Specific Distribution of GJB2 Mutations in Kurdistan Province of Iran; Report of a Relatively Isolated Population

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Abstract

Hearing Loss (HL) represents high genetic heterogeneity with an incidence of almost 1 out of 500 newborns in most populations. Approximately half of the cases have a genetic basis that most of them are autosomal recessive non-syndromic (ARNSHL) with DFNB1-related defect in many worldwide populations. Given the heterogeneity of the trait together with the unique infrastructure of Iranian population, made it necessary to study other loci than GJB2 in various ethnic groups in order to elucidate the genetic etiology of HL in Iran. The present study was designed to determine the contribution of four DFNB loci in Kurdistan Province, west Iran. In this connection, we performed GJB2 analysis and homozygosity mapping using STR markers covering the DFNB21, DFNB4 and DFNB7/11 loci in 20 pedigrees with ARNSHL. GJB2 mutations were the cause of HL in 20% of the patients. However, surprisingly, we only observed 35delG and IVS1+1G>A mutation among the study population. We also did not find any family linked to DFNB21, DFNB4 and DFNB7/11. Our results indicate the contribution of other loci in etiology of HL in Kurdistan that needs to be carefully investigated.

Keywords: ARNSHL; Iranian Kurdish people; GJB2; DFNBs.

Introduction

Hearing Loss (HL) is the most prevalent sensorineural disorder, affecting one out of every 500 newborns [1]. The majority (~70%) of cases are non-syndromic (non-syndromic hearing loss, NSHL) with the remaining 30% being associated with other clinical findings (syndromic hearing loss, SHL). The autosomal recessive form of NSHL (ARNSHL) accounts for over 80% of NSHL and is expected to be even more prevalent in families from consanguinity belt, including Iran [2, 3].

Identification of over 100 loci and 60 genes (www.hereditaryhearingloss.org), emphasizes the heterogenic nature of ARNSHL. In spite of this heterogeneity, DFNB1 (GJB2) accounts for up to 50%
of ARNSHL in many populations [4-7], with more than 300 variants described so far (www.hgmd.org). GJB2 mutations also remain the major cause of ARNSHL in Iranian population but only approaching an average of ~16-18% of cases [8, 9]. On the other hand, analysis of GJB2 mutations in different Iranian ethnic groups shows a remarkable difference. Therefore, it worth considering the molecular etiology of ARNSHL differs considerably among different Iranian ethnic groups.

SLC26A4 which encodes for the anion exchanger pendrin, is expressed in cells bordering the endolymph and spiral ganglion [10]. Mutations in the SLC26A4 at DFNB4 locus cause both ARNSHL and Pendred syndrome, which is characterized by thyroid enlargement and HL. The HL phenotype is associated with abnormal inner ear development, identifiable by temporal bone imaging [10, 11]. To date, over 200 mutations have been reported in SLC26A4 [MORL homepage. http://www.medicine.uiowa.edu/pendredandbor], making it the second cause of congenital HL after GJB2.

TMC1 gene encodes for transmembrane channel-like 1 protein which is a member of the subfamily A of transmembrane channel-like (TMC) gene family. TMC1 mutations can cause both Autosomal recessive (DFNB7/11) and autosomal dominant (DFNA36) mode of hearing impairment [12, 13].

The TECTA gene encodes α-tectorin, which is one of the main noncollagenous parts of the tectorial membrane in the inner ear. Mutations in TECTA have been detected in both autosomal recessive and autosomal dominant HL (DFNB21 and DFNA8/12) [14, 15].

Iranian population is heterogeneous with high rate of consanguineous marriage; however, few investigations have ever addressed the prevalence of DFNB loci other than DFNB1 in individual ethnic groups. This study investigates the contribution of GJB2 and the prevalence of 3 frequent DFNB loci including DNB4, DFNB7/11 and DFNB21 in 20 Iranian ARNSHL families from Kurdistan province, west of Iran. These results give a preliminary overview of the molecular epidemiological characteristics of hereditary HL in this region and will provide data for better genetic diagnostics and counseling.

