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# High Frequency of the p.R34X Mutation in the *TMC1* Gene Associated with Nonsyndromic Hearing Loss Is Due to Founder Effects

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Founder mutations, particularly 35delG in the *GJB2* gene, have to a large extent contributed to the high frequency of autosomal recessive nonsyndromic hearing loss (ARNSHL). Mutations in transmembrane channel-like gene 1 (*TMC1*) cause ARNSHL. The p.R34X mutation is the most frequent known mutation in the *TMC1* gene. To study the origin of this mutation and determine whether it arose in a common ancestor, we analyzed 21 polymorphic markers spanning the *TMC1* gene in 11 unrelated individuals from Algeria, Iran, Iraq, Lebanon, Pakistan, Tunisia, and Turkey who carry this mutation. In nine individuals, we observed significant linkage disequilibrium between p.R34X and five polymorphic markers within a 220 kb interval, suggesting that p.R34X arose from a common founder. We estimated the age of this mutation to be between 1075 and 1900 years, perhaps spreading along the third Hadramaout population movements during the seventh century. A second founder effect was observed in Turkish and Lebanese individuals with markers in a 920 kb interval. Screening for the *TMC1* p.R34X mutation is indicated in the genetic evaluation of persons with ARNSHL from North African and Southwest Asia.

## Introduction

Hearing loss is the most common sensory disorder worldwide. In children, mutation in a single gene is a major cause, and many different responsible genes have been identified. Most frequently the disorder is nonsyndromic and of autosomal recessive inheritance. Founder effects have been reported for some of the common mutations associated with autosomal recessive nonsyndromic hearing loss (ARNSHL) (Abe *et al*., 2000; Dreyer *et al*., 2001; Borck *et al*., 2003; Ouyang *et al*., 2003; Yan *et al*., 2003; Rodríguez-Ballesteros *et al*., 2008; Joseph and Rasool, 2009). Some ancient founder mutations, such as 35delG in the *GJB2* gene, have been described in many parts of the world (Gasparini *et al*., 2000; Van Laer *et al*., 2001; RamShankar *et al*., 2003; Belguith *et al*., 2005).

Twenty-nine different mutations of the transmembrane channel-like gene 1 (*TMC1*) gene (GenBank accession number: NM_138691.2) have been reported in 48 families with ARNSHL (Kurima *et al*., 2002; Kalay *et al*., 2005; Meyer *et al*., 2005; Santos *et al*., 2005; Kitajiri *et al*., 2007; Hilgert *et al*., 2008; Tlili *et al*., 2008; Sirmaci *et al*., 2009). The most common recessive mutation for hearing loss in the *TMC1* gene is p.R34X. This nonsense mutation results from a T-to-C transition at position 100 from the first ATG (c.100C $\rightarrow$ T) and is located in exon 7. It accounts for over 30% of mutant alleles of *TMC1* and occurs in populations throughout Asia and North Africa (Kurima *et al*., 2002; Kitajiri *et al*., 2007; Hilgert *et al*., 2008; Tlili *et al*., 2008; Sirmaci *et al*., 2009). The high frequency of the p.R34X mutation in Tunisian and Pakistani populations has been

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related to a common ancestral founder (Kitajiri *et al*., 2007; Tlili *et al*., 2008).

In this study, we performed haplotype analysis in unrelated hearing-impaired individuals carrying the p.R34X mutation originating from Algeria, Iran, Iraq, Lebanon, Pakistan, Tunisia, and Turkey. We analyzed polymorphic markers both within and flanking the coding sequence of the *TMC1* gene and found two distinct p.R34X haplotypes in these populations. We have estimated the age of the more prevalent haplotype to be between 1075 and 1900 years.

## Materials and Methods

### Subjects

The study was performed on 156 unrelated individuals with ARNSHL from seven countries in North Africa and Asia. Among this cohort, 11 individuals carry the p.R34X mutation (Table 1). Blood samples were collected from each subject after written informed consent.

### Mutation analysis

For specific detection of the p.R34X mutation, primers 5′-AAGCACTTTCTGACATTACTC ATTG-3′ and 5′-TGGAA CTTTTGAAAGAATATCAGA-3′ were used to amplify a 250-bp fragment including exon 7 of the *TMC1* gene. After polymerase chain reaction (PCR), the samples were restriction digested with the enzyme *Taq*I (Jena Bioscience, Munich, Germany) followed by electrophoresis on a 3% agarose gel. Digestion cleaves the normal allele into two fragments (79 and 171 bp), whereas the mutant allele remains uncleaved (250 bp).

