

Caryn R. Rothrock · Alessandra Murgia  
Edi L. Sartorato · Emanuela Leonardi · Sainan Wei  
Sarah L. Lebeis · Laura E. Yu · Jill L. Elfenbein  
Rachel A. Fisher · Karen H. Friderici

## Connexin 26 35delG does not represent a mutational hotspot

Received: 25 October 2002 / Accepted: 28 February 2003 / Published online: 9 April 2003

© Springer-Verlag 2003

**Abstract** Non-syndromic hearing impairment (NSHI) is the most common form of deafness and presents with no other symptoms or sensory defects. Mutations in the gap junction gene *GJB2* account for a high proportion of recessive NSHI. The *GJB2* gene encodes connexin 26, which forms plasma membrane channels between cochlear cells. In Caucasian populations a single mutation, 35delG, accounts for most cases of NSHI. This mutation appears to be most prevalent in individuals of Mediterranean European descent, with carrier frequencies estimated as being as high as one in thirty. The 35delG region may be a mutational hotspot. The mutation arises from the deletion of a guanine from a six-guanine stretch and nearby microsatellite markers show little evidence for linkage disequilibrium. We believe that 35delG is an old mutation in a chromosomal region of high recombination. The genetic context of the 35delG mutation was examined to distinguish between an old or a recurring mutation. We identified two single-nucleotide polymorphisms (SNPs) immediately upstream of the first exon of *GJB2*. Polymerase chain reaction/restriction fragment length polymorphism analysis determined the SNP genotype of 35delG containing chromosomes from

various populations, including Italy, Brazil, and North America. We found the same, relatively rare, polymorphism associated with the 35delG mutation in all populations studied. We have also examined microsatellite markers D13S175, which is 80 kb telomeric to *GJB2*, and D13S1316, which is 80 kb centromeric to *GJB2*. D13S175 appears to be in weak linkage disequilibrium with 35delG, while D13S1316 is less so. SNPs located between the 35delG mutation and the microsatellite markers show strong evidence of linkage disequilibrium. Taken together, these results indicate there has been substantial recombination near the 35delG mutation; however, we present evidence that the 35delG mutation arose in European and Middle Eastern populations from a single mutational event on a founder chromosome.

### Introduction

Mutations in the connexin 26 (*Cx26*) gene may account for nearly 50% of recessive non-syndromic deafness in Caucasian populations (Cohn and Kelley 1999). To date, more than 50 mutations have been described in this gene (<http://www.crg.es/deafness>), but one mutation, 35delG (also called 30delG), is the cause of deafness in the majority of cases. This mutation is the deletion of one guanine from a string of six guanines near the amino terminus of the gene product and results in a frameshift and termination of the protein at amino acid 13 (Zelante et al. 1997). Further studies have revealed that the carrier frequency of the 35delG mutation in Caucasian populations, especially those of Mediterranean descent, is as high as 1 in 31 (Estivill et al. 1998). The frequency of mutations in the *GJB2* gene appear to be associated with the ethnicity of the populations studied (Gasparini et al. 2000).

Connexins have diverse distribution in tissues, where the same connexin can be present in multiple tissues, and a single tissue can have many types of connexins. *Cx26* is expressed in a wide variety of tissues (Zhang and Nicholson 1989). Gap junctions are formed by connexin proteins and are involved in cell-to-cell communication by allowing the

C. R. Rothrock · S. Wei · S. L. Lebeis · L. E. Yu  
K. H. Friderici (✉)  
Department of Microbiology and Molecular Genetics,  
Michigan State University,  
5163 Biomedical and Physical Sciences Building,  
East Lansing, Michigan, 48824-4320, USA  
Tel.: +1-517-3556463 ext.1558, Fax: +1-517-3538957,  
e-mail: frideric@msu.edu

R. A. Fisher · K. H. Friderici  
Department of Pediatrics and Human Development,  
Michigan State University, East Lansing, Michigan, USA

J. L. Elfenbein  
Department of Audiology and Speech Pathology,  
Michigan State University, East Lansing, Michigan, USA

A. Murgia · E. Leonardi  
Department of Pediatrics, University of Padua, Padua, Italy

E. L. Sartorato  
CBMEG, Laboratorio de Genetica Humana,  
Universidade Estadual de Campinas, SP Campinas, Brazil

passage of ions and small molecules between the cytoplasm of neighboring cells. Connexins assemble into hexamers called connexons and form homo- or heterodimers with a connexon of an adjacent cell, creating a functional gap junction. Gap junctions play a major role in tissue development and differentiation. In the ear, gap junctions are proposed to regulate the cycle of potassium ions between cells during auditory transduction (Kikuchi et al. 1995). It is interesting to note that the 35delG mutation results only in non-syndromic hearing impairment (NSHI) and does not appear to affect other tissues. Additional connexin proteins may be able to substitute for Cx26 in these tissues and to compensate for the loss of Cx26.

The origin and age of the 35delG mutation is unclear. Some arguments have been presented that this mutation is recurrent. The context of the mutation shows homology to a putative mutational hotspot TG(A/G)(A/G)(G/T)(A/C) associated with frameshift mutations (Krawczak and Cooper 1991). In addition, there is no obvious linkage disequilibrium between the 35delG mutation and nearby microsatellite markers (Morell et al. 1998; Carrasquillo et al. 1997; Denoyelle et al. 1997). The high frequency of 35delG has previously been attributed to polymerase slippage on the stretch of six guanine residues in the GJB2 gene (Denoyelle et al. 1997). The absence of a common haplotype for the 35delG allele would argue for multiple origins of the mutation.

