Origins and frequencies of SLC26A4 (PDS) mutations in east and south Asians: global implications for the epidemiology of deafness


Rearrival mutations of SLC26A4 (PDS) are a common cause of Pendred syndrome and non-syndromic deafness in western populations. Although south and east Asia contain nearly one half of the global population, the origins and frequencies of SLC26A4 mutations in these regions are unknown. We PCR amplified and sequenced seven exons of SLC26A4 to detect selected mutations in 274 deaf probands from Korea, China, and Mongolia. A total of nine different mutations of SLC26A4 were detected among 15 (5.5%) of the 274 probands. Five mutations were novel and the other four had seldom, if ever, been identified outside east Asia. To identify mutations in south Asians, 212 Pakistani and 106 Indian families with three or more affected offspring of consanguineous matings were analyzed for cosegregation of recessive deafness with short tandem repeat markers linked to SLC26A4. All 21 SLC26A4 exons were PCR amplified and sequenced in families segregating SLC26A4 linked deafness. Eleven mutant alleles of SLC26A4 were identified among 17 (5.4%) of the 318 families, and all 11 alleles were novel. SLC26A4 linked haplotypes on chromosomes with recurrent mutations were consistent with founder effects. Our observation of a diverse allelic series unique to each ethnic group indicates that mutational events at SLC26A4 are common and account for approximately 5% of recessive deafness in south Asians and other populations.

The east Asian study subjects included one large Korean family (K-87) segregating severe to profound prelingual deafness. Fraser15 used this test to estimate that Pendred syndrome accounts for 5.6–7.8% of hereditary deafness, but it is now clear that molecular genetic techniques can provide a more accurate estimate of the prevalence of SLC26A4 deafness.

Most published studies of SLC26A4 mutations have dealt with western populations.1–10 There are only a few reported cases from Asia11–21 with no estimates of mutation or phenotype frequencies in deaf Asian populations. The epidemiology of SLC26A4 deafness may vary among Asian and western populations, as has already been reported for recessive GJB2 (Cx26) mutations at the DFNB1 locus.22, 23 Up to 50% of NSRD is associated with GJB2 mutations in some western populations,22 whereas GJB2 mutations only account for 5% of deafness in Korea24 and 20–30% in Japan.25–27 Since Asia contains approximately one half of the global population,27 the origins and frequencies of SLC26A4 mutations among its populations have important implications for a global understanding of the genetic epidemiology of deafness.

MATERIALS AND METHODS

Subjects

Approval for this study was obtained from institutional review boards (IRBs) at the National Institutes of Health (NINDS/NIDCD joint IRB), Medical College of Virginia (Western IRB), Ajou University (Suwon, Korea), Shinsu University School of Medicine (Matsumoto, Japan), Hiroasaki University School of Medicine (Japan), All-India Institute of Medical Sciences (Delhi, India), and the Centre of Excellence in Molecular Biology (Lahore, Pakistan). Informed consent was obtained for all participants.

The east Asian study subjects included one large Korean family (K-87) segregating severe to profound prelingual deafness, and 92 Korean, 86 Chinese, and 195 Mongolian
SLC26A4 deafness in Asians

In Korean family K-87 (fig 1A), there are deaf offspring from two different matings between unrelated deaf subjects, suggesting that their hearing loss is caused by mutations of a gene in which mutant alleles are a common cause of recessive deafness in Korea. Computed tomography showed EVA in II.6 and II.7 (fig 1B), and ultrasonography showed a goitre in II.7. STR haplotype (D7S546, D7S2549, and D7S2456; fig 2) and SLC26A4 exons were PCR amplified and sequenced as described previously.1 Novel intronic primers were designed to flank: exon 2, 5'-CTCCGATGCTCTTGCTTCA-3' and 5'-CTCCGATGTCTCTACGCA-3'; exon 4, 5'-GGAGGCTACTAGTGTTTTCA-3' and 5'-GGGACCAGCAGCGAAGATGA-3'; exon 16, 5'-AGCTTTAGGGTGGCCAGGGTTTCA-3' and 5'-AGCTTTAGGGTGGCCAGGGTTTCA-3'; and exon 20, 5'-TTACCTTTCAATGTGCAAAA-3' and 5'-TTACCTTTCAATGTGCAAAA-3'. The genomic deletion mutation IVS2-IVS3del4kb was detected by PCR amplification and sequencing with primers 5'-CTCTGACCCAGGAGAGTTCC-3' and 5'-CTCTGACCCAGGAGAGTTCC-3'.

