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Did the *GJB2* 35delG Mutation Originate in Iran?

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Abstract

Mutations in *GJB2* are a major cause of autosomal recessive non-syndromic hearing loss (ARNSHL) in many populations. A single mutation of this gene (35delG) accounts for approximately 70% of *GJB2* mutations that are associated with ARNSHL in Caucasians in many European countries and also in Iranian. In this study, we used PCR and restriction digestion to genotype five single nucleotide polymorphisms (SNPs) that define the genetic background of the 35delG mutation over an interval of 98Kbp that includes the coding and flanking regions of *GJB2*. Two microsatellite markers, D13S175 and D13S141, were also analyzed in patients and controls. These data suggest that the 35delG mutation originated in north and northwest of Iran.

Keywords

GJB2; 35delG; Iran; ARNSHL

INTRODUCTION

One in every 1000 neonates is born with profound congenital deafness, often the result of autosomal recessive inheritance (autosomal recessive non-syndromic hearing loss, ARNSHL). Although more than 77 genetic loci have been implicated in ARNSHL (known as DFNB loci), mutations in one gene – *GJB2* (MIM *121011) – are the most frequent genetic cause of congenital deafness in many populations. Previous studies have demonstrated that the most frequent *GJB2* mutation in the northern European population is the 35delG mutation, which originated on a specific genetic background consistent with an ancestral founder. [Zelante et al, 1997 ; Chaib et al, 1994 ; Denoyelle et al, 1997 ; Kelsell et al, 1997 ; Morell et al, 1998 ; Park et al, 2000 ; Maw et al, 1995].

The 35delG mutation in *GJB2* is also the most common pathogenic allele in persons with ARNSHL in Iran, especially in provinces that include Turkish and Gilaki ethnicities where

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in aggregate, mutations in *GJB2* account for 22.2% and 38.3% of ARNSHL, respectively. In these two populations, homozygosity for the 35delG mutation is estimated at 32% and 17% respectively.[Najmabadi et al, 2005; Hashemzadeh Chaleshtori et al, 2007].

Based on the historical route of early human migration from Siberia to the Iran plateau and Europe, a report postulating a possible 35delG founder in the Middle East [Van Laer et al, 2001], and the low prevalence of this mutation in Pakistan and Oman (3.7 % and 0%, respectively).[Brown et al, 1996; Simsek et al, 2001a Simsek et al, 2001b] we conducted this study to test the hypothesis that the *GJB2* 35delG mutation may have originated in an Iranian population several thousand years ago.

MATERIALS AND METHODS

PATIENTS

Fifty probands (20 males, 30 females) 16.3 ±11 years of age were selected from unrelated families originating from the Turkish (East Azerbaijan) and Gilaki (Gilan Province) ethnic groups of Iran for inclusion in this study. All persons were homozygous for the *GJB2* 35delG mutation. Controls with wild type *GJB2* sequence were selected from healthy blood donors and matched to the case group by sex, age and ethnicity. Five single nucleotide polymorphisms (SNPs) and two short tandem repeat (STR) markers flanking *GJB2* were used to define the genetic background (Fig 1).

Informed consent was obtained from cases and controls after the study had been approved by the local ethics committee of the Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

MICROSATELLITE ANALYSIS

Two dinucleotide repeat microsatellite markers – D13S141(UniSTS:147652) and D13S175(UniSTS:19888) – flanking *GJB2* were amplified in a volume of 20 µl containing 100 ng of template DNA, 1x PCR buffer (F. Hoffmann–La Roche Lt –Applied), 200 mM each dNTP(Roche -Applied), one unit of Taq DNA polymerase (F. Hoffmann–La Roche Lt –Applied) and 10 pmol of each primer. Amplification of D13S141 was performed using primers: forward, 5'-ACC ACG GAG CAA AGA ACA GA-3' and reverse, 5'-GTC CTC CCG GCC TAG TCT TA-3'. D13S175 was amplified using primers: forward, 5'-TAT TGG ATA CTT GAA TCT GCT G-3' and reverse, 5'-CAC TTA AAA TCT ACT CTC TCA GCA G-3'. Amplification conditions included a 30-cycle three-step PCR (denaturation at 94 °C for 30 s, annealing 58 °C for 30 s, extension at 72 °C for 30 s) with an initial step at 94° for 5 min and an extra extension time at 72 °C for 3 min. PCR products were resolved using 8% polyacrylamide gel electrophoresis and silver nitrate staining.

