sensorineural hearing loss and optic atrophy were evident in his 16 year old daughter (case 2). We identified heterozygous deletion in exon 8 of WFS1 gene (Wolframin protein) in father and in one of his affected daughters with hearing loss and optic atrophy. The genetic results demonstrate the necessity of screening for the possible point mutation and/or larger deletions in WFS1 gene in cases with non-syndromic mild type sensorineural hearing loss. This study emphasizes the need for careful molecular evaluation in cases with impaired hearing, insulin-dependent diabetes mellitus and optic atrophy for the diagnosis of Wolfram syndrome. Proper genetic counseling must be given accordingly to patients and their other family members since it is important for their next generation.

Key Words: Wolfram Syndrome; WFS1 protein; heterozygote detection; hearing loss; wolframin protein

ÖZET Wolfram Sendrom 1 geninde (WFS1) meydana gelen yapısal mutasyonlar otozomal dominant ve/veya resesif kalıtılan hafif tip sensörinöral işitme kaybına neden olmaktadır. Bu çalışmada sensörinöral işitme kaybı olan aynı aileden iki olguda konneksin 21, 26, 30 ve wolframin gen mutasyonu analizi yapılmıştır. Total genomik DNA izolasyonu için hasta ve aile bireylerine ait periferik kan-EDTA örnekleri kullanıldı. Hedef genlerin mutasyon analizi multipleks polimeraz zincir reaksiyonu destekli çoklu bölge bağlantılı prob amplifikasyon (Multiple Ligated Probe Amplification; MLPA) yöntemi ile yapıldı. Orta derecede sensörinöral işitme kaybı olan 48 yaşında erkek hasta (olgu 1) ve onun sensörinöral işitme kaybı ve optik atrofi bulunan 16 yaşında kızı (olgu 2) çalışmaya dahil edildi. Yapılan mutasyon analizi sonucunda her iki olguda da wolframin proteinini kodlayan WFS1 geni ekson 8 bölgesinde heterozigot delesyon saptanırken, konneksin 21, 26 ve 30 gen bölgelerinde yapısal bir mutasyona rastlanmadı. Bu çalışmadaki iki olgudan elde edilen sonuçlar bize non-sendromik hafif tip sensörinöral işitme kaybı olan olgularda WFS1 gen bölgesinin olası mutasyonlar açısından taranmasının unutulmaması gerektiğini göstermiştir. Wolfram sendromlu olgularda klinik tanı yanı sıra yapılan ileri moleküler incelemeler, hem uygun genetik danışmanlık hem de bir sonraki kuşakta ortaya çıkabilecek mutasyonun gösterilmesi açısından ne kadar önemlidir.

Anahtar Kelimeler: Wolfram Sendromu; WFS1 protein; heterozigot saptamak; işitme kaybı, wolframin proteini

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Hearing loss is the most common sensorineural disorder that present in one of every 1000 newborns. Hearing impairment can be classified as mild, moderate, or severe, including total deafness. Dysfunction or degeneration of elements such as cochlea, neurons, stria vascularis and neural network alone, or in combination, can result in cochlear impairment and sensorineural hearing loss in humans. Sensorineural hearing loss not only involves a reduction in sound level or ability to hear faint sounds, but also affects speech understanding, or ability to hear clearly. Birth injury, viruses, head trauma, autotoxic medications, genetic syndromes, aging and tumors may cause this type of hearing loss.¹ Approximately 0.1% of children are born with profound hearing loss in many populations. The disorder DFNB1 (Deafness, Neurosensory, autosomal recessive 1) is caused by defects of the connexin 31 protein that is coded by *GJB2-6* gene which is located on chromosome 1p^{35.1} region. More than 100 mutations in *GJB2* gene have been described in genetic deafness. The defects of *WFS1* gene which is located on chromosome 4p^{16.1} and encodes the wolframin DFNA protein cause the syndromic hearing impairment. The prevalence of Wolfram syndrome (WFS) is one in 770 000 live births, with carrier frequency of one in 354.² WFS is an uncommon progressive neurodegenerative disorder characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness with an autosomal recessive pattern of inheritance.³⁻⁵ Although some studies have provided evidence for genetic heterogeneity, the genotype-phenotype relations are not clear. Patients are prone to develop diabetes insipidus, deafness, and urinary tract and neurological abnormalities. Approximately 60% of patients with WFS die at age 35, usually due to central respiratory center failure following brain stem atrophy.^{6,7} Eiberg et al.⁸ reported a case of autosomal dominant optic atrophy with hearing impairment and impaired glucose regulation which is associated with a missense mutation in the *WFS1* gene.

