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TJP2 Gene Mutation c.G1012A May Responsible for Congenital Hearing Loss with Incomplete Penetrance in An Iranian Pedigree

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ABSTRACT

Hereditary hearing loss (HHL) comprises half of the congenital deafness which arises from genetic mutations. Mutations in the *TJP2* gene, encoding tight junction protein 2, are one of the gene alterations in HHL resulting in an autosomal dominant nonsyndromic form of the disease. An 11-year-old male patient with clinically approved congenital hearing loss was referred to our laboratory. The molecular genetic analysis detected an atypical heterozygous variant -c.1012G > C, p.G338R in exon 5 of the *TJP2* gene on chromosome 9 position 71836379 (GRch37). Segregation analysis for his parents has uncovered the same mutation in the patient's mother, but without any deafness phenotype. This case report provided evidence to demonstrate for the first time the incomplete penetrance of this *TJP2* mutation and proposed this mutation as a "likely pathogenic" variant in an Iranian pedigree.

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Introduction

According to recent evidence, the incidence rate of congenital hearing loss is approximately 1 in every 1000 live births, comprising the most common birth defect in developed countries (Egilmez and Kalcioglu 2016). Hereditary hearing loss (HHL), which accounts for nearly 50% of congenital forms of the disease, is a result of genetic mutations and can be classified as syndromic and nonsyndromic forms. Syndromic form of the disease, which accounts for approximately 30% of all deafness cases, co-occurs with other symptoms in addition to deafness. However, nonsyndromic deafness comprises 70% of the cases and occurs without any other medical complications (Yang *et al.*, 2019). Nonsyndromic hearing loss can be classified by the mode of inheritance. 1) 75-80% the cases harbor recessive genes; 2) Dominant genes comprise 20-25% of cases; 3) about 1-2% of the patients with deafness inherit the disease by X-linked pattern; and 4) less of 1% of all deafness cases are affected by mitochondrial inheritance (Yang *et al.*, 2019). To date, more than 300 genetic loci have been reported to be linked with nonsyndromic hearing impairment, of which 92 genes are disease-causing and among which 34 genes are known to be associated with the autosomal dominant form of the disease (Wang *et al.*, 2015). Moreover, recent evidence has identified the implication of approximately 70 genes in the autosomal recessive nonsyndromic form of deafness (Shearer *et al.*, 2017). Although several altered genes and their loci have been identified previously for the disease, the detection of mutations within a connexin-encoding gene, known as *GJB2*, that causes 30-50% of

childhood recessive hearing losses, has led to a better understanding of the genetic basis of deafness in the past 15 years (Lenz and Avraham, 2011). It is now obvious that the major causes of severe-to-profound and mild-to-moderate autosomal recessive forms of nonsyndromic hearing loss in most populations are *GJB2* and *STRC* mutations, respectively. However, the pathogenicity of mutated variants of these genes may vary markedly by ethnicity (Zou *et al.*, 2019). For example, more than 30 genes have been reported to cause hereditary non-syndromic hearing loss in the Iranian population. However, *GJB2* mutations are estimated to be the most common causes of diseases (16.7%) in Iran (Ghasemnejad *et al.*, 2017). Moreover, some studies also unraveled that the mutation rates of this gene in the northern populations of Iran are higher than that of southern or eastern parts. They also indicated a gradient in the frequency of *GJB2* mutations from north to south parts of Iran (Koohiyan *et al.*, 2019a; Koohiyan *et al.*, 2019b). *TJP2*, tight junction protein 2 encoding gene, is a gene which is expressed in the inner ear, where it is implicated in apoptosis of hair cells causing age-related deafness (Walsh *et al.*, 2010). A number of alterations, including missense mutation and overexpression of the *TJP2* gene have been established to cause autosomal dominant nonsyndromic hereditary hearing impairments (ADNSHHI) (Wang *et al.*, 2015). The aim of this present case study was to report an atypical heterozygous variant of the *TJP2* gene with its phenotypic appearance in an Iranian pedigree.

