A common founder for the 35delG *GJB2* gene mutation in connexin 26 hearing impairment

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Abstract

Fifty to eighty percent of autosomal recessive congenital severe to profound hearing impairment result from mutations in a single gene, *GJB2*, that encodes the protein connexin 26. One mutation of this gene, the 35delG allele, is particularly common in white populations. We report evidence that the high frequency of this allelic variant is the result of a founder effect rather than a mutational hot spot in *GJB2*, which was the prevailing hypothesis. Patients homozygous for the 35delG mutation and normal hearing controls originating from Belgium, the UK, and the USA were genotyped for different single nucleotide polymorphisms (SNPs). Four SNPs mapped in the immediate vicinity of *GJB2*, while two were positioned up to 76 kb from it. Significant differences between the genotypes of patients and controls for the five SNPs closest to *GJB2* were found, with nearly complete association of one SNP allele with the 35delG mutation. For the most remote SNP, we could not detect any association. We conclude that the 35delG mutation is derived from a common, albeit ancient founder.

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Congenital severe to profound hearing impairment affects 1 in 1000 neonates.[1] In half of these children, the hearing impairment is the result of genetic factors,[2] with autosomal recessive inheritance representing approximately 80% of this total.[1] Although 30 recessive genes have been localised (G Van Camp, R J H Smith. Hereditary Hearing Loss Homepage, http://dnalab-www.uia.ac.be/dnalab/hhh, March 2001), a large proportion of recessive severe to profound hearing impairment has been linked to the DFNB1 locus.[3] In 1997, Kelsell *et al*[4] showed that mutations in *GJB2* lead to hearing impairment at this locus. Mutations in the same gene also are responsible for autosomal dominant hearing impairment at the DFNA3 locus.[5]

GJB2 encodes connexin 26, a gap junction protein that is assumed to be a component of the potassium recycling pathway in the inner ear. Loss or malfunction of these gap junctions, as might be reflected by mutations in *GJB2*, may disrupt potassium movement from the hair cells through the supporting cell network to the endolymph, leading to hearing impairment. The resultant hearing loss involves all frequencies, is of variable severity (from mild to profound) even within sibships, rarely progresses, and most frequently is symmetrical in the two ears.[6–8]

To date, 48 recessive and seven dominant disease causing *GJB2* mutations have been identified (R Rabionet, P Gasparini, X Estivill. Connexins and Deafness Homepage, http//www.iro.es/cx26deaf.html, March 2001). One mutation, the deletion of one guanosine residue from a stretch of six between nucleotide positions 30 and 35 (35delG), which results in a frameshift and premature protein truncation at codon 13, is the most common deafness causing allelic variant of *GJB2* in sporadic patients and autosomal recessive families from Italy, Spain, Portugal, France, UK, Israel, Lebanon, Tunisia, Algeria, New Zealand, and in white American families of northern and southern European origin.[9–13]

Carrier frequencies of the 35delG mutation have been determined in several countries (table 1), and in some populations this mutation is more frequent than the ΔF508 cystic fibrosis mutation. However, in non-white populations, the 35delG mutation is either not found or is very rare, with other "common" mutations prevailing, like the 235delC in the Japanese and Koreans,[14–16] the 167delT in the Ashkenazi Jewish,[17 18] and the R143W mutation in an African village.[19] Most investigators have assumed that the high frequency of the 35delG mutation reflects the presence of a mutational hot spot within *GJB2*,[9 11 17 20 21] but others have suggested a founder effect.[13] To resolve the issue, we performed genotyping in the immediate vicinity of the *GJB2* gene. From these data, we present evidence that the high frequency of the 35delG *GJB2* allelic variant in the white population is the result of a founder effect rather than a mutational hot spot.

Subjects and methods

SUBJECTS

In this study, the following subjects were genotyped: 35 Belgian, 30 British, and 49 American white patients with non-syndromic hearing impairment who were homozygous for the 35delG mutation, and 70 Belgian, 30 British, and 50 American white random normal hearing controls. The American control samples were screened and selected for the absence of the 35delG mutation. In the Belgian and British control samples, no 35delG genotyping was performed.