SNP GENOTYPING

The five SNPs flanking the 35delG mutation were genotyped by conventional PCR and restriction digestion. Amplification of SNP1(rs3751385) was performed as previously described [Tekin et al, 2005] with genotyping completed by digestion of the amplification product using MaeI (F. Hoffmann–La Roche Lt –Applied). SNP2(rs9552101 or SNP 1285)

and SNP3(SNP 1245) were included in the same amplicon and resolved by digestion with BanI (New England Biolabs) and HaeIII (F. Hoffmann–La Roche Lt –Applied), respectively. SNP4(rs877098) and SNP5(rs747931) were also amplified using a single primer pair and resolved by digestion with EcoRI and PstI, respectively [Tekin et al, 2005] (Fig 2).

PCR was completed using genomic DNA amplified (120 ng) and 1 U of FastStart Taq polymerase (Roche-Applied), 1.5 mM of MgCl₂, dNTP (0.2 mM) and 10 pmol of the appropriate primers. For amplification, hot start PCR with 40 cycles of 3 steps including: 94 °C, 30 s; 58 °C, 30 s; and 72 °C, 45s was performed with an initial denaturation for 5 min and a final extension of 7 min.

RESULTS

Results of genotyped SNPs and STR markers and final analysis in patients and controls by SPSS version 13.0 are demonstrated in Table 1. With the exception of SNP5, which is located far from 35delG, allele distribution is significantly different between cases and controls. The 35delG mutation segregates most commonly on a T-C-G-T genetic background (SNP4-SNP3-SNP2-SNP1, respectively) ; in contrast, genotypes from control samples are in Hardy-Weinberg equilibrium (PLINK software) (Table 1).

Eighty five percent of Gilaki cases carry the T-C-G-T haplotype, as do 80% of Turkish cases. Eight of 50 individuals(16%) did not carry the most common block, consistent with a recombination event after the 35del mutation arose [Rothrock et al. 2003] (Table 2).

DISCUSSION

Although mutations in *GJB2* are a frequent cause of hearing loss in many populations, there are some populations – like the Pakistani and Arab – in which *GJB2* – related deafness is rare [Brown et al, 1996; Simsek et al, 2001a Simsek et al, 2001b]. In this study, we investigated the origins of the 35delG mutation in *GJB2* by studying flanking polymorphisms and found that 41 of 50 probands with severe-to-profound deafness and homozygous for the 35delG mutation shared a common haplotype T-C-G-T for SNP4-SNP3-SNP2-SNP1, respectively. These results are consistent with the presence of a 35delG founder in northern Iran. As SNP5 is more distant from the 35delG mutation, recombination is more possible [Rothrock et al, 2003].

In multiple northern European populations reported by Van Laer and colleagues, significant differences between the genotypes of patients and controls were found for five SNPs close to *GJB2*, with nearly complete association of one SNP allele (SNP1 in their paper) with the 35delG mutation. They concluded that the 35delG mutation may have originated somewhere in the Middle East and then spread throughout Europe [Van Laer et al, 2001]. This hypothesis is in agreement of another study which presented- by application of different markers - evidence for a founder in Caucasian and Middle Eastern population.[Rothrock et al. 2003]

Based on this statement, and reported carrier frequencies for the 35delG mutation of 1/35.2 and 1.78% in the Turkish population, Tekin and colleagues sought to determine whether the 35delG mutation has single origin in Anatolia. They found the prevalence of haplotype (T-C-G-T) to be 43% and postulated a single 35delG origin in Anatolia [Gasparini et al. and Tekin et al. 2001 and Tekin et al. 2004]

Our analysis of 35delG mutation-carrying chromosomes - using the same STR and SNP markers shows that 42 of 50 probands (84%) share the common haplotype (T-C-G-T), in agreement with data in the Anatolian population [Tekin et al, 2004]. The comparison of these haplotypes in different populations is shown in Table 3.