Materials and Methods

Subjects

Totally 20 large pedigrees compatible with autosomal recessive mode of inheritance, with at least 2 affected members were recruited. The families were referred to the Center for Genetic Counseling, Center of the Welfare and Rehabilitation Organization, Sanandaj, Kurdistan province. All patients had bilateral prelingual hearing loss. The detailed history could not attribute HL to known environmental factors (such as CMV, trauma, aminoglycoside drugs). In addition, the possibility of SHL was excluded by detailed clinical examination. HL was confirmed with pure tone audiometry. After the Informed consent was obtained, peripheral blood samples were collected in 0.5 M EDTA containing tubes and genomic DNA was extracted using the salting out method [16]. Concentration and purity of DNA was measured (Nanodrop®TM1000-Thermo Scientific) and approximately 50 ng of the genomic DNA was used to genotype each sample. The study was approved by the Ethics committee of Tehran University of Medical Sciences (TUMS).

PCR-Sequencing and Screening of GJB2 Exons

From each pedigree, one affected member was sequenced for exon1 and 2 of GJB2 gene. Following primers were used for amplification of exon1 forward: 5’CGACCACAGCCATCCCCGACC-3’ and reverse: 5’-GGAGCGTGTTGTGGCTCCAGCC-3’ which yielded a 684 bp band. Each PCR reaction condition was: : 0.8µl of each of the primers (10 PM), 7µl Mastermix (amplicon® Mastermix Contains MgCl2, Taq PCR buffer, Taq DNA polymerase and dNTPs) and 1µl DNA (50ng/ul) adjusted to 25 µ by ddH2O. PCR reactions were performed under the following condition: initial denaturation at 95°C for 2 minutes, followed by 33 cycles of denaturation at 95 °C for 30 s, 72 °C for 60 s, and final extension at 72 °C for 5 min. Exon 2 was amplified based on the protocol described before. PCR products were run in 1/5% agarose gel electrophoresis. Bands were visualized by EtBr staining. Afterward, the PCR-amplified products were sequenced on an ABI 3130 automated sequencer (Macrogen®, South Korea). Sequencing data were analyzed by chromas software. After identification of each variant, ensemble.org, dbSNP (http://www.ncbi.nlm.nih.gov/snp), 1000 genome databases (http://browser.1000genome.org) and HGMD (http://www.hgmd.cf.ac.uk/ac/index.php) were searched for previously known variants. Sequence variant numbering was based on the transcript ENST00000382848 for GJB2.

Genotyping and linkage analysis

At least, three informative short tandem repeat (STR) markers were analyzed for each locus. The STR markers were chosen based on their physical distance found at NCBI UniSTS and Map Viewer. Further markers were examined upon encountering an uninformative marker.
The used primer sequences are shown in Table 1. STR markers were amplified by touch down PCR in an ABI thermal cycling 2720 (Applied Biosystems®, USA). PCR protocol and thermal cycling program are available upon request. Generally, PCR products were run on 8-12% PAGE at 15 mA for 4-6 h. Bands were visualized by silver staining. Alleles were assigned by visual assessment.

Two-point and multi-point parametric LOD scores were calculated by Superlink version 1.6 and Simwalk version 2.91 options of Easylinkage plus version 5.05 software [17]. After linkage analyses, haplotypes were reconstructed via Simwalk and were visualized by Haplopainter software version 029.5 [18].

### Results

#### Screening of GJB2 Exons

Totally 20 large pedigrees including 46 affected and 60 healthy members were recruited, among which four pedigrees showed GJB2 mutations. Interestingly, three of four were compound heterozygous for 35delG and splice site mutation of IVS1+1G>A, while one pedigree was homozygous for 35delG. Pedigrees of the families positive for GJB2 mutations and electropherograms of the mutations are depicted in Figure 1.

#### Genotyping STR Markers and Homozygosity Mapping

Families negative or heterozygote for DFNB1 mutations were screened for DFNB4, DFNB21 and DFNB7/11 loci by linkage analysis which no family showed to be linked to the mentioned loci.

#### Discussion

HL is the most common sensory defect with high clinical and genetic heterogeneity [19]. Up to now, over 100 loci have been mapped and about 60 genes identified for ARNSHL (http://www.hereditaryhearingloss.org). GJB2 mutations in the DFNB1 locus
are the main cause of ARNSHL in many ethnic groups, including Iran [20-22]. The prevalence of HL in Iran is 2–3 times greater than other populations [19] while GJB2 mutations are responsible for 16.7% of ARNSHL cases and this prevalence may vary among different ethnic groups.

