Genetics of hereditary hearing loss in east Iran population: A systematic review of GJB2 mutations

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Summary

Mutations in the GJB2 gene are the most common cause of pre-lingual hearing loss (HL) worldwide. Previous studies have shown the frequency of GJB2 mutations to be 16% in Iran, but varies among different ethnic groups. Here, we have reviewed results from previous published mutation reports to provide a comprehensive collection of data for GJB2 mutations and HL in eastern Iran. We conducted a systematic literature review of PubMed, Google Scholar, Web of Science, and Science Direct databases for articles published before March, 2019. The literature search was performed by 2 independent researchers. The primary data of these studies including the number of samples, allelic frequency, and so on were extracted. Six studies involving 812 unrelated families from four different eastern provinces were included and analyzed for the type and prevalence of GJB2 mutations. A total of 19 different genetic variants were detected. GJB2 mutations were 8.8% in the studied eastern provinces, which was lower than that reported in northern populations of Iran. Moreover, a gradient in the frequency of GJB2 mutations from north to south Iran was observed. c.35delG was the most frequent mutation, accounting for 48.5% % of the populations studied. However, this mutation was absent in the Baluchi population. This review shows that particular rare mutations are frequent in some Iranian ethnic groups, and should be considered for genetic counselling.

Keywords: Iranian population, genetic counseling, GJB2, non-syndromic hearing loss

1. Introduction

Hearing Loss (HL) is the most common sensory disorder, affecting 1 in every 500-1,000 newborns (http://hearing.screening.nhs.uk/nationalprog). It is estimated that 50-70% of HL is related to genetic causes. Almost two thirds of cases include non-syndromic forms (NSHL), since hearing impairment is the only

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common mutation with carrier frequency as high as 2-4% (17). However, c.235delC, c.167delT and c.71G>A are the most frequent mutations in the Japanese (18), Ashkenazi Jewish (19), and Indians (20), respectively. The Iran population is genetically heterogeneous and a mixture of several ethnicities; consanguineous pedigrees affected with ARNSHL have helped to identify many HL associated genes. Over the past 15 years, tremendous amounts of epidemiologic data have been collected on the Iranian population in order to determine the mutation spectrum and frequency of \textit{GJB2} mutations (21-30). It was shown that the mutation frequency of \textit{GJB2} varies between 0 and 35% among different regions of Iran and c.35delG is the most common mutation reported (31,32). In this paper, we summarized the published data on the frequency and profile of the \textit{GJB2} gene mutations in 812 unrelated families from 4 different provinces; namely Khorasan, Sistan & Baluchestan, Kerman and Hormozgan in east Iran compared to other parts of this country.

2. Methods and Analysis

2.1. Publication search

A systematic literature review of PubMed, Google Scholar, Web of Science, and Science Direct databases was conducted in English for articles published before April, 2019. The following keywords and medical subheadings were used simultaneously in each set: ("hearing loss" or "deafness" or "hearing impairment") and ("GJB2") or ("connexine26") or ("35delG") and ("Iran"). Alternative spellings were also considered. The literature search was performed by 2 independent researchers.

2.2. Inclusion criteria and data extraction, data analysis

Eligible studies included in our review met the following inclusion criteria: \textit{i}) performed on non-syndromic HL subjects, \textit{ii}) described ethnicity of tested subjects, and \textit{iii}) detected all \textit{GJB2} mutations. Studies were excluded if HL was a result of environmental factors such as infection, trauma, rubella, meningitis, mumps, ototoxic drugs and premature birth. The flow diagram of the selected eligible studies is shown in Figure 1. The necessary data were extracted from the final eligible articles as follows: first author, publication year, subject ethnicity and number of cases. The frequency and mutation type of \textit{GJB2} were extracted from relevant studies and categorized, corresponding with geographical boundaries. \textit{In silico} analyses were also performed with available software tools to predict pathogenicity of the mutations.