Table 1 Carrier frequency of the 35delG mutation in various populations

Population	Subjects tested	Carriers 35delG	Carrier frequency (%)	Reference
Europe				
Norway	190	1	0.5	22
Denmark	95	2	2.1	22
Estonia	113	5	4.4	22
Germany	200	4	2.0	22
UK	119	0	0.0	22
Holland	89	2	2.2	22
Belgium	190	1	0.5	22
Belgium	360	9	2.5	23
France (Brittany)	96	1	1.0	22
France	200	1	0.5	22
France	68	0	0.0	9
France	512	14	2.7	24
Bulgaria	157	1	0.6	22
Czech Republic	195	4	2.1	22
Slovenia	182	1	0.5	22
Italy (Sardinia)	116	4	3.4	22
Italy	255	8	3.1	22
Italy	150	6	4.0	12
Spain	200	5	2.5	22
Spain	130	3	2.3	22
Portugal	179	4	2.2	22
Malta	144	4	2.8	22
Greece	400	12	3.0	22
Greece	395	14	3.5	25
Turkey	150	4	2.7	22
North America				
White Americans	173	1	0.6	17
White Americans	100	1	1.0	26
White Americans	96	2	2.0	11
White Americans	560	14	2.5	27
African Americans	173	0	0.0	17
African Americans	190	0	0.0	22
Asian Americans (Indian, Japanese, Koreans)	53	0	0.0	17
Ashkenazi Jewish Americans	551	4	0.7	17
Other populations				
Egyptians	95	0	0.0	22
Arabs	58	1	1.7	22
Koreans	100	1	1	15

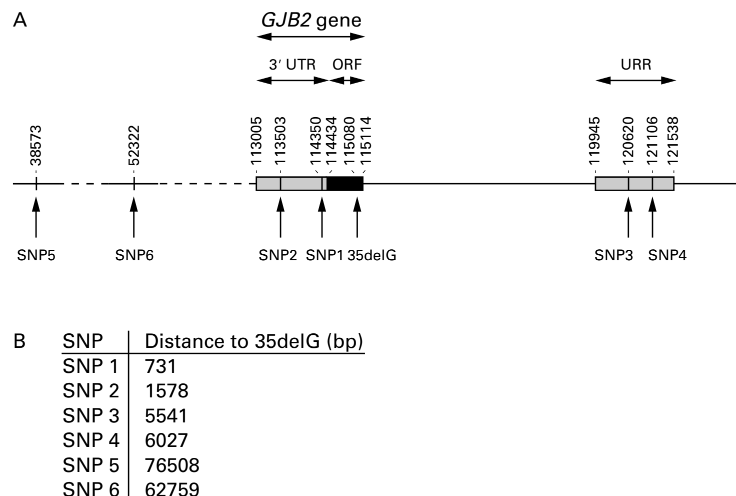

Figure 1 (A) Positions of the SNPs relative to the GJB2 gene. The GJB2 open reading frame (ORF) is represented by the black box. The 3'UTR and the URR are represented by shaded boxes. The borders of the ORF, UTR, and URR, the positions of the six SNPs, and the 35delG mutation are indicated with their respective nucleotide numbers derived from the genomic sequence of clone RP11-264J4 (Genbank accession number AL138688). (B) The distance from each SNP to the 35delG mutation in the GJB2 gene (given in bp).

SNP IDENTIFICATION

To identify SNPs (single nucleotide polymorphisms) in regions adjacent to the *GJB2* gene, the 3'UTR (untranslated region, Genbank accession number M86849), and the URR (upstream regulatory region, Genbank accession number AF091526) were sequenced in six 35delG carriers. Four SNPs were discovered, two in the 3'UTR (SNP 1 and 2) and two in the URR (SNP 3 and 4). The relative positions of the SNPs with regard to the *GJB2* gene are depicted in fig 1, where the SNPs are placed on the complete nucleotide sequence of clone RP11-264J4 (Genbank accession number AL138688). Several SNPs more distant from the *GJB2* gene were chosen from the SNP Consortium LTD (http://snp.cshl.org/) and analysed in random subjects. Two informative SNPs, SNP 5 (TSC1102325) and SNP 6 (TSC0127952), were selected for further analysis and positioned on the nucleotide sequence of clone RP11-264J4 (fig 1).