### Genotyping

To examine haplotypes associated with the p.R34X mutation, genotyping was performed for 5 microsatellite markers (D9S273, D9S1806, D9S1837, D9S1876, and D9S175) and 16 single-nucleotide polymorphisms (SNPs) (rs7030498, rs2278669, rs12553768, rs1417619, rs2589619, rs1663742, rs2589614, rs1444829, rs348471, rs10869211, rs4256646, rs10781135, rs7874184, rs10746936, rs10869406, and rs2769058) flanking the *TMC1* gene. Positions and average heterozygosity of the markers are listed in Table 1. For the microsatellite markers, heterozygosity varied from 73% to 89% (www.cephb.fr/en/cephdb/browser.php). For SNPs, it varied from 35% to 50% (www.hapmap.org/cgi-perl/gbrowse/hapmap20_B35/). PCR for the microsatellite markers was performed using fluorescently labeled forward primers. Genotyping was done using an ABI 3100 Genetic Analyzer and analysis was performed using Genotyper (version 3.5). The SNPs were genotyped by sequencing using Big Dye Terminator Sequencing V3.1 Kit (Applied Biosystems, Foster City, CA).

### Estimation of the age of p.R34X mutation

Calculation of the age of the p.R34X mutation was performed using a C program developed by Genin *et al.* (2004). When compared with other methods using haplotype information, this approach is efficient with a very small number of affected individuals. Results are presented as the mean number of generations (with empirical 95% confidence interval) estimated over 5000 replicates.

## Results

Using PCR–restriction fragment length polymorphism, the screening of the p.R34X mutation was performed in 72 Tunisian, 54 Algerian, and 22 Pakistani unrelated cases with ARNSHL. Two Algerian and one Pakistani patients carrying the p.R34X mutation were detected. In addition to these three cases, eight hearing-impaired subjects carrying the p.R34X mutation and originating from Tunisia ($n=4$), Iraq ($n=1$), Iran ($n=1$), Lebanon ($n=1$), and Turkey ($n=1$) were included into this study (Scott *et al*., 1996; Hilgert *et al*., 2008; Tlili *et al*., 2008; Sirmaci *et al*., 2009).The p.R34X mutation in all family probands from these populations was confirmed by direct sequencing.

To explore whether the high frequency of the p.R34X mutation of *TMC1* in North African and Asian populations is the result of a founder effect or a mutational hot spot, we searched for evidence of a shared common haplotype of p.R34X with flanking polymorphisms. We analyzed 5 microsatellite markers and 16 SNPs in all probands. In the four Tunisian individuals, an identical haplotype was identified with marker loci D9S273 to rs348471 (centromeric to telomeric) (Table 1). The two Algerians shared 13 of 14 alleles with the Tunisians, while the Iraqi, Iranian, and Pakistani individuals shared 12 of 14, 11 of 14, and 7 of 14 alleles with the Tunisians, respectively (Table 1). The common haplotype in these individuals spans the 220 kb interval between rs2589619 and rs348471. For the two families from Turkey and Lebanon, a second haplotype was observed within the 920 kb interval between rs7030498 and rs348471 (Table 1).

Using the method of Genin *et al.* (2004), we estimated the age of the p.R34X mutation in the first haplotype to be about 43 generations when the marker mutation rate is set at $10^{-3}$. Assuming that one generation is 25 years, this corresponds to 1075 years. When marker mutations are set at $10^{-6}$, the estimated age is 76 generations corresponding to about 1900 years.

## Discussion

Mutations in *TMC1* are a common cause of ARNSHL in India, Pakistan, Tunisia, and Turkey where they account for the hearing-loss phenotype in 3–6% of families (Kurima *et al*., 2002; Kalay *et al*., 2005; Santos *et al*., 2005; Kitajiri *et al*., 2007; Hilgert *et al*., 2008; Tlili *et al*., 2008; Sirmaci *et al*., 2009). Of the 29 reported mutations in this gene, the p.R34X mutation is the most frequent and accounts for over 30% of all *TMC1* ARNSHL-causing mutations. This mutation has been reported in hearing-impaired persons originating from Iran, Iraq, Lebanon, Pakistan, Tunisia, and Turkey (Scott *et al*., 1996; Kurima *et al*., 2002; Kitajiri *et al*., 2007; Hilgert *et al*., 2008; Tlili *et al*., 2008; Sirmaci *et al*., 2009), and we have also identified this mutation in Algeria. Its detection in normal control samples of African-American and northern European origin raises the probability that p.R34X is a prevalent contributor to the genetic load of hearing loss in multiple world populations (Kitajiri *et al*., 2007).