3. Results

A total of 6 studies comprising 812 unrelated families from 4 provinces were included for analysis. The

Figure 1. Flow chart of review process.
groups studied consisted of 296 families from Khorasan province (36.45%), 292 families from Sistan & Baluchestan (35.9%), 121 families from Hormozgan (14.9%) and 103 families from Kerman province (12.6%). Among these families, 78.7% reported parental consanguinity while in 21.3% close consanguinity was denied (Table 1). The GJB2 mutation allele frequencies of each studied group included 13.7%, 8.4%, 7%, 6.3% of total studied families (n = 812) of Khorasan, Hormozgan, Kerman, and Sistan & Baluchestan provinces, respectively. A total of 19 different variants were identified, 14 of which were reported as pathogenic. These include: c.-23+1G>A, c.35delG, c.71G>A, c.336G>T, c.167delT, c.235delC, c.29delT, c.358-360delGAG, c.238G>A, c.427C>T, c.269T>C, c.511G>A, c.229T>C, c.465T>A. The GJB2 mutation spectrum and frequency revealed in this review is listed in Table 2. In the studied populations, c.35delG was the most frequent mutation accounting for 48.5% of the populations studied. The highest rate of c.35delG mutation was detected in Khorasan province with an allele frequency of 10.1% while we did not find any c.35delG mutations in Sistan & Baluchestan (Figure 2). A specific combination of GJB2 mutation types and frequencies were found in different studied provinces (Table 2). A higher GJB2 mutation diversity (9 types) was observed in Khorasan province while the lowest diversity was identified in Sistan & Baluchestan (4 types).

4. Discussion

The identification of genes causing non-syndromic hearing Loss (NSHL) has partially resolved the puzzle of clinical and genetic heterogeneity of HL (33-35). Among these genes the gene with the most significant impact on population genetics and genetic counselling is the GJB2 gene with the mutation c.35delG that accounts for the majority of mutations in deaf Caucasians (12). Studies published so far have reported the differences in frequency of the mutation in different populations, even from neighboring countries. The Iranian population is composed of many different ethnic groups. According to this fact, it is necessary to discuss ethnic specific data. This study reviews the prevalence and type of the GJB2 gene mutations of 812 deaf families from four different provinces of this country. Here, the most consistent finding was the reduction of GJB2 mutation frequencies of north to south Iran. Our data showed a north to south gradient among Iranian populations with a GJB2 mutation frequency of 13.7% for Khorasan province and 6.3% for Sistan & Baluchestan province. In 2007, Hashemzadeh et al. stated that the frequency of GJB2-related HL is in 14.6% of deaf families (130 of 890 families). They also found the highest percentage of GJB2 related HL in the north and northwest regions of Iran (27%), while it was less than 4% in the Southeast region (36). The findings of our study shows that the contribution of GJB2 mutations to ARNSHL is 13.7% in Khorasan province, which is similar to the presented data from Semnan province (37). Bazazzadegan et al. screened 111 ARNSHL families from Semnan province in center Iran for GJB2 mutations. They reported that GJB2 mutations were detected in 11.5% of the ARNSHL families studied.

Another finding of this study was the mutation spectrum of southeast Iran, which was different from those of other Iranian population regions. Two hundred and ninety two unrelated Baluchi deaf families were reviewed, with a frequency of 6.2% for GJB2 gene mutations. Interestingly, c.71G>A was the most frequent GJB2 mutation, while c.35delG was absent in this ethnicity. Results obtained for the carrier frequency of c.71G>A mutation was 70% in the Baluchi population whereas 4.2% in the rest of eastern provinces. However, the Baluchi population is ethnically distinct from the rest of Iran. Besides, 121 unrelated ARNSHL families from Hormozgan province were reviewed, with a frequency of 8.4% for GJB2 gene mutations. This rate of GJB2 mutation has been reported in some populations of the south of Iran like Khuzestan province (37). In the study performed by Bazazzadegan et al. (37) on 103 ARNSHL families indicated that GJB2 related HL accounted for 7% in Kerman province. This is about one third of the frequency of the GJB2 mutations in Isfahan province. In the previous study, we showed that GJB2 mutations explain the cause of ARNSHL in 22.5% of patients from Isfahan province in the center of Iran (38). In addition, Davarnia et al. (39) screened 50 NSHL families from Ardebil province in northwest Iran for GJB2 mutations. They reported that GJB2 mutations were found in 26% of the NSHL families studied. On the basis of these results, it can be concluded that the frequency of GJB2 mutations decreases gradually both west to east and north to south (Figure 3), drawing the migration pathway of the initial founders.

