ORIGINAL ARTICLE

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

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J Med Genet 2003;**40**:242–248

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Revised version received 14 January 2003
Accepted for publication 17 January 2003

Recessive 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 analysed 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.

Approximately one half of childhood hearing loss is thought to have a genetic aetiology, the majority of which is non-syndromic and not associated with abnormalities of other organ systems. Estimates from clinical and epidemiological studies suggest that 80-85% of hereditary, non-syndromic, prelingual deafness is autosomal recessive, 15% is autosomal dominant, and a few percent is inherited as an X linked trait or via matrilineal transmission.[1] There are at least 30 distinct genetic loci (known as DFNB loci) at which mutations can cause non-syndromic recessive deafness (NSRD).[2] In the absence of syndromic features to guide genetic diagnosis, efficient molecular diagnosis requires a detailed knowledge of the distribution of mutant alleles at specific loci for individual populations.

Recessive mutations of the *SLC26A4* (*PDS*) gene on chromosome 7q can cause sensorineural deafness with goitre[3] (Pendred syndrome, OMIM 274600) or NSRD without goitre[4] (at the DFNB4 locus, OMIM 600791). These mutations disrupt in vitro transmembrane anion/base exchange activity of the SLC26A4 polypeptide, pendrin.[5] Pendrin is expressed in non-sensory epithelia of the inner ear,[6] thyroid folliculocytes,[7] renal cortical collecting ducts,[8 9] placental trophoblasts,[10] and uterine endometrium.[11] In vivo, pendrin is likely to mediate iodide transport across the apical membrane of thyroid folliculocytes[7] and bicarbonate secretion by intercalated cells of renal cortical collecting ducts,[8 9] but its critical transport substrate(s) in the inner ear has not been defined.[12]

Both DFNB4 and Pendred syndrome phenotypes are associated with enlargement of the vestibular aqueduct (EVA) as detected by radiological imaging of the temporal bones.[13] It is unknown whether the occurrence of goitre is attributable to the underlying *SLC26A4* genotype,[5] to modifying genetic or environmental factors, or to a combination of these mechanisms. Moreover, deaf subjects may have goitre that is unrelated to Pendred syndrome,[14] although these phenocopies can often be distinguished with a perchlorate discharge test. Fraser[15] 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 diagnostic techniques can provide a more accurate estimate of the prevalence of *SLC26A4* deafness.

Most published studies of *SLC26A4* mutations have dealt with western populations.[3 16–18] There are only a few reported cases from Asia[19–21] 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,[24] whereas *GJB2* mutations only account for 5% of deafness in Korea[22] and 20-30% in Japan.[25 26] 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), Shinshu University School of Medicine (Matsumoto, Japan), Hirosaki 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

probands with sporadic or familial severe to profound prelingual deafness. The south Asian subjects comprised 106 Indian and 212 Pakistani families with three or more affected subjects segregating severe to profound, prelingual, recessive deafness. Subjects were ascertained through schools and outpatient referral clinics for the deaf in Korea,[22] Ulaanbaatar, Mongolia,[28] Sichuan province, China,[29] the Maharashtra, Karnataka, and Tamil Nadu provinces of India, and Punjab province, Pakistan. With the exception of goitre in Korean family K-87, a subset of Pakistani families, and eight Mongolian probands, subjects with syndromic features or known exposure to ototoxic agents (for example, aminoglycosides) were excluded. Korean, Mongolian, and Chinese probands with known mitochondrial or biallelic *GJB2* mutations, comprising 2-15% of the original cohorts, were also excluded from this analysis.

Japanese probands known to have either the splice site mutation IVS7-2A>G or the missense mutation H723R of *SLC26A4*[19] were included in STR genotype and haplotype analyses to detect evidence of founder effects for these alleles. Samples from sibs and parents were analysed, when possible, in order to assign meiotic phase for *SLC26A4* mutations and linked marker genotypes. Control DNA samples were obtained from normal hearing adults from Korea, Mongolia, China, India, and Pakistan. Indian and Pakistani control samples were grouped and analysed according to province of origin to match the origin of deaf subjects.