Lucotte et al. compared 35delG frequencies in different European and Middle Eastern populations and found a lower-to-higher gradient from northern to southern Europe, with the highest 35delG frequencies in southern Europe and the Mediterranean regions. Based on the high 35delG carrier frequencies in southern European countries (3.19%) such as Italy, Greece and south of France compared to the relatively lower carrier frequency in Middle East, they concluded that ancient Greece might be the centre of origin for 35delG mutation and not Turkey. [Lucotte et al. 2005 and Belguith et al 2005]

In another study, by using the same markers as used by Van Laer (2001), Kokota et al. found a common haplotype in the Greek population. Based on the high carrier frequency, a common haplotype, and some historical documents, they concluded that ancient Greece might be the origin of the GJB2 35delG mutation. [Kokota et al. 2008 and 2010]

The 35delG mutation is one of the most prevalent ARNSHL-causing mutations in the north and northwest provinces of Iran amongst the Gilaki and Turkish populations [Najmabadi et al, 2005 ; Hashemzadeh Chaleshtori et al, 2007](Fig 3). These populations (Turk and Gilaki) are bounded by the Caspian Sea in the North and remain relatively isolated by mountains from other parts of Iran. [Najmabadi et al, 2005]

A gradual decrease in the frequency of the 35delG mutation can be seen as we move from the northwest-to-south and east through the Persian Gulf countries.[Najmabadi, 2005]. It is noteworthy that the frequency of the 35delG mutation in resident populations in the Middle East varies, while more eastward, in Pakistan (southeast of Iran), the frequency of *GJB2*-related deafness is very low (3.7%)[Brown et al, 1996]. Southward in Oman, no *GJB2* pathogenic alleles have been reported [Simsek et al, 2001a Simsek et al, 2001b].

Our result (82% for a common haplotype) supports the presence of founder in northern Iran (as compared to 43% in Anatolia). Based on the population frequencies of the 35delG mutation and migration routes from central Asia to Anatolia 10 centuries ago, we suspect that the 35delG mutation arose in or near northwest Iran [Tekin et al, 2004].

In ancient Iran Aryans were, at one time, the only residents of the Iran Plateau. The route of presumed migration of ancient humans from this area was to Europe. Consistent with this migratory route, as one moves from Iran to the east or south, the spectrum and prevalence of *GJB2* mutations changes. In aggregate, our data place the origin of the 35delG mutation in the Middle East and more specifically in northern Iran.

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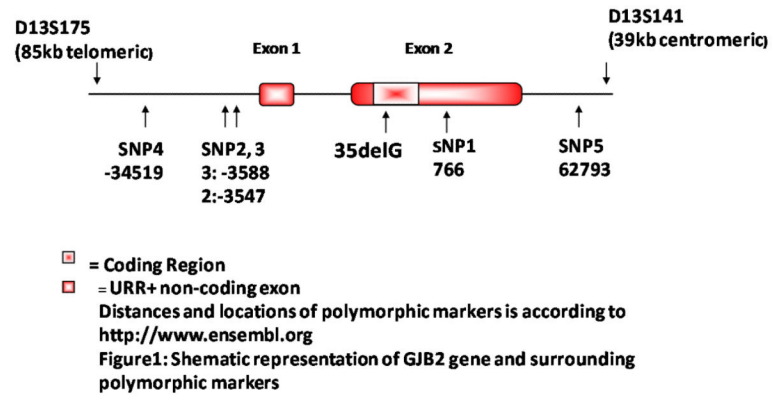


Figure 1.
SNPs and STRs located near and within *GJB2*.

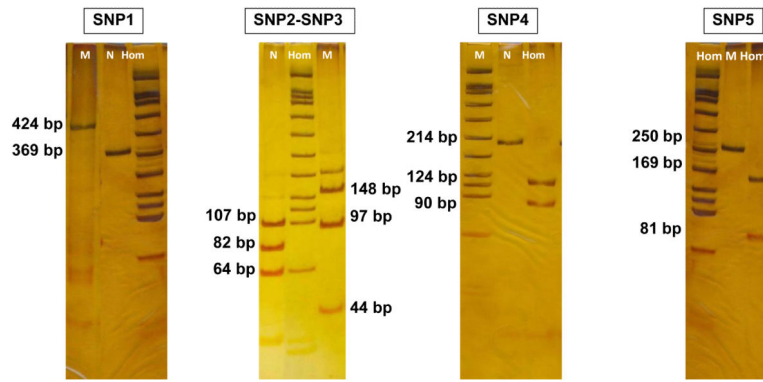


Figure 2. Restriction digests to resolve SNPs 1-5 (Hom: homozygous for the mentioned SNP; N: SNP wild type; M: marker).



Figure 3.
GJB2-related deafness of studied in the Middle East.