In our studied populations, the most common mutation was c.35delG, accounting for 48.5% of GJB2 mutations. The c.35delG mutation (deletion of guanine in position 30-35; rs80338939) is the most common
| Mutations | Kerman (37) | Hormozgan (44) | Hormozgan (45) | Sistan & Baluch (37) | Sistan & Baluch (46) | Sistan & Baluch (47) | Khorasan (41) | Khorasan (37) | Mutation type | Classification | Functional effect |
|-----------|-------------|----------------|----------------|---------------------|-------------------|---------------------|----------------|----------------|---------------|---------------|------------------|
| c.29delT  | 2 (6.25)    | .               | .               | .                   | .                 | .                   | .              | .              | Frameshift    | T              | Disease causing |
| c.35delG  | 4 (1.94)    | 3 (1.42)        | .               | .                   | .                 | .                   | 25 (11.16)    | 33 (8.96)     | Frameshift    | T              | Disease causing |
| c.71G>A   | 2 (0.97)    | 2 (0.95)        | 12 (5.5)        | 2 (1.19)            | 10 (5)           | .                   | .              | .              | Missense      | NT             | Disease causing |
| c.167delT | .           | .               | 2 (0.92)        | 2 (1)               | 2 (1)            | .                   | 1 (0.27)       | .              | Frameshift    | T              | Disease causing |
| c.229T>C  | .           | .               | .               | .                   | .                 | .                   | .              | .              | Missense      | NT             | Disease causing |
| c.235delC | .           | .               | .               | .                   | .                 | .                   | .              | .              | Missense      | NT             | Disease causing |
| c.269G>A  | 2 (6.25)    | .               | .               | .                   | .                 | .                   | .              | .              | Frameshift    | T              | Disease causing |
| c.358-360delGAG | 4 (1.94) | .   | .   | 1 (0.5) | 1 (0.5) | 1 (0.45) | 1 (0.27) | . | Missense | NT | Disease causing |
| c.336G>T  | .           | .               | 1 (0.5)         | 1 (0.5)             | .                 | .                   | 1 (0.27)       | .              | Missense      | NT             | Disease causing |
| c.231G>A  | 1 (0.48)    | .               | .               | .                   | .                 | .                   | 2 (0.54)       | .              | Missense      | NT             | Disease causing |
| c.465T>A  | 1 (0.48)    | .               | .               | .                   | .                 | .                   | .              | .              | Missense      | NT             | Disease causing |
| c.551G>C  | .           | .               | 1 (3.2)         | 1 (3.2)             | .                 | .                   | 2 (0.54)       | .              | Missense      | NT             | Disease causing |
| c.397G>A  | 1 (0.48)    | .               | 6 (2.85)        | 1 (0.59)            | .                 | .                   | 8 (3.57)       | .              | Missense      | NT             | Polymorphism |
| c.101T>C  | .           | .               | .               | 1 (0.59)            | .                 | .                   | 1 (0.45)       | .              | Missense      | NT             | Polymorphism |
| c.341A>G  | .           | .               | 1 (0.59)        | 3 (1.34)            | .                 | .                   | 3 (1.34)       | .              | Missense      | NT             | Polymorphism |
| c.380G>A  | 2 (0.97)    | 4 (1.9)         | 4 (1.85)        | 2 (1.19)            | 4 (2)            | .                   | 3 (1.34)       | .              | Missense      | NT             | Polymorphism |
| c.457G>A  | .           | 3 (1.42)        | 8 (4.76)        | .                   | 3 (1.34)         | .                   | .              | .              | Missense      | NT             | Polymorphism |
| Normal    | 192 (28)    | 192 (198)       | 154 (182)       | 175 (326)           | .                 | .                   | .              | .              | .             | .              | . |
| Total     | 206 (32)    | 210 (216)       | 168 (200)       | 224 (368)           | .                 | .                   | .              | .              | .             | .              | . |