METHODS

Peripheral venous blood samples were obtained for preparation of genomic DNA. DNA preparations and genotype analyses were performed as described previously.[30] 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.[3] Novel intronic primers were designed to flank: exon 2, 5′-CTCCGATCGTCCTCGCTTA-3′ and 5′-CTCCGCTTCTCTCTACGCA-3′; exon 4, 5′-CGCTTAGGCTATC CTACCTGAAATGTGC-3′ and 5′- CACTGAAATCCCATTTCC CTGACAACA-3′; exon 6 , 5′-GGCAGGCTACTAGTGTTTTCA-3′ and 5′-GGCCCAGACTCAGAGAATGA-3′; exon 16, 5′-AGCTTT AGGTGCCAGGCATT-3′ and 5′-GACCCTCTAACTGCTCTCA TCA-3′; and exon 20, 5′-TTCACCTTTCAATGTGCAAAA-3′ and 5′-TGCATTTGGGGGAATTATGT-3′. The genomic deletion mutation IVS2_IVS3del4kb was detected by PCR amplification and sequencing with primers 5′-CTCTTGACCAGGAG GAGTCC-3′ and 5′-CCTCTTCCTCCCTGGAAAATGC-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 (D7S496, D7S2459, and D7S2456; fig 2) and *SLC26A4* mutation analyses showed the deafness cosegregates with homozygosity or compound heterozygosity for two *SLC26A4* missense substitutions, L676Q (exon 17) and H723R (exon 19), in a subset of family members that includes the subjects with EVA (fig 1A). Deafness was not linked to *SLC26A4* in II.3 and III.1, who do not have EVA and are thus deaf from another aetiology. Nucleotide sequence analysis of *GJB2* did not identify pathogenic mutations in II.3 or III.1 (not shown).

SLC26A4 mutations in East Asians

To determine the contribution of L676Q, H723R, and other *SLC26A4* mutations to recessive deafness in east Asia, exons 17 and 19 were selected for sequence analysis of DNA samples from deaf probands from Korea, China, and Mongolia (table 1). We did not analyse all *SLC26A4* exons in all of the probands owing to limitations of DNA sample volumes and the expense of nucleotide sequencing. One H723R homozygote and three H723R heterozygotes were detected among the 92 deaf Korean probands, and two heterozygous H723R carriers were identified among 120 normal hearing Korean controls, confirming that H723R is a common allele in the Korean population. Four L676Q heterozygotes were identified among 195 deaf Mongolian probands and one L676Q carrier was observed in the Mongolian normal hearing controls, indicating that L676Q is a common allele in this population.

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 Mongolian probands. Four novel mutations (1548insC, IVS14-7A>G, S252P, and N392Y) and two previously reported mutations[16 20 21 32] (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 T416P, table 1) most commonly reported among western patients.[16 17] 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 of 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). I455F 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; I490L] mutant allele was ascertained through this same study.[4] If ISL-1 is included, seven (6.6%) of 106 Indian families segregated *SLC26A4* mutations (95% CI

