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High carrier frequency of the GJB2 mutation (35delG) in the north of Iran

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Summary

Objective: Mutations in the GJB2 gene are a major cause of autosomal recessive and sporadic non-syndromic hearing loss in many populations. A single mutation of this gene (35delG) accounts for approximately 70% of mutations in Caucasians with a carrier frequency of 2–4% in Europe. This study aims to determine the rate of 35delG carrier frequency in Iran.
Methods: Genomic DNA was extracted from a total of 550 unaffected unrelated subjects from 4 provinces of Iran following the standard phenol chloroform procedure. The one base pair deletion (35delG) was analysed using a nested PCR procedure; 35delG mutation carriers were subsequently confirmed by sequence analysis. Moreover, using the Binomial probability distribution, we compared the 35delG carrier frequency of Iranian population with the various Middle Eastern and overall European populations.
Results: Of the four populations studied, we found a high carrier frequency of 2.8% in Gilan province in the north of Iran. The overall 35delG carrier frequency was found to

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be 1.25% in the populations studied (our present and previous data) which is similar to the overall 35delG carrier frequency detected in Middle Eastern populations, but Significantly lower than that identified in European populations.

1. Introduction

Genetic deafness is a very common disorder which occurs in approximately 1/2000 live births [1]. Genetic deafness is highly heterogeneous and more than 100 genes are predicted to cause this disorder in humans [2]. In spite of this large genetic heterogeneity, mutations in the GJB2 gene alone, encoding connexin 26, are responsible for approximately half of the severe to profound autosomal recessive non-syndromic deafness in many populations [1,3]. A single mutation of this gene, namely 35delG, is of great importance due to its high pathogenicity and frequency. 35delG homozygosity is associated with more severe deafness compared with other homozygote or compound heterozygote GJB2 mutations [4,5]. Moreover, 35delG accounts for about 70% of the GJB2 mutations in Caucasians with a carrier frequency of 2—4% in Europe [3,6,7]. Conversely it is less frequent or even absent in other ethnic groups where other common mutations prevail such as 235delC which displays a carrier frequency of 1—2% in the Japanese population [8,9], 167delT in the Ashkenazi Jews population with carrier frequency of 4.03% [10] and V37I in Taiwan with carrier frequency of 8.5% [11]. The previous studies revealed a specific combination of GJB2 mutations types and frequencies in different populations of Iran [12—15]. In the present study, we have investigated the carrier frequency of 35delG mutations among 550 unaffected unrelated subjects from four provinces of Iran.

2. Materials and methods

In total, 550 unaffected unrelated subjects with different ethnic origin (Gilak, Azeri, Persian and Lur) from four provinces of Iran including Gilan in north, Azarbaijan Sharqi in north west, Hamadan in west and Chaharmahal va Bakhtiari in south west, were studied (Fig. 1). Informed consent was obtained from all subjects. Genomic DNA was extracted from 0.5 ml peripheral blood following the standard phenol chloroform procedure. The 35delG one base pair deletion was assayed using a nested PCR procedure. The entire coding sequence of GJB2 gene was amplified using primers CX124F 5′ CTC CCT GTT CTG TCC TAG CT 3′/CX929R 5′ CTC ATC CCT CTC ATG CTG TC 3′ (806 bp) at an annealing temperature of 60 °C. The amplified product was then diluted and used as a template for a second round of PCR using primers CX210F 5′CAC GCT GCA GAC GAT CC3′/CX252R 5′GGT GGA GTG TTT GTT CAC3′ (43 bp) at an annealing temperature of 56 °C. The amplified products were then separated by electrophoresis on a 15% Polyacrylamide gel (40% 19:1 Acrylamide: Bisacrylamide) at 35 mA for 2.5 h

Fig. 1 35delG heterozygotes were collected from four provinces of Iran. A dark shade corresponds to a higher carrier frequency (2.8%), while the pale shades representing the lower frequency (1%).

and the products were then visualised by silver staining. The 35delG heterozygotes were detected as two separate bands of 43 bp for the wild type and 42 bp for the mutant allele and confirmed by direct sequencing of the amplified products (806 bp). Sequencing was performed using BigDye terminator V3.1 cycle sequencing kit (Applied Biosystems) on an ABI 3100 DNA sequencer (Applied Biosystems).