Statistical analysis

Differences in STR genotype and haplotype distributions between mutant and wild type chromosomes from ethnically matched, normal hearing control DNA samples were analysed by Fisher's exact test. Some genotype distributions were compared among subjects, not chromosomes. The 19 remaining SLC26A4 exons were analysed in the eight deaf mutation carriers since their deafness was possibly associated with a second SLC26A4 mutation in trans configuration. Mutations were detected in exons 6, 8, 10, 14, and 15 or their adjacent splice sites (table 1). These exons were sequenced in additional probands from each cohort, including a final total of 96 Korean probands. Four novel mutations (1548insC, IVS14-7A>G, S252P, and N392Y) and two previously reported mutations2 3 21 (IVS7-2A>G, T410M) were identified. None of these mutations was detected in 44 to 120 ethnically matched, normal hearing control samples. The pathogenic potential of IVS14-7A>G is unknown since its effect on splicing has not been experimentally determined. All of the missense mutations detected in the probands and south Asian families are non-conservative substitutions of amino acid residues that are conserved in the rat and mouse orthologues of SLC26A4. We did not detect any of the four mutations (L236P, IVS8+1 G>A, E384G, and T410P) commonly reported among western patients.2 3 Six (6.5%) of 92 Korean, five (5.8%) of 86 Chinese, and four (2.1%) of 195 Mongolian probands had at least one detected SLC26A4 mutation (table 1).

SLC26A4 mutations in south Asians

In south Asia, we ascertained 212 families from Pakistan and 106 families from India with three or more deaf offspring from consanguineous matings or, in one case, six affected offspring of a non-consanguineous Indian mating (family DKH-5). All 21 SLC26A4 exons were sequenced in affected probands from 15 families cosegregating deafness with homozygosity for STR markers linked to SLC26A4 (table 2). Homozygous SLC26A4 mutations were identified in all of these probands, and nucleotide sequence analysis of the mutated exons in remaining family members confirmed cosegregation of the mutations with deafness. The affected subjects of a sixteenth family, DKH-5, cosegregated deafness with compound heterozygosity for the nonsense mutation S57X and IVS2, IVS3del4kb, a 4 kb genomic deletion encompassing exon 3. The existence of this novel deletion was initially manifested by hemizygosity for the trans mutant allele S57X in the exon 3 PCR product from affected members of family DKH-5 (not shown).

A total of 10 different mutations, all of which are novel, were detected among the south Asian families (table 2). J455F was detected in 2/90 Pakistani normal hearing control samples, but otherwise none of the other nine mutations were identified in any of 53 or more ethnically matched control samples. The previously reported Indian ISL-1 family segregating the [G497S; 1490L] mutant allele was ascertained through this same study. If ISL-1 is included, seven (6.6%) of 106 Indian families segregated SLC26A4 mutations (95% CI

METHODS

Peripheral venous blood samples were obtained for preparation of genomic DNA. DNA preparations and genotype analyses were performed as described previously.20 Genotypes of STR markers linked to known NSRD (DFNB) loci were determined for affected members of Indian and Pakistani families, and all members of Korean family K-87. SLC26A4 exons were PCR amplified and sequenced as described previously.1 Novel intronic primers were designed to flank: exon 2, 5'-CTCCGATGCTCTTGCTTCA-3' and 5'-CTCCGATGTCTCTACGCA-3'; exon 4, 5'-GGAGGCTACTAGTGTTTTCA-3' and 5'-GGGACCAGCAGCGAAGATGA-3'; exon 16, 5'-AGCTTTAGGGTGGCCAGGGTTTCA-3' and 5'-AGCTTTAGGGTGGCCAGGGTTTCA-3'; and exon 20, 5'-TTACCTTTCAATGTGCAAAA-3' and 5'-TTACCTTTCAATGTGCAAAA-3'. The genomic deletion mutation IVS2-IVS3del4kb was detected by PCR amplification and sequencing with primers 5'-CTCTGACCCAGGAGAGTTCC-3' and 5'-CTCTGACCCAGGAGAGTTCC-3'.

Statistical analysis

Differences in STR genotype and haplotype distributions between mutant and wild type chromosomes from ethnically matched, normal hearing control DNA samples were analysed by Fisher's exact test. Some genotype distributions were compared among subjects, not chromosomes.