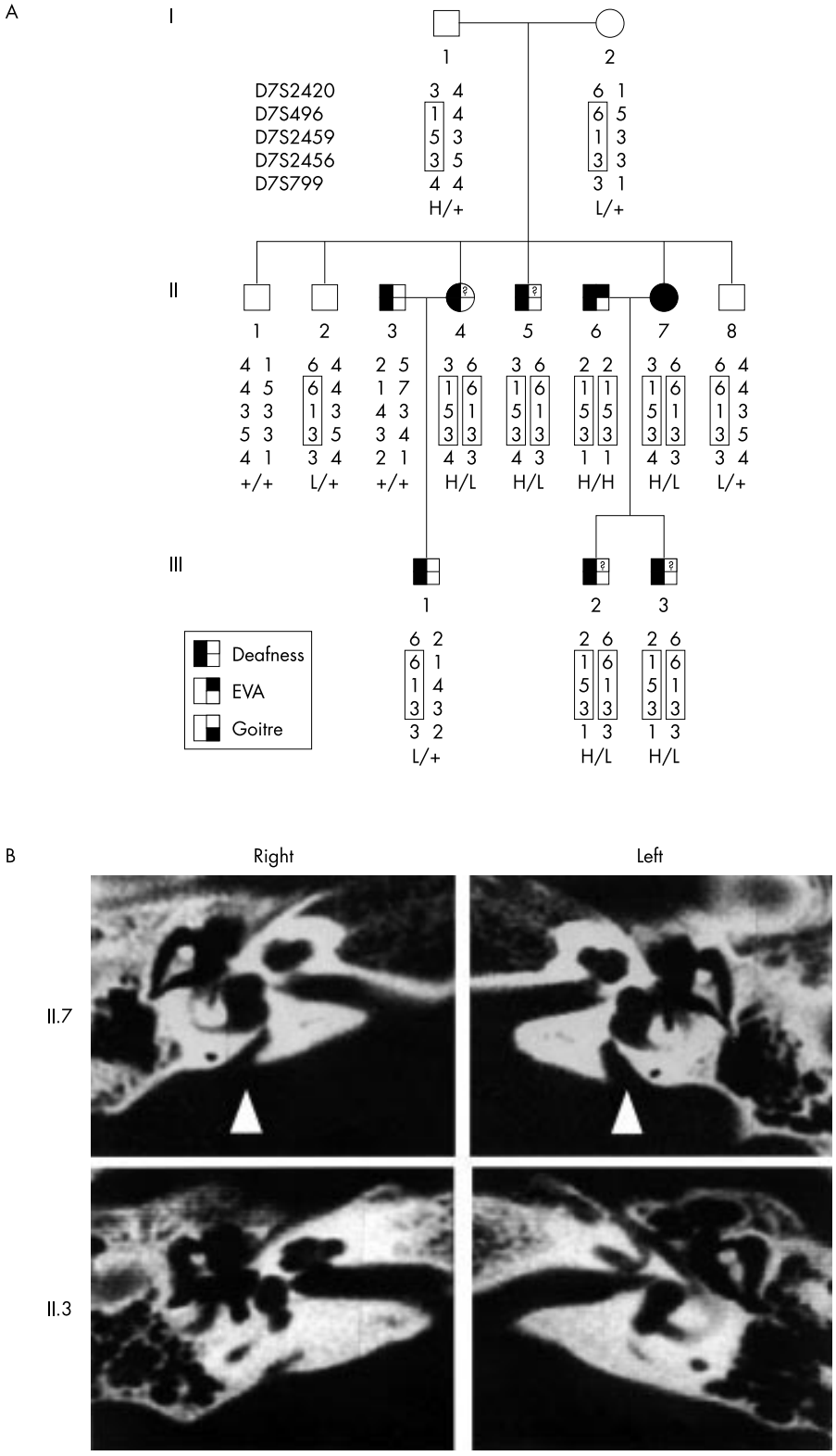

Figure 1 Korean family K-87 segregating recessive deafness. (A) Pedigree showing segregation of severe prelingual deafness, enlarged vestibular aqueducts (EVA), chromosome 7q31 STR genotypes, and *SLC26A4* mutations. STR haplotypes linked to missense mutations of *SLC26A4*, H723R (H) and L676Q (L), are boxed. In II.3 and III.1, deafness is not linked to *SLC26A4* and must have another aetiology. (B) Axial computed tomographic images of the temporal bones showing bilateral EVA (indicated by white arrowheads) in II.7 and normal appearing vestibular aqueducts in II.3.

2.9 to 13.6%). In combination with 10 (4.7%) of 212 Pakistani families (95% CI 2.4 to 8.8%), *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 *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.

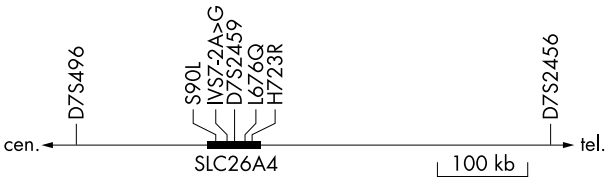

Figure 2 Physical map of *SLC26A4* mutations and linked STR markers on chromosome 7q.

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

Table 1 Numbers of east Asian probands with detected *SLC26A4* mutations

			Korean		Mongolian		Chinese		
Mutation	Exon	Genotype	Deaf (n=92)*	Control (n=120)	Deaf (n=195)	Control (n=94)	Deaf (n=86)	Control (n=44)	Reported ethnic origins
S252P	6	Homo	0	–	0†	–	0	0	
(754T>C)		Hetero	0	–	0†	–	1	0	
IVS7-2 A>G	(8)	Homo	1	0	0†	–	1	0	China[21]
		Hetero	2	0	0†	–	2	0	
N392Y	10	Homo	0	0	0†	–	0	0	
(1174A>T)		Hetero	0	0	0†	–	2	0	
T410M	10	Homo	0	0	0†	–	0	0	Japan,[20] Pakistan,[16] Italy[32]
(1229C>T)		Hetero	1	0	0†	–	0	0	
1548insC	14	Homo	0	–	0†	0	0	–	
(frameshift)		Hetero	0	–	1†	0	0	–	
IVS14-7A>G‡	(15)	Homo	0	–	0†	0	0	–	
		Hetero	0	–	1†	0	0	–	
L676Q	17	Homo	0	0	0	0	0	–	
(2027T>A)		Hetero	0§	0	4	1	0	–	
T721M	19	Homo	0	0	0	0	0	–	Japan,[19] Italy[32]
(2162C>T)		Hetero	1	0	0	0	0	–	
H723R	19	Homo	1	0	0	0	0	–	Japan,[19 20] Netherlands[17]
(2169A>G)		Hetero	3	2	0	0	0	–	
Totals		Homo	2		0		1		
		Comp hetero	3		2		1		
		Hetero	1		2		3		
		Total	6		4		5		
		Percentage (95% CI)	6.5% (2.7 to 14.2)		2.1% (0.7 to 5.5)		5.8% (2.2 to 13.7)		

The numbers of deaf or normal hearing control subjects who are homozygous (Homo), heterozygous (Hetero), or compound heterozygous (Comp hetero) for *SLC26A4* mutations in exons 6, 8, 10, 14, 15, 17, or 19 and adjacent splice sites are indicated.
*Does not include family K-87 owing to lack of a single representative *SLC26A4* genotype among affected family members.
†n=96.
‡Unknown pathogenicity.
§Detected in family K-87.