The carrier rate of the 35delG in Iranian population was compared with that of the various Middle Eastern and overall European populations using the 95% confidence interval of carrier rate based on Binomial probability distribution [16].

3. Results

Ten of 550 subjects (1.8%) were found to be carriers of the 35delG mutation (95% Binomial confidence interval (CI), 0.88—3.32%). Table 1 shows carrier frequencies of the 35delG mutation by province. A rate of 1% carrier frequency was detected in Azarbaijan Sharqi, Hamadan and Chaharmahal va Bakhtiari in north west, west and south west of the country. The highest 35delG carrier frequency (2.8%) was detected in Gilan province in north of Iran. 35delG electropherograms and polyacrilamide gel electrophoresis of wild type and mutant allele are shown in Fig. 2.

The 35delG carrier frequency found in this study (1.8%) is statistically similar to that of the other Middle Eastern populations. However, the overall 35delG carrier rate of 1.30% (95% Binomial CI, 0.96—1.72%) detected in Middle Eastern populations is significantly lower than that: 2.69% (95% Binomial CI, 2.18—3.27%) identified in European populations (Table 2).

4. Discussion

The 35delG mutation is responsible for 10% of all childhood hearing loss and for 20% of all childhood hereditary hearing loss in American Caucasians with northern and southern European origin [17]. Moreover, 35delG accounts for about 70% of the GJB2

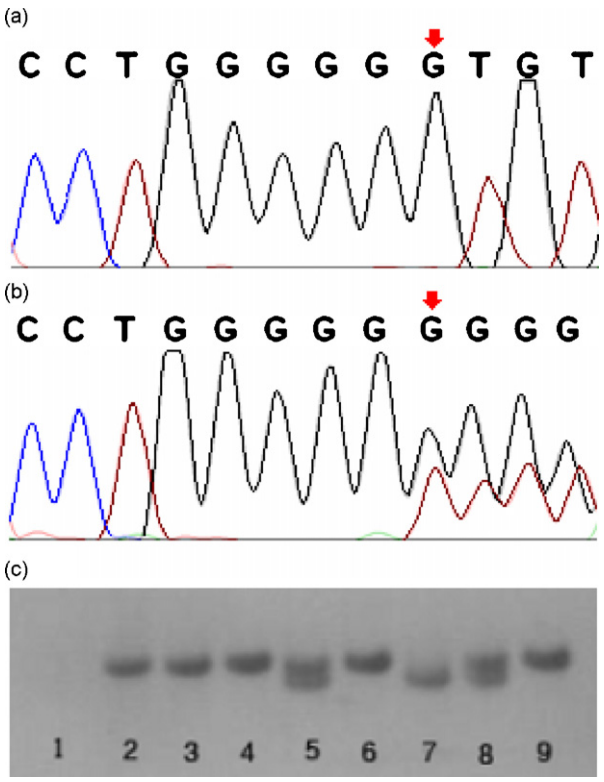

Fig. 2 Electropherograms and polyacrilamide gel electrophoresis of wild type and mutant allele of 35delG. (a) Nucleotide sequences of wild type and (b) heterozygous (carrier) 35delG identified in this study. (c) Nested PCR analysis of the 35delG mutation; lane 1: negative control, lanes 2, 3, 4, 6, 9: wild type, lanes 5, 8: 35delG heterozygous, lane 7: positive control (35delG homozygous).

mutations in Caucasians with a carrier frequency of 2—4% in Europe [3,6,7]. The 35delG mutation is of great importance concerning its high pathogenicity, severity of disease and frequency [4,5].