High carrier rates for recessive mutations are associated with high rates of consanguinity and endogamy (Ben Arab *et al*., 2004), thereby conserving the haplotype flanking those mutations. Consistent with this founder effect, the p.R34X mutation has been reported in 10 Pakistani families where it segregates on a common haplotype (Kitajiri *et al*., 2007). A

Table 1. Haplotypes Associated with the p.R34X Alleles in 11 Unrelated Patients of Geographically Diverse Origins

| | | | Origin and code of affected individuals | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Tunisia* | | | | *Algeria* | | *Iraq* | *Iran* | *Pakistan* | *Lebanon* | *Turkey* |
| *Marker* | *Distance from* p.R34X (bp) | *Heterozygosity*[a] *(%)* | *Yah6* | *Tl1* | *St2* | *Gds241* | *C49* | *S1* | *V5* | *281* | *DEM17-5* | *1203* | *Burak* |
| D9S273 | 2.769.891 | 74 | 211 | 211 | 211 | 211 | 215 | 215 | 201/215 | 211 | 209 | 213 | 215 |
| D9S1806 | 1.107.806 | 73 | 262 | 262 | 262 | 262 | 262 | 262 | 254/262 | 262 | 258 | 260 | 258 |
| rs7030498 | 709 .843 | 48 | G | G | G | G | G | G | A | A | G | A | A |
| D9S1837 | 124.043 | 77 | 251 | 251 | 251 | 251 | 251 | 251 | 251 | 249 | 223 | 241 | 241 |
| rs2278669 | 97.276 | 49 | A | A | A | A | A | A | A | A | G | A | A |
| D9S1876 | 76.533 | 89 | 142 | 142 | 142 | 142 | 142 | 142 | 142 | 142 | 140 | 144 | 144 |
| rs12553768 | 44.435 | 44 | C | C | C | C | C | C | C | C | T | C | C |
| rs1417619 | 23.177 | 44 | T | T | T | T | T | T | T | T | A | T | T |
| rs2589619 | 1.011 | 49 | T | T | T | T | T | T | T | T | T | C | C |
| rs1663742 | 351 | 49 | T | T | T | T | T | T | T | T | T | C | C |
| p.R34X | 0 | | | | | | | | | | | | |
| rs2589614 | 352 | 49 | G | G | G | G | G | G | G | G | G | A | A |
| rs1444829 | 647 | 50 | G | G | G | G | G | G | G | G | G | A | A |
| rs348471 | 210.481 | 49 | A | A | A | A | A | A | A | A | A | G | G |
| rs10869211 | 340,328 | 49 | C | C | C | C | C | C | C | C | C | C | C |
| rs4256646 | 488.341 | 50 | A | G | G | G | G | G | G | G | A | A | G |
| rs10781135 | 595.654 | 35 | T | T | T | T | T | T | T | T | T | T | T |
| rs7874184 | 997.946 | 45 | G | G | G | G | G | G | G | G | G | G | T |
| rs10746936 | 1.476.803 | 49 | T | T | T | T | T | T | T | T | C | C | T |
| rs10869406 | 1.804.780 | 48 | G | G | G | G | G | G | G | G | G | A | G |
| rs2769058 | 2.253.593 | 46 | T | T | T | T | C | C | C | C | C | C | T |
| D9S175 | 2.638.391 | 85 | 264 | 264 | 264 | 264 | 264 | 256/264 | 264 | 264 | 264 | 274 | 264 |

The p.R34X mutation-associated haplotypes are boxed. The marker rs10869211 was not considered because we found allele C in the 11 affected individuals.
[a]Available from Hapmap (www.hapmap.org/cgi-perl/gbrowse/hapmap20_B35/) and CEPH (www.cephb.fr/en/cephdb/browser.php).