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

Mutation	Exon	Family	Country	Linked STR haplotype D7S496	D7S2459	D7S2456
S28R (84C>A)	2	DKMy10	India			
S90L (269C>T)	3	PKDF026	Pakistan	131	145	240
		PKDF074	Pakistan	133	145	240
		PFDF106	Pakistan	133	145	240
		PKSRE	Pakistan	131	145	240
		PKSN23	Pakistan	131	145	240
S57X (170C>A)	3	DKH-5	India			
IVS2_IVS3del4kb*	3	DKH-5	India			
V239D (716T>A)	6	I-56	India			
		PKDF032	Pakistan			
		DKH-10	India			
IVS8+4A>G	8 (splice donor)	PKSR21	Pakistan			
R409P (1226G>C)	10	DKMy-8	India			
I455F (1363A>T)	12	PKSR5	Pakistan			
		PKSR6	Pakistan			
N457K (1371C>A)	12	DKH-9	India			
1863delT†	17	PKSRD	Pakistan			

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

Haplotype	STR marker genotype* D7S497	D7S2459	D7S2456	Korean chromosomes H723R (n=6)	Control (n=52)	Japanese chromosomes H723R (n=20)	Control (n=9)
1	119	147	240	6*	0	10†	0
2	119	145	240	0	8	0	0
3	131	143	240	0	7	0	0
4	135	145	240	0	6	0	0
5	135	143	143	0	6	0	0
6	119	143	143	0	4	0	0
7	121	145	240	0	3	0	0
8	121	147	240	0	2	0	0
9	119	147	242	0	0	3	0
10	119	147	238	0	0	3	0
11	131	147	238	0	0	2	0
12	137	145	240	0	0	0	3
13–35‡	–	–	–	0	16	2	6

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.

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.[19 20 33] In contrast, the carrier frequency was reported to be 1/96 in normal hearing Japanese control subjects,[19] 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

overestimates in the Chinese and Mongolian populations. The presence of an undetected *trans* mutation would be supported by radiological evidence of EVA, but CT and MRI imaging data were not available for those subjects. Conversely, the estimate of *SLC26A4* deafness in east Asia may be low, since we analysed only seven of 21 *SLC26A4* exons in those panels of samples, and the K-87 family was not included in the estimate for the Korean cohort owing to the lack of a single representative genotype for the affected members of the family.

The countries sampled in this study contain nearly one half (42%) of the global population.[27] It is possible that ethnic heterogeneity in the two most populous countries, India and China,[34] led to sampling bias. Our study subjects were from south west China and south and west India, where the specific mutations may differ from those in other provinces inhabited by distinct but related ethnic groups.[34] Moreover, we did not attempt to differentiate Pendred syndrome from NSRD since the goitrous phenotype is incompletely penetrant and not usually evident until adolescence,[35] phenocopies are common,[14] and intermediate perchlorate discharge results are non-diagnostic.[33]

The epidemiology of *SLC26A4* deafness is different from that of *GJB2* mutations at the DFNB1 locus, which is the most common cause of hereditary deafness in many western populations.[24] DFNB1 deafness in those populations is most frequently associated with a single ancient founder allele of *GJB2*,[24 36 37] whose high prevalence has been postulated to have arisen through assortative mating among the deaf.[38] *GJB2* mutations are much less common in Koreans,[22] Middle Eastern Arabs,[39] and African-Americans.[40] *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.

ACKNOWLEDGEMENTS

The first two authors contributed equally to this work. H-J Park ascertained Korean subjects, performed the molecular analyses of east Asian and some south Asian subjects, statistically analysed and interpreted the overall data, and wrote the paper with AJG. S Shaukat ascertained and performed molecular analyses of some Pakistani subjects. We thank the subjects for their participation, Dr Tami Ben-Yosef for advice and technical training, and Drs Tom Friedman, Dennis Drayna, Rob Morell, and Shannon Pryor for comments and critical review of the manuscript. This study was supported by research grant HMP-00-CH-05-0005 from the Ministry of Health and Welfare, Korea (HJP), NIH grants R01 DC 05575 (XZL), R01 DC 02530 and R01 DC 04293 (WEN), and NIDCD/NIH intramural research funds (EW, AG).

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

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