SNP ANALYSIS

SNP analysis was performed using the ABI Prism SNaPshot ddNTP Primer Extension Kit (ABI) according to the manufacturer's guidelines with minor modifications. PCR was performed using standard conditions and the primers and annealing temperatures listed in table 2. PCR products were treated with 0.164 U/µl shrimp alkaline phosphatase (Promega) and 0.164 U/µl exonuclease I (New England Biolabs) for one hour at 37°C, followed by the deactivation of the enzymes for 15 minutes at 72°C. SNaPshot thermal cycling was performed with the respective SNaPshot primer (table 2), the matching PCR product, and the SNaPshot Ready Reaction Premix for 25 cycles of 10 seconds at 96°C denaturation, five seconds at 50°C annealing, and 30 seconds at 60°C extension. SNaPshot products were dephosphorylated by incubation at 37°C (one hour) with 0.15 U/µl calf intestine alkaline phosphatase (Gibco BRL Life Technologies). The enzyme was deactivated as described above and SNaPshot products were analysed on an ABI3100 automated DNA sequencer (ABI).

STATISTICAL ANALYSIS

Differences between groups were tested with χ^2 statistics, if at least 80% of the expected frequencies exceeded 5 and all the expected frequencies exceeded 1. If the expected values did not meet the criteria mentioned above, the numbers of the two least frequent alleles were added and Yates's correction for continuity for two by two tables was used. If the expected values of the two by two table still did not meet the criteria mentioned above, Fisher's exact test was used. Both the χ^2 test and Fisher's exact test were calculated using the SigmaStat 2.0 software (Jandel Corporation). All p values were taken to be significant at <0.05.

Results

In order to determine whether the high frequency of the 35delG allele variant of *GJB2* is the result of a mutational hot spot or a founder effect, SNPs adjacent to (SNPs 1 to 4, fig 1) and more distant from (SNP 5 and 6, fig 1) *GJB2* were analysed. Table 3 summarises the genotypes observed in this study. SNP 1 is very informative and results, even with small sample numbers, in significantly different genotypes for controls and patients. SNPs 2 and 3 are less informative, as the allele that is linked to 35delG (G for SNP 2 as well as for SNP 3) is also the most frequent allele in the

Table 2 PCR and SNaPshot primers

SNP	Primer	Primer sequence	Annealing temperature (°C)	Length of PCR product (bp)
SNP 1	F	aggcactggtaactttgtcc	59	589
	R	gtgtctggaatttgcatcctgc		
	SNaPshot	agctgtcaaggctcagtcgc		
SNP 2	F	caatgctattcttgacaacagg	59	901
	R	ccacgttaaaggtgaacattgg		
	SNaPshot	acctctttaaatcagcgttctttcc		
SNP 3	F	ctgtgtgatgttggctagtcg	56	676
	R	gacaccacagtgagttttcc		
	SNaPshot	ctgccctagaggagtcctgagcctacttgg		
SNP 4	F	gcttaggatagtactgctgc	56	502
	R	cacggtgccattccatcagg		
	SNaPshot	ctcattgacgtgcagagactgaccagatagagaag		
SNP 5	F	ctgagtaccaatctcaccac	56	230
	R	tgcacagggataataaccgc		
	SNaPshot	gggccccttaaacatgaggc		
SNP 6	F	taacaaggcctccaagtgac	55	381
	R	gcttgccatcaagaaaggag		
	SNaPshot	tcagcgtcatctctttggactgca		

general population. However, when using larger sample numbers even these less informative SNPs show significant differences between controls and patients. SNP 4 is informative, giving significant differences even when using smaller sample numbers. SNP 6, which is approximately 63 kb away from the *GJB2* gene, only shows significant differences between patients and controls in the total test population (fig 1, table 1). For SNP 5, which is the most remote SNP we investigated (approximately 76 kb away from *GJB2*, fig 1), no significant association with the 35delG mutation could be found. We conclude that the high frequency of the 35delG mutation in *GJB2* is the result of a founder effect.