Current study aimed to find out the possible role of the mutations in the *GJB2-6* and *WFS1* genes with MLPA technique in two first degree rela-

tive probands with non-syndromic sensorineural hearing impairment and mild type optic atrophy. Another aim of the current study was to investigate the family members of patients with mild sensorineural hearing loss for possible point mutations in *WSF1* gene region for early clinical diagnosis and genetic counseling and to put forward the importance of genetic counseling.

AUDIOLOGIC EVALUATION PROTOCOL

We evaluated two Turkish patients with WS from the same family: Father (case 1) who had diabetes mellitus, hearing impairment and optic atrophy and his two daughters, one of them (case 2) had a unilateral sensorineural hearing loss in her left ear. Genetic analysis was performed, endocrinologic parameters were tested and ultrasonography, threshold tonal audiometry and pure tone audiometric analysis (Interacoustic AC40) were performed and some other clinical parameters were investigated in the current probands. The hearing involvement was monitored by successive audiometric examinations: on the day of presentation, and every two days after presentation.

Hearing was classified as normal, conductive hearing loss, mixed hearing loss and sensorineural hearing loss according to Santos & Russo.⁹ The pure tone average of 500, 1000, 2000, and 4000 Hz was calculated. Classification of the degree of hearing loss was also established according to the threshold, as recommended by Shames and Wing:¹⁰ <15dBHL normal; 16-40 dBHL slight; 41-55 dBHL mild; 56-70 dBHL moderate; 71-90dBHL severe; >91 dBHL profound hearing loss.¹⁰

CASE 1

A 43-year-old man presented with an eight year history of progressive hearing loss and tinnitus in his left ear started 10-20 days ago. Ear, nose and throat examination revealed tympanosclerosis on the right eardrum of the patient. His personal history included an operation on the right ear (simple mastoidectomy and tympanoplasty) that was performed in another institution two years ago. The hearing loss was determined with pure tone audiometry. The audiometric results showed the