Case report

An 11-year-old male patient with clinically proven congenital hearing loss was referred to our laboratory. His parents were second cousins with no detected family history of deafness. His mother had a history of two previous miscarriages, but the patient was born after a normal and full-term pregnancy with normal vaginal delivery without any use of ototoxic medications by mother during pregnancy. The informed consent form was signed by his parents and other family members to participate in this study. The whole blood from the patient was taken to extract DNA and whole exome enrichment using the Twist human core exome kit (CeGaT GmbH, Germany). After that, the cDNA library was sequenced using the Illumina Genome Analyzer II platform with mean on-target coverage 117X. Upon receiving the Whole Exome Sequencing (WES) data, 110 genes involved in the hearing loss (71 genes involved in AR and X-linked form and 39 genes involved in AD and X-linked form) were filtered based on the patient's phenotype, non-syndromic hearing loss, and hearing loss panel of the Cegat Company. Variants related to the patient's phenotype were selected based on the American College of Medical Genetics and Genomics (ACMG) Criteria. Finally, the variant c.G1012C of the *TJP2* gene (NM-001170416.1), which had a likely pathogenic effect was selected as the candidate gene for deafness in the patient. Two primer TJP2-F (5'-GAGCATTGACCAGGACTA-3') and TJP2-R (5'-GGATGACAGAGCAACACT-3') were used for amplification of the *TJP2* gene fragments.

For segregation analysis and mutation confirmation, we extracted the DNA from Whole blood samples of patient and his family using Roche kit and PCR was performed on this DNA specimens. Thereafter, electrophoresis of DNA samples and confirming a 324 -base-pair-long BP, Cycle sequencing and manual cleanup was performed, then and the product was sequenced by using the Sequencer ABI 3500. Data analysis was performed using FINCH TV software. This molecular genetic analysis showed an atypical heterozygous variant -c.1012G > C, p.G338R in exon 5 of the *TJP2* gene on chromosome 9 position 71836379 (GRch37), which has previously been noted “uncertain significance” variant in the Clinvar database.

To confirm whether this mutation in *TJP2* is responsible for the deafness phenotype, which was observed in the patient, the segregation analysis was performed for his parents, his healthy brother, grandparents and his aunt (Fig. 1). The results revealed the same mutation in the patient’s mother with no deafness phenotypic trait. His father, grandparents, healthy brother, and aunt didn’t reveal any change in the mentioned position of the *TJP2* gene (Fig. 2).