*The pathogenic mutations and benign variants were separated in the two parts. The mutations were arranged in numerical order. T, Truncated protein; NT, Non-Truncated protein; NA, Not Available.
mutation in many world populations as well as many countries in the Middle East such as Turkey, north and northwest of Iran (40). The study of the geographical distribution of the GJB2 mutations showed less allelic heterogeneity in the east compared to the north of Iran. The four most frequent mutations of the GJB2 gene in the west of Iran, namely, c.35delG, c.71G>A, c.358_360delGAG and c.167delT are responsible for ~79.8% of all pathogenic alleles in east Iran (Table 2). The c.35delG mutation, which is the most common (up to 85%) among northern regions (41), makes up 48.5% of GJB2 mutations in the eastern populations. The c.71G>A, c.358_360delGAG and c.167delT are the second, third and fourth most common mutations, with a sum of 20.9%, 5.2% and 5.2% of all pathogenic alleles. The p.Trp24*, a nonsense mutation is the result of c.71G > A transition, changing the TGG codon for Trp residue to a stop codon, which leads to a truncated protein with probably no functional properties. In silico analyses are consistent with the pathogenicity of the mutation (Table 1). Sistan & Baluchestan province is located on the western border of Pakistan and is populated mainly by Sistani & Baluchi ethnicities. The c.71G > A mutation is the most common mutation in Pakistan and Indian populations. The rate of carriers of c.71G > A mutation is 4.08% in the Pakistan population (42,43). This mutation shows a high frequency in the Baluchi group, where the population is related to neighboring Pakistan. This review showed a particular combination of GJB2 mutations diversity in different provinces of east Iran. A higher GJB2 mutations diversity (9 types) was detected in Khorasan province, suggesting the co-existence of several different ethnic groups and immigrations to big cities such as Mashhad during the last century. In addition, historical background like occurrence of different wars with foreign nations, immigration, and location of the route of the Silk Road could support this diversity. In contrast with the high diversity of Khorasan province, we found a very low rate of diversity in some populations such as the Baluchi population who are probably isolated with cultural and geographical barriers. The limitation of this study is a small number of studies especially for Kerman province (103 families). Therefore, more screening programs in the east population are warranted.

5. Conclusion

The critical and specific position of Iran and the existence of various ethnic groups with different cultures suggest high heterogeneity throughout Iran but specific intra ethnic traditions such as intragroup marriages may give rise to a high homogeneity in some loci and mutations within groups. Our study revealed a frequency of 8.8% for GJB2 mutations, relatively low compared to other studies of central populations with estimated frequencies between 13-15%. Referring to the GJB2 mutations, one section of this cohort had the c.35delG mutation, so this mutation is the most common mutation that is tested first. In studied populations, specific mutations are common, which are detected in each group; for example, the frequency of the c.71G>A shows a high rate in Sistan & Baluchestan province, accounting for 70% of the mutant alleles. In addition, the causes of HL in some populations such as Baluchi are likely more homogenous than other parts of east Iran. This review highlights the importance of GJB2 mutations in development of HL in eastern parts of Iran and are of great importance for successful disease management and interventions, mainly for genetic counseling and cochlear implants.

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