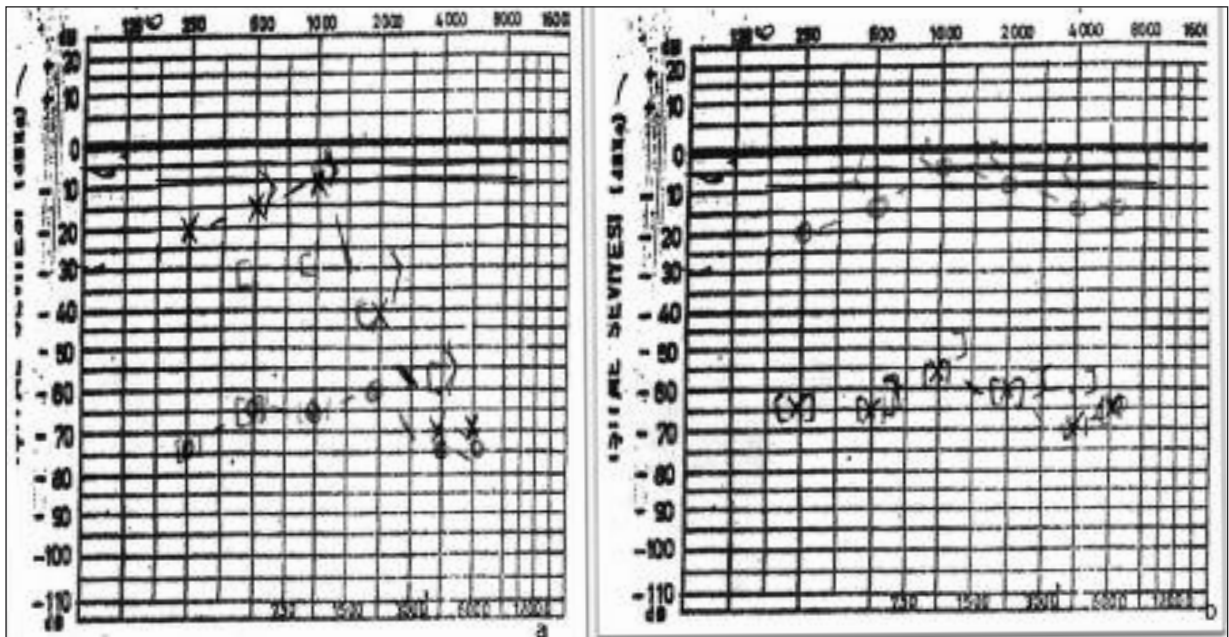


FIGURE 1: The audiometric results of case 1 showed mild mixed type hearing loss in the right ear, acoustic trauma and mild sensorineural hearing loss in the left ear (a). Mild sensorineural hearing loss and hearing impairment were detected in left ear of case 2 (b).

mild mixed type hearing loss in the right ear, acoustic trauma (sensorineural hearing loss after 2000 Hz) and mild sensorineural hearing loss in the left ear (Figure 1a). Hearing impairment in the right ear was progressive and obviously detectable with the audiometry. Patient was at risk for developing insulin dependent diabetes mellitus. His blood glucose levels were measured and they turned to be within the normal limits (fasting blood glucose level is 94 mg/dl, postprandial blood glucose level is 104 mg/dl.) after insulin treatment.

Patient was treated with 2 ml once a day vitamine B (B1, B6, B12, folic acid, nicotinamide and calcium pantothenate) combination; vitamine C, 1 ml once a day; Ginkgo glycoside 100 mg, four times a day and dexamethasone 40 mg once a day, for three days.

CASE 2

The second case was a 16-year-old girl, the daughter of the case 1 (Figure 1b) who showed sensorineural hearing loss and optic atrophy. She had complained of hearing loss for one year. Sensorineural mild hearing loss and hearing impairment were detected in her left ear (Figure 1b). Her right ear was with pure tone audiometry. She was analy-

zed for the similar clinical findings and investigated for the possible mutations in *GJB2-6* and *WFS1* gene regions by MLPA technique, like case 1. Heterozygous deleted mutation was detected in exon 8 of *WFS1* gene in both cases (Figure 2, arrows). No mutation was detected in other gene spam that examined (connexin 21, 26 and 30; Figure 2). Ear, nose and throat examination was normal. Blood

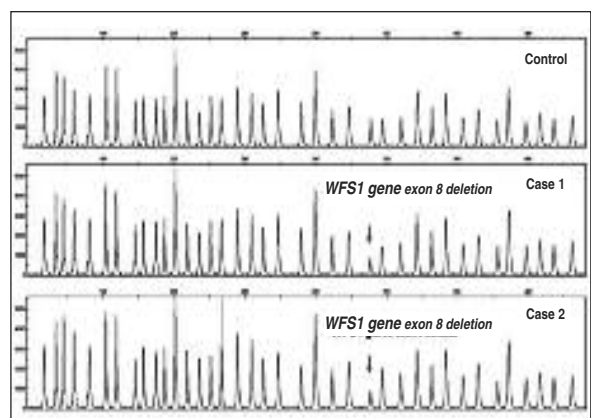


FIGURE 2: Detection of *WFS1* gene exon 8 deletion in normal control and affected cases with MLPA method. Capillary electropherograms are from a normal control (top) and father with sensorineural hearing impairment (case 1) and one of his affected daughter (case 2). The apparent heterozygous deletion with ~50% peak reduction in *WFS1* exon 8 gene region was indicated in both affected cases with sensorineural hearing impairment (red arrows).

glucose levels of this patient were within the normal limits.

Both cases were followed clinically for two years and audiometric measurements indicated progressive hearing loss in both patients. Ophthalmologic examination included the best corrected visual acuity determination, funduscopy, fluorescein retinal angiography, and Goldmann kinetic perimetry.