Kurdistan population is located in the west part of Iran. Kurdish population of Iran is distributed among 4
different provinces including: Kurdistan, Kermanshah, Ilam and Azerbayjan while the population of Kurdistan province remains isolated by culture and religious approach. Findings of the current study showed that contribution of GJB2 mutations in Kurdistan province of Iran is 20% that almost seem to be consistent with results of Mahdieh et al [23] also conducted on a Kurdish population. However, distribution of the variants is not exactly the same. In the current study, only 2 GJB2 variants, including 35delG and IVS1+1G>A, were observed while the other study found six variants including 35delG, R127H, del120E, R32H, IVS1+1G>A and R184P [24]. It worth mentioning that study population of Mahdieh et al. mostly originate from Kermanshah province, which is less isolated in comparison to Kurdistan. This might be a possible explanation for the different mutational spectrum observed. This discrepancy may also arise due to differences in sample size which was larger in Mahdieh’s et al.

In the Iranian Azeri patients, a frequency of 28% of mutant GJB2 have been reported [25]. In HL population of Oman [26], Pakistan [27],Turkey [28], and Slovakia [29], contribution of GJB2 gene mutations is 0%, 6.1%, 25%, and 45.6%, respectively. In addition, some studies indicated 31.7% contribution of GJB2 mutation in Turkey [30], in neighboring of Iranian Azeri Turkish residents.

Many allelic variations have been reported in GJB2 (http://www.hgmd.cf.ac.uk/ac/index.php). Among different deafness-causing alleles, 35delG mutation is the most common one. This frame shift mutation results in a truncated protein [19]. The frequency of this allele differs among different Iranian ethnic groups [21], for example, it is 71.6 % in Iranian Azeri Turkish population [31]. The present findings indicate a frequency of 62.5% and 37.5% for 35delG and IVS1+1G>A among Kurdish people from Kurdistan province of Iran, respectively. R127H, delE120, W24X, V27I+ E114G, R184P, -3170G4A and 235delIC are the other most common mutations of GJB2 in Iranian population [19] which were not observed in this study.

Here, we found no family linked to DFNB4, DFNB21 and DFNB7/11.

SLC26A4 gene, located on the DFNB4, 7q31[32], is considered to be the second leading cause of autosomal recessive HL in many populations [33]. Studies had reported frequencies of 10% and 4.8% for SLC26A4 mutations in Iran [34, 35]. Interestingly, unlike the previous reports, no mutation was found in SLC26A4 in our study population which consequently suggests that DFNB4 may not be a major locus in etiology of HL in Kurdistan Province of Iran.

TMC1 at the DFNB7/11 locus on chromosome 9q31-21 (MIM 606706, GenelID 117531), encodes transmembrane channel-like 1 protein [38]. Mutations of this gene has been reported as a frequent cause of ARNSHL in several populations, including Pakistan, India and also Iran [39]. In a heterogeneous Iranian study population, 9.4% of HL families were linked to DFNB7/11 [40]. However another study failed to replicate this frequency and only a 2% contribution was observed [34]. Here, our results show that TMC1 mutations are not an important cause for ARNSHL in Kurdish population.

TECTA gene which has been mapped on chromosome 11q22–q24 (MIM 602574, GenelID 7007) [41, 42], encodes a protein of 2155 amino acids. TECTA mutations have been found in French, Belgian, Swedish and Austrian families [41]; in Iran, they are responsible for 4.1% of ARNSHL cases [43-45]. However, unlike previous studies, no mutation in TECTA was observed in our study population which suggests its minor contribution in etiology of HL Iranian people from Iran.

Conclusion

In this study, we could find the genetic basis of HL in only four out of 20 pedigrees of the studied population, while the etiology in about 80% remains to be identified. GJB2 mutations were the most common cause of HL, accounting for about 20% of the families. Regarding high frequency of 35delG and IVS1+1G>A, it worth to detect carrier frequency of these two variants among normal population.

Other loci should also be investigated in order to clarify the molecular etiology of hearing loss in Kurdistan province. Our results highlight the heterogeneity of HL and provide clues for a different panel of DFNB loci involved in the etiology of hearing loss in the province.

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References

2. Van C. G., Willems P. J. and Smith RJ. Nonsyndromic


30. Uyguner O., Eмиroglу M., Uzumecu A., Hafiz G., Ghanbari A., Baserar N. and Wollnik B. Frequencies of