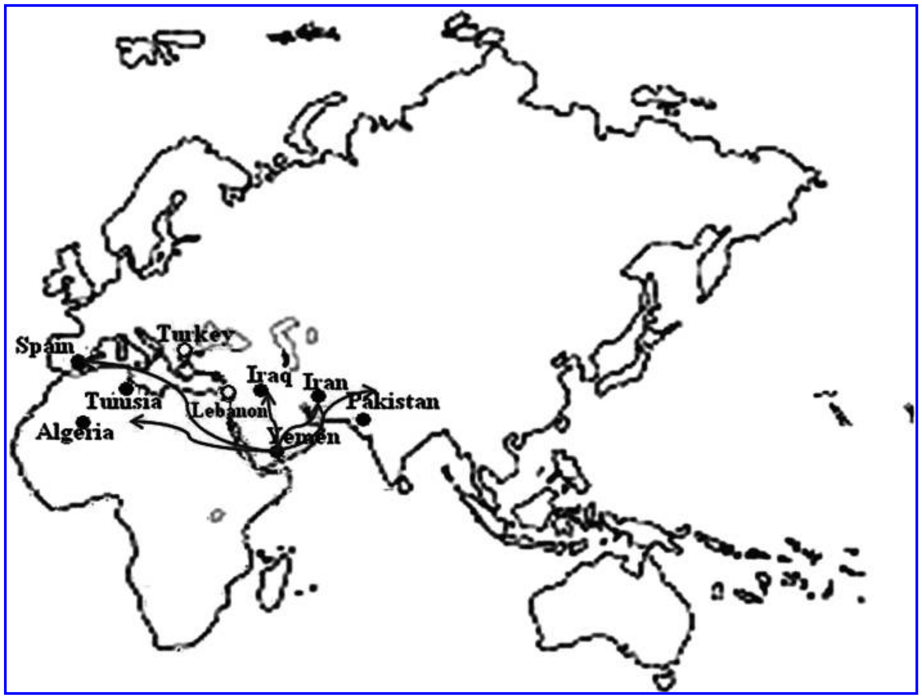


**FIG. 1.** Distribution of hearing-impaired patients carrying the p.R34X mutation. Dots indicate the origin of patients presenting the p.R34X mutation. Arrows correspond to the main Hadramaout population movement routes during the seventh century.

common haplotype has also been observed in four Tunisian families segregating the p.R34X mutation (Tlili *et al.*, 2008).

In this study, we analyzed 21 polymorphic markers surrounding the p.R34X mutation in 11 unrelated individuals from Algeria, Iran, Iraq, Lebanon, Pakistan, Tunisia, and Turkey and identified two disease-associated haplotypes. The hypothesis that the mutation arose a very long time ago in a single individual was not considered. In fact, we detected two different haplotypes at 350 bp from the p.R34X mutation. The first haplotype, a 220 kb interval flanked by markers rs2589619 and rs348471, was detected in nine of 11 individuals from Algeria, Iran, Iraq, Pakistan, and Tunisia. It is the first time that a common haplotype was described in individuals from these different countries. The Algerian, Iraqi, Iranian, and Pakistani haplotypes shared 13 of 14, 11 of 14, 10 of 14, and 6 of 14 alleles, respectively, with the Tunisian founder haplotype. This result shows that there is correlation between genetic diversity and geographic distance from Tunisia. The small chromosomal interval of the first haplotype is consistent with an ancient origin of a single founder mutation. The age of the mutation in this haplotype was estimated to be between 1075 and 1900 years. Possibly, the mutation was spread throughout these countries along the third Hadramaout population movement in the seventh century (Fig. 1). Hadramaout, a historical region of the South Arabian Peninsula, was the focal point for the origins and development of the Islamic faith in the seventh century (Hussain, 2005). The two families originating from Turkey and Lebanon segregate a second haplotype of 920 kb defined by markers rs7030498 and rs348471. The mutation may have spread in the Ottoman Empire that lasted from 1299 to 1922 when many countries including Lebanon and Turkey were part of this empire.

In conclusion, our study shows that the p.R34X mutation in *TMC1* in North African and Asian individuals arose from at least two different founders. Screening for this mutation should be included in the evaluation of North African and Asiatic persons segregating ARNSHL. Its detection would facilitate genetic counseling in these populations.

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## Disclosure Statement

No competing financial interests exist.

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