Patients' pedigree analysis showed dominantly inherited sensorineural hearing loss. Father (case 1, II-4) and his both daughters (case 2- arrow, III-1 and 2) were investigated for mutation in *GJB2-6* and *WFS1* gene regions by MLPA technique. Other family members that were indicated with a question mark in the pedigree (I-1, II-3 and 6) were known as the individuals with sensorineural hearing loss but were not investigated with genetic analysis. The pedigree shows the dominant type of sensorineural hearing loss trait in the current family profile through three generations (Figure 3).

DNA ISOLATION AND MUTATION ANALYSIS

Total genomic DNA was extracted from the 200 µl peripheral blood samples with the Invitex blood (250) DNA isolation procedure (Invitex, Germany). The mutation analysis of *GJB3* exons 1-3 and *WFS1* exons 1 and 8 genes were performed with multiplex polymerase chain reaction (PCR) amplification

technique. The multiplex ligated probe amplification (MLPA) products of target genes were genotyped by capillary electrophoresis of MLPA technique according to the manufacturer's instructions.

All 11 exons of the *GJB3* and *WFS1* genes were amplified. Briefly, 150 ng DNA was denatured and hybridized overnight at 60 °C with SALSA probe mix. After treating the samples with ligase 65 for 15 min at 54 °C, PCR was performed with the specific SALSA FAM PCR primers. Capillary electrophoresis of PCR products was performed using an ABI PRISM 310 and data analysis was performed by exporting the peak areas to a Gene Mapper (AB) and Coffalyzer V8 (MRC, Netherlands). Each result from cases and control were confirmed by two independent tests. A heterozygous deletion was detected in father and his affected daughter with non-syndromic hearing loss (Figure 2, Case 1 and 2). Mutation screening revealed the heterozygous distinct variants in *WFS1* gene of exon 8. Phenomenon was showed autosomal inheritance of Wolfram Syndrome in both cases. No mutation was detected in other non-affected daughter.

The present study was approved by local ethics committee and a written informed consent was obtained from all cases.

DISCUSSION

Hearing loss is one of the most common inherited disorders and up to 50% of the cases with autosomal recessive non-syndromic sensorineural hearing loss are associated with a mutation in the locus DFNB. DFNB contains two connexin genes namely *GJB2* and *GJB6*. Wolfram syndrome is an autosomal recessive disorder with severe neurodegeneration. Affected individuals present with juvenile-onset insulin-dependent diabetes mellitus, optic atrophy, diabetes insipidus, sensorineural deafness, dementia, psychiatric illnesses, renal-tract abnormalities and bladder atony. In addition, dominant mutations in the *WFS1* gene have been shown to play a role in low-frequency sensorineural hearing loss, progressive hearing loss, and deafness with optic atrophy. Cx mutations are now widely accepted as one of the most common human mutations causing severe sensorineural deafness.^{11,12}

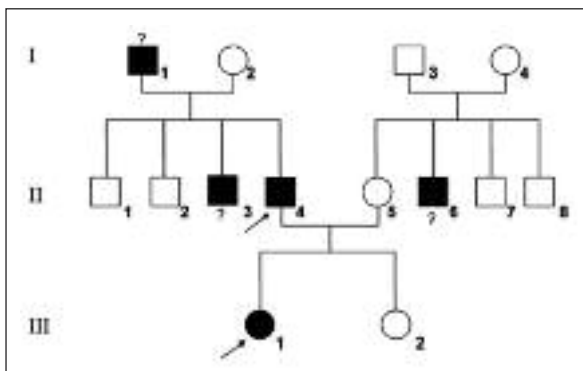


FIGURE 3: Pedigree analysis shows the dominant inheritance type of sensorineural hearing loss in a current large family. The family members that were indicated with question mark (I-1, II-3 and 6) were reported as the patients with sensorineural hearing loss but not investigated with genetic analysis (Arrows indicate the proband cases).