In the present study, the carrier frequency of 35delG mutations among 550 unaffected unrelated Iranian subjects from four provinces ranged from 1% in Azarbaijan Sharqi (north west), Hamadan (west), and Chaharmahal va Bakhtiari (south west) to 2.8% in Gilan (north) with an average carrier frequency of 1.8%. The high rate of 35delG carrier frequency in Gilan is consistent with the previous finding of high 35delG mutation frequency (34 and 27% of all deaf-

Table 1 Carrier frequency of the 35delG mutation in the various provinces investigated

Province	Subjects	Carriers	Carrier frequency (%)
Gilan	250	7	2.8
Azarbaijan Sharqi	100	1	1
Hamadan	100	1	1
Chaharmahal va Bakhtiari	100	1	1
Total	550	10	1.8

Table 2 GJB2 35delG mutation carrier frequencies, observed rates and 95% confidence intervals (Binomial) in 12 Middle Eastern populations compared with an overall 13 European populations (24)

Population	Rate of 35delG heterozygote	Carrier observed rate (%)	95% CI Binomial distribution	Reference
Cyprus (Greek Cypriots)	10/405	2.47	1.19—4.50	[24]
Lebanon	7/300	2.33	0.94—4.75	[26]
Israel (Ashkenazi Jewish)	1/467	0.21	0.01—1.19	[27]
Persian Jews	2/59	3.39	0.41—11.71	[25]
Iraqi Jews	1/115	0.87	0.02—4.75	[25]
Egypt	0/95	0	0—5.67	[25]
Oman	0/280	0	0—1.31	[19]
Turkey	12/674	1.78	0.92—3.09	[22]
Turkey	5/429	1.17	0.38—2.70	[23]
Azerbaijan	0/75	0	0—4.8	[21]
Iran	0/250	0	0—1.47	[18]
Iran	10/550	1.8	0.88—3.32	Present study
Middle East (overall)	48/3699	1.30	0.96—1.72	Present study
Europe (overall)	97/3610	2.69	2.18—3.27	[24]

ness chromosomes investigated) amongst deaf families in north of the country [13,15]. The enrichment of the 35delG mutation in both deaf and normal populations of Gilan is likely to reflect a founder effect within this region perhaps relating to the geographical isolation of this population. Our previous study of 890 families with autosomal recessive or sporadic hearing loss revealed that 35delG is the most prevalent GJB2 mutation accounting for 74.5% of the GJB2 mutations chromosomes accounting for 10.8% of all chromosomes studied in Iran [15].

A lower rate of 35delG carrier frequency was found in the other populations studied and in total a carrier frequency of 1.8% was identified in the four populations studied. This is a higher rate than our studies of Tehran, which represent a very mixed population, in which we were unable to detect the mutation in a control group of 250 blood donors [18]. Taken together our data indicates a lower rate of 35delG mutation in the south and south east compared with north and north west of the country amongst deafness families [13,15], and an overall 35delG carrier frequency in the general population in Iran is estimated to be less than 1.25%. This corresponds to a slightly lower rate of 35delG carrier frequency compared with that of our neighbouring country Turkey (Table 2). A low frequency or absence of 35delG carriers have been reported in other our neighbouring countries including Oman, Pakistan and Azerbaijan [19—23]. This is consistent with a decreasing GJB2 mutation frequency in a north westerly to south easterly gradient across Iran [13,15]. Using the Binomial probability distribution, we analyzed 35delG carrier rate of Iranian population compared with those of European and Middle Eastern populations. Our results are in agreement with the previous meta-analysis studies [7,24].

In conclusion, the overall 35delG carrier rate of 1.25% found in Iranian population represented a similar rate of that detected in Middle Eastern populations, but significantly lower than that identified in European populations. The high carrier frequency of 35delG mutation in Gilan in north of the country is likely to reflect a founder effect, carrier advantage or both. As 35delG accounts for 71.6% of the GJB2 mutations in overall Iranian population, our simple 35delG screening test would facilitate diagnosis of congenital deafness and early treatment of the affected subjects particularly in regions with high carrier frequency like Gilan [15,25].

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