The 35delG mutation must have arisen many centuries ago as haplotype sharing is observed only in a small region. On the basis of the recombination frequency of a more distant SNP, we can estimate the age of the 35delG mutation. From one generation to the next, the probability of having a recombination between a gene and a nearby marker is equal to the recombination fraction θ. In one generation, a fraction θ of all sibs will have a recombination and a fraction $1-\theta$ will not. After N generations, the fraction of descendants that has no recombination equals $(1-\theta)^N$. When examining SNP 6 in the total test population and assuming A was the allele associated with the 35delG mutation in the founder, 161 out of 224 chromosomes showed no recombination in a region of approximately 63 kb (assumed to correspond to $\theta=0.00063$). Consequently, the 35delG mutation is estimated to be about 500 generations, or approximately 10 000 years old. As we do not know the true recombination frequency of this region, it should be emphasised that this calculation represents no more than a rough estimation, and that the range of error for this calculation is undoubtedly large.

Discussion

In this study, we show that the high frequency of the 35delG mutation is the result of a founder effect rather than a mutational hot spot, although the latter hypothesis has been favoured by most authors.[9 11 17 20 21] It has been argued that the deletion of one G from a stretch of six could have arisen frequently through polymerase slippage or the presence of a chi consensus motif in the immediate vicinity.[9 11] A strong argument for a mutational hot spot is certainly the presence of this mutation in several geographically and ethnically distinct populations.[21] However, its absence or low prevalence in other ethnic groups, such as Asians,[14 15] argues against this hypothesis.

Morell *et al*[17] have shown that several different haplotypes surround the 35delG mutation,[17] another strong argument in favour of a mutational hot spot. Their study considered an interval of 2 cM, while we have focused on a region of less than 70 kb or more than 95% smaller. As we have shown, the 35delG mutation is ancient and therefore haplotype sharing will be observed only in a very small chromosomal interval.

The age of the mutation further explains its widespread occurrence across Europe, the Mediterranean region, and the United States. Possibly, the mutation originated somewhere in the Middle East and was spread throughout Europe along the two Neolithic population movement routes. The first route followed the coast of the Mediterranean sea to Italy and Spain, while the other followed the Danube and Rhine valleys to northern Europe. The 35delG mutation would have been brought to the United States by emigration from Europe.

Table 3 Results of the SNP analysis

		Belgian			British			American			Total		
SNP	Genotype	Patient	Control	p	Patient	Control	p	Patient	Control	p	Patient	Control	p
SNP 1	CC	0	47	<0.001	0	18	<0.001	0	31	<0.001	0	96	<0.001
	CT	0	13		2	12		2	18		4	43	
	TT	33	1		27	0		45	1		105	2	
SNP 2	GG	31	20	0.014	27	24	0.025	47	43	0.013	105	87	<0.001
	GA	0	5		0	6		0	7		0	18	
	AA	0	0		0	0		0	0		0	0	
SNP 3	GG	33	29	0.053	29	25	0.052	49	41	0.003	111	95	<0.001
	GA	0	5		0	5		0	9		0	19	
	AA	0	0		0	0		0	0		0	0	
SNP 4	TT	31	22	0.008	28	20	<0.001	48	35	<0.001	107	77	<0.001
	TG	0	6		0	10		0	14		0	30	
	GG	0	0		0	0		0	0		0	0	
SNP 5	GG	26	21	0.611	22	20	0.472	35	31	0.613	83	72	0.358
	GA	6	8		5	10		13	13		24	31	
	AA	2	2		1	0		1	4		4	6	
SNP 6	GG	6	5	0.072	6	3	0.431	3	6	0.224	15	14	0.019
	GA	10	17		6	13		17	22		33	52	
	AA	19	9		17	13		28	22		64	44	

The low carrier frequency seen in other populations, for example, in the Korean and Arab population, could also have originated from the same founder. On the other hand, however, it cannot be excluded that the 35delG mutation has arisen on more then one occasion.

The reason for the high frequency of 35delG remains to be elucidated. By comparison, the ΔF508 cystic fibrosis mutation is by far the best known and studied ancient mutation. Its frequency varies in different populations and estimates of age of origin of the mutation range, from 2940 to 52 000 years, depending on the methods used.[28 29] It has been postulated that ΔF508 carriers have an increased resistance to chloride secreting diarrhoea resulting in selective advantage for heterozygous carriers.[30] At present, it is not known whether the carrier status for a mutation in *GJB2* is associated with increased reproductive fitness.

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