RESULTS

A common locus for recessive deafness in Koreans

In Korean family K-87 (fig 1A), there are deaf offspring from two different matings between unrelated deaf subjects, suggesting that their hearing loss is caused by mutations of a gene in which mutant alleles are a common cause of recessive deafness in Korea. Computed tomography showed EVA in II.6 and II.7 (fig 1B), and ultrasonography showed a goitre in II.7. STR haplotype (D7S546, D7S2549, and D7S2456; fig 2) and SLC26A4 exons were PCR amplified and sequenced as described previously.1 Novel intronic primers were designed to flank: exon 2, 5'-CTCCGATGCTCTTGCTTCA-3' and 5'-CTCCGATGTCTCTACGCA-3'; exon 4, 5'-GGAGGCTACTAGTGTTTTCA-3' and 5'-GGGACCAGCAGCGAAGATGA-3'; exon 16, 5'-AGCTTTAGGGTGGCCAGGGTTTCA-3' and 5'-AGCTTTAGGGTGGCCAGGGTTTCA-3'; and exon 20, 5'-TTACCTTTCAATGTGCAAAA-3' and 5'-TTACCTTTCAATGTGCAAAA-3'. The genomic deletion mutation IVS2-IVS3del4kb was detected by PCR amplification and sequencing with primers 5'-CTCTGACCCAGGAGAGTTCC-3' and 5'-CTCTGACCCAGGAGAGTTCC-3'.
2.9 to 13.6%). In combination with 10 (4.7%) of 212 Pakistani families (95% CI 2.4 to 8.8%), \(\text{SLC26A4}\) mutations were detected in a total of 17 (5.4%) of 318 families from south Asia (95% CI 3.3 to 8.6%).

Origins of recurrent \(\text{SLC26A4}\) mutations

The detection of IVS7-2A>G, L676Q, H723R, and S90L in multiple probands from different Asian populations suggested that they may have arisen on ancestral founder chromosomes.
H723R is the most commonly reported SLC26A4 mutation in Japanese subjects,19, 20 so we analysed H723R linked haplotypes of three STR markers in 26 unrelated probands from Korea and Japan. We observed an association of H723R with a single haplotype by Fisher’s exact analysis in Korean (p=0.00000002) and Japanese chromosomes (p=0.009) (table 3), suggesting its derivation from a common founder. Meiotic phase and chromosome 7q31 haplotypes could not be assigned for some chromosomes with L676Q or IVS7-2A>G owing to a lack of parental DNA samples. Nevertheless, L676Q was significantly associated with the 119 bp allele of the centromeric flanking marker D7S496 (p=0.0006, online supplementary table 1) and the 139 bp allele of the intragenic marker D7S2459 (p=0.00005, online supplementary table 1). The 240 bp allele of the telomeric flanking marker D7S2456 was present in all six subjects with L676Q (online supplementary table 1), but its detection in 54-65% of controls precluded statistical significance (p=0.057 for Mongolian subjects).

IVS7-2A>G was weakly associated with the 145 bp allele of D7S2459 on Korean chromosomes (p=0.046, online supplementary table 2), but similar comparisons of Chinese or Japanese chromosomes or subjects with IVS7-2A>G did not reach statistical significance (not shown). S90L was associated with a single two marker haplotype comprising the 145 bp allele of D7S2459 and the 240 bp allele of D7S2456 in all five unrelated Pakistani families in which it was detected (p=0.0001, table 2).

**DISCUSSION**

There are over 50 published mutant alleles of SLC26A4,18 most of which originated in western countries. Our study shows that overall frequencies and diversities of SLC26A4 mutations are similar in western and various Asian populations, although the mutations are different. This conclusion is consistent with recent reports of diverse, novel mutant SLC26A4 alleles in small numbers of Japanese,19 20 Mexican,31 Spanish, and Italian patients.32 We observed several examples of more closely related Asian ethnic groups sharing common SLC26A4 mutations, which may have been derived from shared ancestral founders. Mutations arising before the divergence of related ethnic groups could have been vertically transmitted to each of the groups or, alternatively, mutant alleles may have been shared through more recent genetic admixture.

H723R and IVS7-2A>G are prevalent alleles accounting for a majority of observed SLC26A4 mutations in our Korean study.
population. H723R is also prevalent among the Japanese, in whom it has been identified in 12 of 19 patients (17 of 38 chromosomes) with hearing loss and EVA.

In contrast, the carrier frequency was reported to be 1/96 in normal hearing Japanese control subjects, which is consistent with our results in Koreans and confirms the specific association of H723R with hearing impairment. IVS7-2A>G is another recurrent SLC26A4 mutation in multiple east Asian study populations. We detected IVS7-2A>G in multiple probands from the Korean and Chinese cohorts (table 1), as well as Japanese patients with hearing loss and EVA (SU, unpublished observations). The lack of a common STR haplotype linked to IVS7-2A>G on different chromosomes may reflect that this is a hot spot for recurrent mutational events, although this allele has not been observed in western populations. Alternatively, IVS7-2A>G may be an older founder mutation which has undergone ancestral recombination events with the flanking STR markers D7S496, D7S2459, and D7S2456 (fig 2). Analysis of single nucleotide polymorphisms more proximal to IVS7-2A>G might be required to identify a smaller region of linkage disequilibrium.