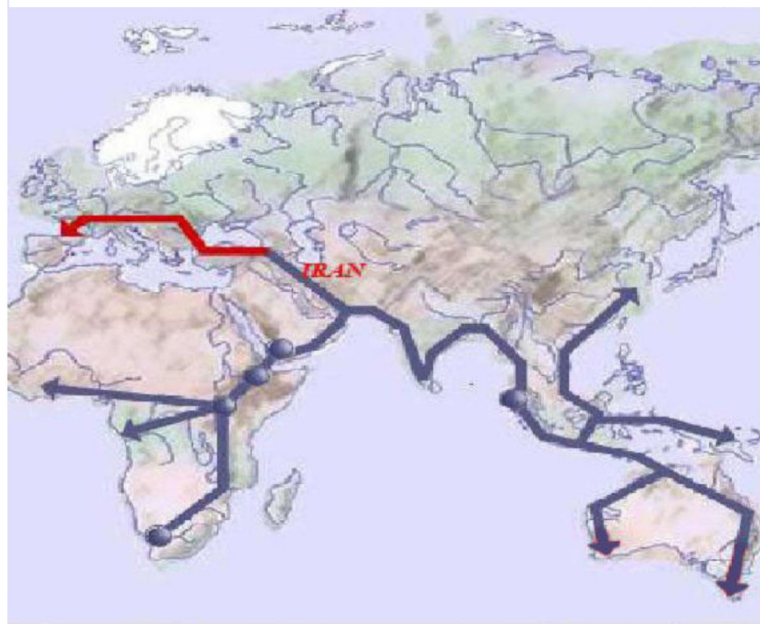


Figure 4. Journey of mankind presented by Bradshaw Foundation (<http://www.bradshawfoundation.com/journey/>). The red color shows the migration path from Iran to Europe.

Table I

Results of genotyping analysis by SPSS version 13.0

Marker	Alleles	Patients homozygous for 35delG mutation (Chr=100)	Controls (Chr=100)	P value	
D13S175(84785 bp telomeric)	1(107)	14	4	0.00000	☆
	2(109)	1	14		
	3(111)	94	51		
	4(113)	1	11		
	5(115)	0	14		
	6(117)	0	6		
SNP4(34519 bp telomeric)	C	7	59	0.00000	☆
	T	93	41		
SNP3(3588 bp telomeric)	C	100	24	0.00000	☆
	T	0	76		
SNP2(3547 bp telomeric)	A	0	6	0.029	☆
	G	100	94		
SNP1(765 bp centromeric)	C	8	84	0.00000	☆
	T	92	16		
D13S141(39262 bp centromeric)	1(116)	0	0	0.00000	☆
	2(118)	0	2		
	3(120)	2	6		
	4(122)	4	76		
	5(124)	86	10		
	6(126)	8	6		
	7(130)	0	0		
SNP5(62793 bp centromeric)	C	35	39	0.661	
	T	65	61		

☆ shows the significant difference (P value < 0.05) of the variable between cases and controls for those specific alleles.

Table II

Eight of 50 individuals (16%) did not carry the most common block, consistent with a recombination event after the 35delG mutation arose

SNP4	SNP3	SNP2	SNP1	Ethnicity	Frequency
C	C	G	T	Turk-Gilaki	(3/50)6%
T	C	G	C	Turk-Gilaki	(5/50)10%

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Table III

Comparison of haplotypes in different populations (according to the last published data in the references)

Allele	Iran		Turkey		Italy		Brazil	Us	Ashkenazi	Greece	Belgian	British
	Deaf	Normal	Deaf	Normal	Deaf	Normal						
SNP1 (rs3751385)	C	84	0	89	—	—	—	0	—	1	0	0
	T	92	16	89	29	—	—	45	—	117	33	27
SNP2 (rs9552101 or SNP1285)	G	100	94	91	87	100	100	100	100	—	—	—
	A	0	6	0	3	3	0	0	0	—	—	—
SNP3 (SNP1245)	C	100	24	92	5	99.3	100	100	100	—	—	—
	T	0	76	0	67	0.7	0	0	0	—	—	—
SNP4 (rs877098 or Hcv1813042)	T	93	41	86	55	95.4	89.2	—	—	—	—	—
	C	7	59	6	69	4.6	10.7	—	—	—	—	—
SNP5 (rs747931)	C	35	39	35	49	—	—	—	—	39	—	—
	T	65	61	57	87	—	—	—	—	71	—	—