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ORIGINAL INVESTIGATION

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Evidence of a founder effect for the 235delC mutation of *GJB2* (connexin 26) in east Asians

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Abstract Mutations in the *GJB2* gene encoding connexin 26 (Cx26) are a major cause of autosomal recessive and sporadic cases of congenital deafness in most populations. The 235delC mutation of *GJB2* is the most frequent known mutation in some east Asian populations, with a carrier frequency of approximately 1%. In order to study the origin of 235delC among east Asians, we analyzed single-nucleotide polymorphisms (SNPs) within the coding region of *GJB2* and flanking the 235delC mutation. We observed significant linkage disequilibrium between 235delC and five linked polymorphic markers, suggesting that 235delC arose from a common founder. The detection of 235delC only in east Asians, but not in Caucasians, and the small chromosomal interval of the shared haplotype suggest that 235delC is an ancient mutation that arose after the divergence of Mongoloids and Caucasians. Similarly, the finding that this mutation appears on a single haplotype provides no support for the possibility that recurrent mutation is the explanation for the high frequency of the allele.

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Introduction

Congenital hearing loss occurs in approximately 1 in 1,000 live births (Fortnum et al. 2001; Liu et al. 2001), over 50% of these cases being hereditary. About 70% of prelingual hereditary deafness is non-syndromic. Autosomal recessive forms (DFNB) account for 75%–80% of these cases, autosomal dominant forms (DFNA) comprise about 20%–25%, and 1%–2% are inherited as X-linked forms (DFN). To date, at least 80 human loci for non-syndromic hearing loss have been mapped, and more than 30 have been cloned (http://dnalab-www.uia.ac.be/dnalab/hhh/). In spite of this high degree of genetic heterogeneity, recessive mutations in *GJB2* are responsible for nearly half of the genetic cases of childhood hearing loss in many populations. Dominant mutations of *GJB2* are much less common but can lead to nonsyndromic hearing loss (Denoyelle et al. 1998) or deafness associated with ectodermal abnormalities sometimes accompanied by corneal epithelial defects (Maestrini et al. 1999; Kelsell et al. 2000; Richard et al. 2002).

GJB2 encodes connexin 26 (Cx26), a member of a large family of transmembrane gap-junction proteins that mediate electrical and metabolic coupling between adjacent cells. In the cochlea, Cx26 is expressed in the supporting cells of the neurosensory epithelium and in cochlear duct fibrocytes (Lautermann et al. 1998) and is presumed to be involved in potassium recycling from depolarized hair cells back to the endolymph. To date, more than 70 *GJB2* mutations have been reported to cause deafness, with a significant difference in the frequency and distribution among different populations (http://www.iro.es/cx26deaf.htlm).

One mutation, 35delG, is the most common variant in many populations, accounting for up to 70% of all *GJB2* pathologic alleles in Caucasians from northern and southern Europe and north America, with a carrier rate ranging from 1.3% to 2.8% (Green et al. 1999; Gasparini et al. 2000). The 35delG mutation is either absent or very rare in non-Caucasian populations, with other mutations prevailing, such as 167delT in Ashkenazi Jews, where the carrier frequency is 3%–4% (Morell et al. 1998; Sobe et al. 2000), and R143W, a common mutation in a village in eastern Ghana (Brobby et al. 1998). The high frequency of the 35delG *GJB2* allelic variant in the white population has been shown to be the result of a founder effect, rather than a mutational hot spot (Van Laer et al. 2001). The 167delT mutation prevalent in Ashkenazi Jews has also been attributed to a founder effect (Morell et al. 1998).

Both the 35delG and 167delT mutations are absent or exceptionally low in some Asian populations, in which another mutation, the deletion of cytosine at position 235 (235delC), is the most prevalent; this mutation accounts for up to 80% of pathogenic *GJB2* alleles among Japanese (Fuse et al. 1999; Abe et al. 2000; Kudo et al. 2000), Koreans (Park et al. 2000), and Chinese (Liu et al. 2002), with carrier rates ranging from 1.0% to 1.3%. Interestingly, 235delC has not been detected in south Asian populations from India, Pakistan, Bangladesh, and Sri Lanka, in whom the prevalent *GJB2* mutations are W24X and W77X (Kelsell et al. 1997; Scott et al. 1998; Richard et al. 2001). The high frequency of the 235delC mutation in multiple east Asian populations raises the possibility that it results from recurrent deletion at a mutation hotspot, or that it is derived from a common ancestral founder. We have previously postulated a possible founder effect in Chinese based upon a common three-marker haplotype linked to 235delC (Liu et al. 2002), and a recent study by Ohtsuka et al. (2003) has lead to the conclusion that Japanese chromosomes with 235delC arose from a single founder. In order to determine whether 235delC in different east Asian populations is derived from a single ancestral founder, we have analyzed polymorphic markers both within and flanking the coding sequence of *GJB2* on 235delC chromosomes from Mongolia, China, Korea, and Japan. Our data indicate that the high frequency of the 235delC mutation in east Asian populations is the result of a founder effect, rather than a mutational hot spot.