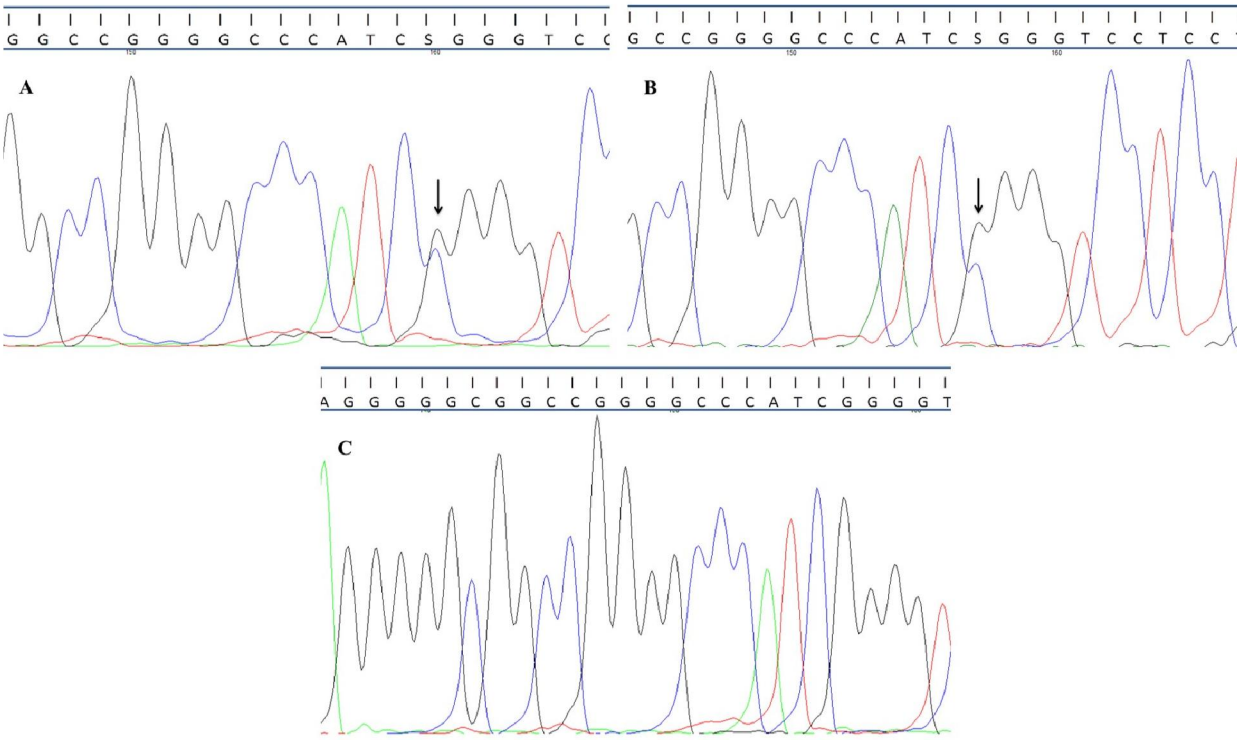

Fig. 1. Electropherogram profiles of *TJP2* gene in this family: Segregation analysis *TJP2* gene for patient (A); His mother (B); Father (C); The arrows in both figures A and B show the same mutation in patient and his mother; but no such a mutation is detected in his father.

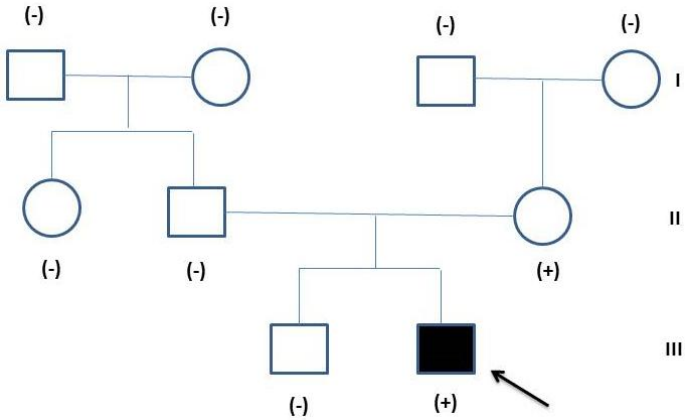

Fig. 2. Pedigreegram in this family: Schematic pedigree for our studied Family with an atypical heterozygous variant [c.1012G > C, p.G338R in exon 5 of *TJP2* gene on chromosome 9 position 71836379 (GRch37)]. Circles indicate females; Squares indicate men; Filled symbols indicate affected individuals; Black arrow=proband; (+) =mutation identified; (-) =no mutation identified.

Discussion

Several lines of previous evidence have demonstrated that alterations in the *TJP2* gene can cause non-syndromic hearing loss (Baux *et al.*, 2017; Walsh *et al.*, 2010; Wang *et al.*, 2015). It has been proven that the expression levels of the *TJP2* gene usually show an age-dependent decrease from the embryonic period to adulthood. However, its overexpression in the individuals with hearing loss has been confirmed to diminish the phosphorylation of glycogen synthase kinase 3β (GSK-3β) causing cochlear hair cell death and hearing loss (Op de Beeck *et al.*, 2011). Missense and gain-of-function mutations in the *TJP2* gene have also been detected in patients with autosomal dominant