Our results indicate that SLC26A4 mutations account for approximately 5% of all prelingual deafness in east Asia (table 1) and 5% of recessive deafness in south Asia. These approximations include heterozygotes that may not have a trans SLC26A4 mutation, which would have led to significant

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**Table 2** SLC26A4 mutations and chromosome 7q31 haplotypes in south Asian families

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Exon</th>
<th>Family</th>
<th>Country</th>
<th>Linked STR haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>S28R (84C&gt;A)</td>
<td>2</td>
<td>DKMy10</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>S90L [269C&gt;T]</td>
<td>3</td>
<td>PKDF026</td>
<td>Pakistan</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PKDF074</td>
<td>Pakistan</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PFDF106</td>
<td>Pakistan</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PKSRE</td>
<td>Pakistan</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PKSN23</td>
<td>Pakistan</td>
<td></td>
</tr>
<tr>
<td>S57X (170C&gt;A)</td>
<td>3</td>
<td>DKhS.5</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>IVS2_IVS3del4kb*</td>
<td>3</td>
<td>DKhS.5</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>V239D [716T&gt;A]</td>
<td>6</td>
<td>IS6</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PKDF032</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DKh10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVS8+4A&gt;G</td>
<td>8</td>
<td>(splice donor)</td>
<td>PKSR21</td>
<td>Pakistan</td>
</tr>
<tr>
<td>R409P (1226G&gt;C)</td>
<td>10</td>
<td>DKhM.8</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>I455F [1363A&gt;T]</td>
<td>12</td>
<td>PKSRE</td>
<td>Pakistan</td>
<td></td>
</tr>
<tr>
<td>N457K [1371C&gt;A]</td>
<td>12</td>
<td>DKhH.9</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>1863delT†</td>
<td>17</td>
<td>PKSRD</td>
<td>Pakistan</td>
<td></td>
</tr>
</tbody>
</table>

STR genotypes are given as allele sizes (bp). Underlines indicate the haplotype significantly associated with S90L (p=0.0001).

*4017 bp genomic deletion of IVS2-713 to IVS3+3164, with 3 bp insertion of CAT.
†Predicted to result in a frameshift and premature translation termination.

**Table 3** Chromosome 7q31 STR haplotypes linked to H723R

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>STR marker genotype</th>
<th>Korean chromosomes</th>
<th>Japanese chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D7S497</td>
<td>D7S2459</td>
<td>D7S2456</td>
</tr>
<tr>
<td>1</td>
<td>119 147 240</td>
<td>6* 0 10† 0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>119 145 240</td>
<td>0 8 0 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>131 143 240</td>
<td>0 7 0 0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>135 143 240</td>
<td>0 6 0 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>135 143 143</td>
<td>0 4 0 0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>119 143 143</td>
<td>0 4 0 0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>121 145 240</td>
<td>0 3 0 0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>121 147 240</td>
<td>0 2 0 0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>119 147 242</td>
<td>0 0 3 0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>119 147 238</td>
<td>0 0 2 0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>131 147 238</td>
<td>0 0 2 0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>137 145 240</td>
<td>0 0 0 3</td>
<td></td>
</tr>
<tr>
<td>13–35‡</td>
<td>– – –</td>
<td>0 16 2 6</td>
<td></td>
</tr>
</tbody>
</table>

STR genotypes are given as allele sizes (bp). Numbers of mutant (H723R) and wild type chromosomes with each STR haplotype are shown.

*Haplotype 1 is significantly associated with Korean H723R chromosomes (p=0.00000002).
†Haplotype 1 is significantly associated with Japanese H723R chromosomes (p=0.009).
‡Haplotypes each observed on one chromosome.
mutations are much less common in Koreans, Eastern Arabs, and African-Americans. The specific genomic deletion mutations, such as IVS2-IVS3del4kb, in populations where they are identified. Mutation analyses should include strategies to detect non-diagnostic mutations at the DFNB1 locus, which is the most common cause of hereditary deafness in many western populations. DFNB1 deafness in those populations is most frequently associated with a single ancient founder allele of GJB2, whose high prevalence has been postulated to have arisen through assortative mating among the deaf. GJB2 mutations are much less common in Koreans, Middle Eastern Arabs, and African-Americans. SLC26A4 mutation frequencies may be more constant among different populations since they arise from multiple, newer mutational events. The expense and inaccessibility of temporal bone radiology and perchlorate discharge testing are prohibitive for the clinical diagnosis of SLC26A4 deafness in many populations. In those cases, SLC26A4 mutation analysis may be the only available diagnostic test since blood or other tissues can be collected locally and sent elsewhere for testing. Our results indicate that rigorous molecular diagnosis will require an analysis of all SLC26A4 exons unless the patient’s ethnicity and the SLC26A4 mutation spectrum for that population are well defined. Mutation analyses should include strategies to detect specific genomic deletion mutations, such as IVS2-IVS3del4kb, in populations where they are identified. Future studies are therefore needed to define further the identities and distributions of SLC26A4 mutations in Asian and other global populations.

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Supplementary tables 1 and 2 can be found on our website at www.jmedgenet.com/supplemental

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