Subjects and methods

Subjects

In this study, we included DNA samples from 45 unrelated patients carrying the 235delC mutation, 26 of whom were homozygous and 19 heterozygous for the 235delC mutation of *GJB2*. Deaf patients known to carry at least one 235delC allele from the following east Asian populations were genotyped: nine Mongolian, 20 Chinese, 13 Japanese, and three Korean. The control group consists of samples from 105 Mongolian, 100 Chinese, 63 Japanese, and 94 unrelated Korean subjects without apparent hearing loss.

Polymorphic markers analysis

The positions of the polymorphisms with their respective nucleotide numbers (derived from the genomic sequence of clone RP11-264J4; GenBank accession no. AL138688) are shown in Fig. 1. The distance from each polymorphism to the 235delC mutation is shown in Table 1. Three common sequence changes within the *GJB2* gene coding sequence, viz., V27I (79G→A; rs2274084), E114G (341A→G; rs2274083), and I203T(608T→C), and four single-nucleotide polymorphisms (SNPs) were analyzed. SNP1 (rs3751385) is located in the 3′ UTR (untranslated region; GenBank accession no. M86849), whereas SNP2 and SNP3 (rs912404) are intronic. SNP6 (TSC0127952) is 63 kb downstream of *GJB2*. A combination of single strand conformation polymorphism (SSCP) analysis and direct sequencing was applied for genotyping with a total of eight primer pairs (Table 2). Polymerase chain reaction (PCR) was performed in a 12.5-µl reaction with 40 ng genomic DNA, 10 pmol each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$, and 1 U TaqDNA polymerase. The amplification conditions were 95°C for 5 min, then 30 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. For sequence analysis, the PCR products were initially run on a 1-mm-thick nondenaturing 8% polyacrylamide gel (acrylamide; N, N′-methylene) bisacrylamide (49:1) at 4°C. SSCPs were detected by using silver staining as previously described (Liu et al. 1997). Direct sequencing was performed by using an ABI PRISM Big Dye Terminator Cycle Sequencing Reaction Kit and an automated sequencer (ABI 3100).

Statistical analysis

Differences in the frequencies of the three genotypes between cases and controls for each polymorphic site were tested with chi-square statistics. When necessary, the two least-frequent genotypes were pooled, yielding expected frequencies greater than 5 for 80% of the comparisons, and expected frequencies greater than 1 for all comparisons. For expected values of less than 5, Yates's correction for continuity was applied. If the expected values were still too low, Fisher's exact test was used. SigmaStat 2.0 software was used

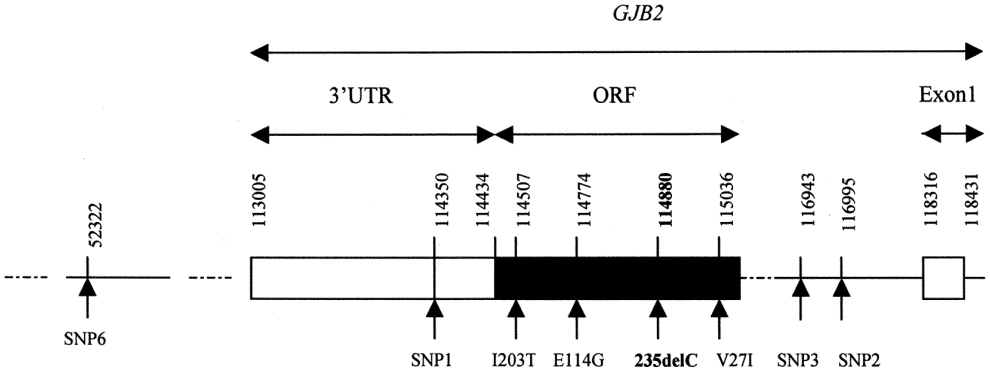

Fig. 1 Position of the polymorphic sites relative to the *GJB2* gene. The gene consists of two exons: a 5′ non-conding (exon 1) and a coding exon (exon 2). The borders of the open reading frame (*ORF*), 3′untranslated region (*3′UTR*), the positions of the polymorphic sites, and the 235delC mutation are shown with their respective nucleotide numbers derived from the genomic sequence of clone RP11-264J4 (Genebank accession no. AL138688)