forms of hearing loss (Lenz and Avraham, 2011). However, knockout of *TJP2* could hamper embryonic development and leads the experimental mice to die (Xu *et al.*, 2008). In the present study, we presented two cases in this present case report; an 11-year-old boy with a clinically proven congenital deafness, and his mother with the same variant, but with no deafness presentation. This variant has been reported and classified as "uncertain significance" in the Clinvar database, but it can be classified as likely pathogen according to the American College of Medical Genetics and Genomics (ACMG) criteria as follows: 1). The same amino acid change as a previously established pathogenic variant regardless of nucleotide change (p.G311R); 2) located in a mutational hot spot and/or critical and well-established functional domain (*e.g.*, active site of an enzyme) without benign variation; 3) absent from controls in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium; 4). This variant is absent in the Iranome project (800 genomes of the healthy Iranian people project). As we found no alteration in other gene variants related to hearing loss in WES of the patient and observed the same variant with no apparent phenotype in his mother, therefore we propose two possible explanations to describe this situation; 1). The mutation of the *TJP2* gene may be "non-penetrant" in mother or 2). Late onset of the disease. Human autosomal dominant diseases have generally heterozygous genotypes with a rare occurrence in which the individuals with heterozygous genetic display a phenomenon called incomplete or non-penetrance (Otto and Horimoto, 2012). Several autosomal dominant diseases have been reported to exhibit the non-penetrant trait. Otosclerosis, presented by a progressive overgrowth of the bony ossicles, is an example of late-onset dominant hearing loss with decreased penetrance. This reduced penetrance causes the appearance of the symptoms of otosclerosis in only 60% of subjects with the gene alterations (Marschark and Spencer, 2003). Different forms of autosomal dominant deafness have been reported to have reduced penetrance. For example, *MYH9* gene involved in DFNA17 form, *MYO1A* gene implicated in the DFNA48 form, and specific mutations in *GJB2* like M34T and V371I involved in DFNA3 form of deafness (Pollak *et al.*, 2007; Vona *et al.*, 2014; Wasano *et al.*, 2016). Estimation of precise penetrance for assessing the risks of recurrence of the genetic-based disease has been identified to have great importance in families with incompletely penetrant diseases or for affirming genetic map locations by linkage analysis (Horimoto *et al.*, 2010). Penetrance generally is defined as the percentage of individuals having a specific genotype who exhibit the expected disease trait under particular environmental conditions (Cohn *et al.*, 2007). Penetrance rate is therefore defined as the possibility of a heterozygote individual manifesting a full set of disorder-related symptoms or some of them (Otto and Horimoto, 2012). The mechanism by which a non-penetrant phenotype occurs has not been understood. However, most previous evidence has suggested that the genetic factors alone are not able to describe the penetrance phenomenon, but the involvement of both genetic and environmental modalities is needed to estimate the susceptibility to disease (Kremer, 2019). The other possible explanation for our observed genetic alteration regarding the phenotype was a phenomenon called expressivity, which is the range of phenotypes manifested by a particular genotype under certain environmental factors (King *et al.*, 2007). Accordingly, we proposed that the expressivity may be a possible explanation for the manifestation of the deafness phenotype in the patient and the lack of this trait in his mother. However, expressivity and penetrance may sometimes be considered as same. For example, variable expressivity may be a function of incomplete penetrance (Thiselton *et al.*, 2002; Zlotogora, 2003). In spite of the fact that the majority of patients with autosomal dominant HHL have a deaf parent, various factors such as the failure in diagnosing the disease in family members, late-onset in a parent, and incomplete penetrance of the disease-causing mutation in an asymptomatic parent may lead to a negative family history (Shearer *et al.*, 2017).

Conclusion

We described the case of an 11-year-old boy who presented with clinically diagnosed

congenital hearing loss. However, further analyses revealed that the patient and his mother had a previously unknown mutation in *TJP2* gene, but his mother had no deafness signs. Our study reported for the first time two cases with the same *TJP2* gene variant but with incomplete penetrance in the parent. This case report sheds new light on the interplay between genetic and non-genetic modulators in appearing a phenotype and could aid in the understanding the possible mechanisms of the occurrence of congenital hereditary hearing loss.

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Conflicts of interest

The authors have no conflict of interest to declare.

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