Gasparin et al.¹³ reported novel mutations in exon 8 of *WFS1* gene in 27 Brazilian patients with WFS. Homozygous and/or compound heterozygous *WFS1* mutations were reported in WFS in Lebanon population.¹⁴ Evirgen et al.¹⁵ showed that 35delG mutation was the most frequent one which led to congenital hearing loss in Turkey. A wide range of gene mutations such as *PAX*, *Connexin 26* (*GJB2*), *Connexin 30* (*GJB6*), *Connexin 31* (*GJB3*), *Wolframin protein* (*DFNA*) and *SLC26A4* were reported in patients with hearing loss.¹⁶⁻¹⁸ Dai et al.¹⁷ showed *SLC26A4* gene variation in patients with sensorineural hearing loss in Chinese population. Mutations in mitochondrial DNA (mtDNA) are the major cause of hearing loss. Bae et al.¹⁶ performed a systematic mutational screening of 12S rRNA, tRNA Ser (UCN), tRNA Lys and tRNA Leu (UUR) genes in 227 unrelated patients with nonsyndromic hearing impairment in Korean population. On the other hand Lee et al.¹⁹ reported that *GJB2-6* and *SLC26A4* mutations together were the major cause of congenital hearing loss in the Korean population. In current report, no mutation was detected in the *GJB2-6* genes in both cases. Structural gene deletion in *Connexin 30* (*GJB6*) is frequently seen in Spain, France, the United Kingdom, Israel and Brazil. No point mutations were detected in *GJB* genes (*Connexin 26*, *30* and *31*) in the current three family members with a non-syndromic hearing loss. Same results of wild type *Connexin 30* gene profiles were also reported by Del Castillo et al.²⁰ in cases with non-syndromic hearing loss.

Progressive hearing impairment is a form of non-syndromic, non-congenital sensorineural hearing loss, which has an autosomal dominant trait with variable age of onset and progression rates. Phenotype/genotype correlation is difficult and the same mutation may result in different individual phenotypes. All types of dominantly inherited progressive hearing losses eventually progress to severe or profound hearing loss, as in our current case 1 (father). Asymmetric and mild to severe progressive sensorineural hearing loss was detected in case 1 and an early-onset symmetric hearing loss was detected in one of his affected daughters (case 2).

Case 1 and case 2 were diagnosed as WS due to their clinical findings of hearing loss, insulin dependent diabetes mellitus (only case 1), optic atrophy and *WFS1* gene mutation. Case 2 showed a less severe Wolfram phenotype of optic atrophy and unilateral hearing loss. Genetic studies in patients with WS reported a wide spectrum of mutations in coding sequence of *WFS1* gene. In our both cases, deleted mutation was detected in only one allele (heterozygous) in exon 8 of *WFS1* gene. The *connexin* genes that examined were in normal structure in both cases.

WFS1 mutations at the *DFNA6/14/38* locus were first described in 2001 in six families with low frequency sensorineural hearing loss (LFSNHL) that was non-progressive below 2000 Hz; the causative mutations all clustered in the C-terminal domain of the wolframin protein.²¹ Autosomal dominant LFSNHL appears highly predictive of a *WFS1* mutation, and causes of LFSNHL include retrocochlear lesions and cochlear otosclerosis.²²

Most possibly, the current cases are the first reported examples of an autosomal dominant progressive hearing loss which is associated with a heterozygous *WFS1* exon 8 gene mutation. In this study, two members of the family with autosomal dominant LFSNHL had mutations in the *WFS1* gene, indicating a non-syndromic low frequency hearing loss. However, the nature of hearing loss in case 1 was progressive. Here we recommend the systematic analysis of *WFS1* gene sequences in patients with parentally inherited diabetes mellitus and non-syndromic deafness. Our data also showed that *WFS1* exon 8 which encodes a protein with an extended C-terminal domain is the major gene involved in WS probands.

CONCLUSION

Genetic mutations are an important causes of hearing loss. Two cases presented in this study and their genetic results indicate that possible point mutation and/or larger deletions in *WFS1* gene is associated with autosomal dominant non-syndromic mild type sensorineural hearing loss. Technologic developments, new examination

techniques, and genetic tests have allowed earlier and more accurate diagnosis, providing further clarification on the etiology of the condition. In conclusion, families which have more than one case with clinical findings of Wolfram Syndrome must be screened for target GJB2-6 and WSF1 genes and appropriate genetic counseling must be

performed. This study emphasizes the need for a careful molecular evaluation in cases with impaired hearing, insulin-dependent diabetes mellitus and optic atrophy for the diagnosis of Wolfram syndrome. Proper genetic counseling given to patients and their family members is important for their next generation.

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