Table 1 The distance (bp) from each polymorphism site to the 235delC *GJB2* mutation

Polymorphic sites	Distance to 235delC (bp)
V27I	155
E114G	106
I203T	372
SNP1	529
SNP2	2114
SNP3	2062
SNP6	62557

for calculating both chi-square and Fisher's exact tests. All *P*-values were taken to be significant at <0.05.

Results

In order to explore whether the high frequency of the 235delC mutation of *GJB2* in east Asian populations is the result of a founder effect or a mutational hot spot, we searched for evidence of a shared common haplotype of 235delC with flanking polymorphisms. We analyzed three non-synonymous polymorphisms within the coding region of *GJB2* (V27I, E114G, I203T) and four flanking SNPs (Fig. 1) in a total of 26 homozygous and 19 heterozygous 235delC patients and compared them with panels comprised of a total of 362 controls (Tables 3, 4). I203T and SNP3 were found to be insufficiently informative for our study. In each population, the 235delC mutation was in significant disequilibrium with allele A of SNP2, which was relatively uncommon among control groups. The genotypes of this highly informative SNP, located 2114 bp from nucleotide position 235 of *GJB2*, were significantly different between patients and controls, even with small sample sizes (Table 3). SNP1, V27I and E114G were less informative in our study populations. For these polymorphisms, the alleles linked to 235delC (G and A alleles for V27I and E114G, respectively, and C allele for SNP1) are also the most common alleles in the general population. However, with the larger sample size available for the Chinese population, and in an overall comparison in which the homozygous and heterozygous subjects are combined, a significant difference was obtained between patients and controls even for these less informative polymorphisms (Table 4). The genotypes of SNP6, located approximately 62,550 bp from nucleotide position 235 of *GJB2*, were only significantly different between patients and normal controls in the pooled total test groups (Table 3). The polymorphic marker allele frequencies exhibited no significant differences among Mongolian, Chinese, Japanese, and Korean control individuals. No significant departure from Hardy-Weinberg equilibrium was found, except for V27I and E114G in the Mongolian control group (data not shown).

In 235delC homozygotes, 235delC was associated with one core SNP2-V27I-E114G-SNP1 haplotype, A-G-A-C. In contrast, four major haplotypes were observed in the normal controls: G-G-A-C and A-G-A-C were the most common with frequencies of 30% and 21%, respectively. The small chromosomal interval of haplotype sharing is consistent with an ancient origin of a single founder mutation. SNP6, the most distant marker in our study, was used to estimate the number of generations that have elapsed since the putative ancestral mutational event. Based upon the distance from SNP6 to 235delC (63 kb), and assuming a linear relationship of 1 cM=1,000 kb, the calculated recombination rate is 0.00063 per generation. Assuming that T was the SNP6 genotype of the founder 235delC chromosome, and that 35/228 (=0.154; Table 3) of wild-type chromosomes currently have the C allele, the recombination frequency (between nt 235 and SNP6) is 0.00063×0.154=0.0000967 per generation. Hence, the frequency of allele T of SNP6 on wild-type chromosomes

Table 2 PCR and SNP primers

Polymorphic sites	Primer	Primers sequence	PCR product size (bp)
V27I	F	CATTCGTCTTTTCCAGAGCA	323
	R	CAC GTG CAT GGC CAC TAG	
G114E	F	CGTGTGCTACGATCACTAC	201
	R	AGCCTTCGATGCGGACCTT	
I203T	F	TATGTCATGTACGACGGCT	237
	R	TCTAACAACTGGGCAATGC	
SNP1	F (for SSCP)	TAGGGGAGCAGAGCTCCAT	265
	R (for SSCP)	TGCATCCTGCTGAATGTCAC	
	F (for sequencing)	AGGCACTGGTAACTTTGTCC	589
	R (for sequencing)	GTGTCTGGAATTTGCATCCTGC	
SNP2	F	CCTGTCCAGTTACCATCAGC	185
	R	ACTTCGTCCTGCTTCTGAGC	
SNP3	F	GCCCTCACTGAGTGGAAAAC	180
	R	TTCTCAGCTGATGGTAACTGGA	
SNP6	F	TCCTGACCTCACATGATCCA	297
	R	GAAAATACCCTGCGCTTTGA	

Table 3 Results of the polymorphisms analysis in patients with the homozygous 235delC mutation. All *P*-values were taken to be significant at <0.05 (*ND* not determined)

Polymorphisms	Genotype	Chinese			Japanese			Korean			Mongolian			Total		
		Patient	Control	*P*	Patient	Control	*P*	Patient	Control	*P*	Patient	Control	*P*	Patient	Control	*P*
V27I	AA	0	6	0.0098	0	8	0.002	0	12	0.1665	0	5	0.1137	0	31	<0.001
	AG	0	41		0	29		0	45		0	56		0	171	
	GG	11	53		10	26		3	36		2	22		26	137	
E114G	AA	11	70	0.0994	10	47	0.197	3	61	0.6171	2	62	0.3557	26	240	<0.001
	AG	0	27		0	15		0	28		0	43		0	113	
	GG	0	3		0	1		0	5		0	0		0	9	
I203T[a]	TT	11	ND		10	55	0.64	3	ND		ND	104		24	159	0.65
	TC	0			0	7		0				1		0	8	
	CC	0			0	1		0				0		0	1	
SNP1	CC	11	8	<0.001	5	7	0.04	3	28	0.039	2	16	1.0	21	59	<0.001
	CT	0	13		1	11		0	49		0	6		1	79	
	TT	0	6		0	7		0	16		0	1		0	30	
SNP2	AA	11	0	<0.001	3	1	0.0064	3	5	<0.001	2	0	0.002	19	6	<0.001
	AG	0	11		0	7		0	47		0	12		0	77	
	GG	0	9		1	16		0	42		0	18		1	85	
SNP3[a]	CC	0	0		0	0		0	0		0	0		0	0	
	CG	0	0		0	0		0	0		0	0		0	0	
	GG	11	24		6	26		3	24		2	21		22	95	
SNP6	CC	0	0	1.0	0	0	0.0782	0	0	1.00	0	0	0.4891	0	0	<0.05
	CT	0	2		0	10		0	6		2	17		2	35	
	TT	11	28		7	17		3	18		0	16		21	79	

[a]Non informative

Table 4 Results of the polymorphisms analysis in patients with the homozygous and heterozygous 235delC mutation. All *P*-values were taken to be significant at <0.05 (*ND* not determined)

Polymorphisms	Genotype	Chinese			Japanese			Korean			Mongolian			Total		
		Patient	Control	*P*	Patient	Control	*P*	Patient	Control	*P*	Patient	Control	*P*	Patient	Control	*P*
V27I	AA	0	6	0.01	0	8	<0.001	0	12	0.1665	0	5	<0.001	0	31	<0.001
	AG	2	41		0	29		0	45		0	56		2	171	
	GG	18	53		13	26		3	36		9	22		43	137	
E114G	AA	19	70	0.0128	13	47	0.124	3	61	0.6171	9	62	0.0132	44	240	<0.001
	AG	0	27		0	15		0	28		0	43		0	113	
	GG	0	3		0	1		0	5		0	0		0	0	
I203T[a]	TT	19	ND		13	55	0.455	3	ND		ND	104		35	159	0.467
	TC	0			0	7		0				1		0	8	
	CC	0			0	1		0				0		0	1	
SNP1	CC	14	8	0.0022	5	7	0.04	3	28	0.039	8	16	0.7409	30	59	<0.001
	CT	3	13		1	11		0	49		1	6		5	79	
	TT	0	6		0	7		0	16		0	1		0	30	
SNP2	AA	12	0	<0.001	4	1	0.0012	3	5	<0.001	4	0	<0.001	23	6	<0.001
	AG	4	11		0	7		0	47		4	12		8	77	
	GG	0	9		1	16		0	42		1	18		2	85	
SNP3[a]	CC	0	0		0	0		0	0		0	0		0	0	
	CG	0	0		0	0		0	0		0	0		0	0	
	GG	17	24		6	26		3	24		9	21		35	95	
SNP6	CC	0	0	0.1793	0	0	0.3875	0	0	1.00	0	0	0.0603	0	0	0.69
	CT	4	2		1	10		0	6		8	17		13	35	
	TT	14	28		7	17		3	18		1	16		25	79	

[a]Non informative

increases by 0.9999033 per generation. Since 44/46 (=0.957) of modern 235delC chromosomes carry the T allele, the number of generations since 235delC arose is *n*, where $(0.9999033)^n=0.956521$; *n* is thus calculated to be 460 generations, or approximately 11,500 years, if the mean generation time is estimated to be 25 years.

Discussion

Mutation 235delC is predominant a mutation of *GJB2* among east Asian populations (Fuse et al. 1999; Abe et al. 2000; Kudo et al. 2000; Park et al. 2000; Liu et al. 2002). It is predicted to cause a frameshift at codon 79 and to result in a truncated Cx26 polypeptide, unless the mutant allele transcript is first degraded via nonsense-mediated mRNA decay. Recently, a study by Ohtsuka et al. (2003) concluded that Japanese chromosomes with 235delC arose from a single founder. Our data indicate that the 235delC among all east Asian populations was derived from a common ancestral founder. Although we cannot formally rule out the possibility that 235delC independently arose many times on the same specific haplotype, the finding that 235delC is present only in east Asians (Fuse et al. 1999; Abe et al. 2000; Kudo et al. 2000; Park et al. 2000; Liu et al. 2002) argues against this hypothesis. Therefore, the 235delC mutation, with a widespread geographic distribution, was probably derived from a founder mutation that spread throughout north/northeast Asia. Furthermore, our data indicate that 235delC arose in an ancient northern Mongoloid progenitor, sometime after the divergence of east Asians from Caucasians, which took place about 15–35,000 years ago (Cavalli-Sforza and Feldman 2003). We have estimated the age of the 235delC mutation to be about 460 generations, or approximately 11,500 years old, based on the assumption that 1 cM is equivalent, on average, to 1000 kb throughout the human genome. This assumption is open to question (Weiss 1993). As we do not know the true recombination rate, the range of error for this calculation is probably very large.

Previous studies of the distribution of immunoglobulin G (IgG) heavy-chain allotypes (Gm markers) in eastern Asia have led to the hypothesis that "northern Mongoloids" represent a founding population in Asia. These studies show that populations of northern China, Korea, and Japan have Gm types that are compatible with a "northern Mongoloid" origin, presumably from the Baikal area of the southern Soviet Union (Matsumoto et al. 1986; Matsumoto 1988a, 1988b). Superimposing our data onto these observations, it is tempting to speculate that the 235delC mutation might also have arisen in the Baikal area and then spread to Mongolia, China, Korea, and Japan through subsequent migration. There are a number of other mutations, such as the R413P mutation of phenylalanine hydroxylase [EC 1.14.16.1] in classic phenylketonuria (MIM 261600), R95X in complement component 9 deficiency (C9D; MIM 120940), and the H723R mutation of *SLC26A4* (*PDS*) in recessive deafness, which are also unique to east Asians, and for which founder effects in Japanese, Chinese, and Korean populations have been demonstrated (Wang et al. 1991; Khajoee et al. 2003; Park et al. 2003).

The 35delG and 167delT mutations of *GJB2* are also prevalent among certain populations and are thought to have arisen from single ancestral mutations (Morell et al. 1998; Van Laer et al. 2001). The underlying genetic mechanism(s) accounting for the high prevalence of certain founder DFNB1 alleles of *GJB2* is unknown. We (Nance et al. 2000) have suggested the hypothesis that improved reproductive fitness combined with intermarriage among deaf people may have contributed to the high frequency of *GJB2* deafness in the USA and could represent a novel mechanism for maintaining specific genotypes at unexpectedly high frequencies. However, this seems less likely to be applicable to Asian populations, where there has not been a long tradition of intermarriages among the deaf. However, little data is available about changes in the fitness and use of sign language or in the incidence of linguistic homogamy among the deaf in many of these populations. Alternatively, a selective biological advantage conferred by *GJB2* mutation carrier status could also explain the observed 235delC prevalence among Asian populations. Meyer et al. 2002 have postulated that R143W of *GJB*2, which is common in Africa, might counterbalance an evolutionary disadvantage of deafness, because the epidermis was found to be significantly thicker in individuals heterozygous or homozygous for R143W than in wild-type family members. In addition, sodium and chloride concentrations in sweat were higher among homozygotes. Functionally, these changes could confer resistance against pathogens, trauma, or insect bites. Nevertheless, the reason that different mutations might have a selective advantage in different populations remains unknown. If it holds true that changes in the fitness and mating structure of the deaf population has contributed to an increase in the frequency of connexin deafness, this mechanism would be expected to amplify the frequency of whichever allele is the most frequent in each population. Future studies may distinguish between these and other mechanisms underlying the increased prevalence of some *GJB2* founder mutations, such as 235delC.

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