In contrast, there is powerful epidemiological evidence that the 35delG mutation does not recur frequently, since it is largely absent from non-European populations (Gasparini et al. 2000). The 35delG mutation has not been detected in other populations, such as those of Japanese origin, although other mutations in Cx26 are present (Fuse et al. 1999; Abe et al. 2000; Kudo et al. 2000). The carrier frequency of the 35delG mutation is 1/35 in southern Europe, decreases to 1/79 in central and northern Europe (Gasparini et al. 2000), and is estimated to be 1/40 in US Caucasians (Green et al. 1999). We believe 35delG is an old mutation in a chromosomal region of high recombination. When examined more closely, evidence for linkage disequilibrium with the 35delG mutation has been found in a number of populations (Tekin et al. 2001; Van Laer et al. 2001; Shahin et al. 2002). However, in populations such as those of the United States and Europe where relaxed selection and assortative mating have occurred, apparent linkage disequilibrium may result from amplification, in the population, of a few chromosomes with independent mutations and a similar haplotype (Nance et al. 2000). In this report, we present evidence that the 35delG mutation arose in European and Middle Eastern populations from a single mutational event on a founder chromosome.

## Materials and methods

### Subjects

We analyzed DNA samples from patients from several different countries, including Italy, North America, and Brazil. DNA samples of 69 unrelated patients from Italy with NSHI, all of whom

were homozygous for the 35delG mutation, and 100 DNA samples of Italian patients with no 35delG mutation were tested. Patients from Brazil included 15 homozygous for 35delG and 11 heterozygous for 35delG. We had two DNA samples of patients of Ashkenazi Jewish descent, both of whom were homozygous for 35delG. The samples from the US included an extended family of German heritage and consisting of 143 DNA samples, 12 of whom were homozygous for 35delG (with five independent 35delG chromosomes in the family), 23 of whom were heterozygous for 35delG, and 102 of whom had no 35delG mutation. All samples were obtained with the patients' consent.

### Microsatellite genotyping

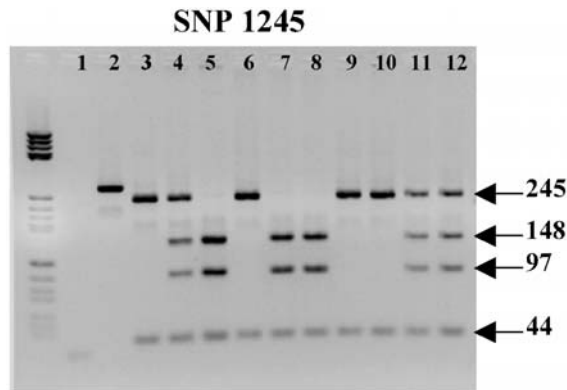
Two microsatellite repeat polymorphisms were used as genetic markers: D13S175 and D13S1316. Reactions were carried out in a volume of 15  $\mu$ l containing 20 ng DNA, 1 $\times$  PCR buffer (Perkin Elmer, Roche Molecular Biochemicals, Indianapolis, Ind.), 10 mM dATP, 10 mM dGTP, 10 mM dTTP, 1 mM dCTP, 0.03 U *Taq* polymerase (Gibco, Life Technologies, Rockville, Md.), 0.7  $\mu$ Ci  $\alpha$ -<sup>32</sup>P-dCTP (Amersham Pharmacia Biotech, Piscataway, N.J.), and 0.25  $\mu$ M each of the forward primer and reverse primer (Macromolecular Structural Facility at Michigan State University, East Lansing, Mich.). The primers for amplifying D13S175 were CTG GTG ACC TAA TGT ACA GT and AC TAT GCA TCA CCT CAC AT, and for amplifying D13S1316 were CTA CTG GGG AGG CTG G and CAT GTC TCT GAA TCG CTT TT. Polymerase chain reaction (PCR) amplification included a denaturing step of 5 min at 94°C, followed by 35 cycles of 94°C for 30 s, annealing for 30 s at 57°C for D13S175 or 53°C for D13S1316, and a 30 s extension at 72°C. There was a final extension at 72°C for 5 min. After denaturation, the samples were separated on 6% polyacrylamide gels (SequaGel, National Diagnostics, Atlanta, Ga.) followed by drying and autoradiography.

### Single-nucleotide polymorphism genotyping and 35delG mutation screening

Sequence analysis of several patients revealed two single-nucleotide polymorphisms (SNPs) in the promoter region of GJB2, immediately upstream of the first exon (Kiang et al. 1997). The first (SNP1245) positioned 186 bp upstream from the putative transcriptional start site (or 3556 bp from the AUG) is a T to C transition. The second polymorphism (SNP1285) is a G to A variation located 146 bp upstream from the transcriptional start site. The primers designed to amplify the region containing both SNP1245 and SNP1285 produced a 289-bp product and were GGG TCC CGA CTC TCA GC and GCC TCT TCC CTC GGA GAC (Macromolecular Structural Facility at Michigan State University). The 25- $\mu$ l PCR consisted of 1 $\times$  GC Buffer (Clontech), 5  $\mu$ l GC melt (Clontech), 1.1 mM MgOAc, 1 $\times$  GC Genomic Polymerase (Clontech), 0.2 mM dNTP mix (Gibco, Life Technologies), 1  $\mu$ M each primer, and 20 ng genomic DNA. Amplification conditions were: denaturation for 5 min at 94°C, followed by 40 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 45 s. There was a final extension at 72°C for 7 min.

To test for SNP T1245C, PCR products were digested with *BanI* (New England Biolabs) at 37°C for 2 h, by using 10  $\mu$ l PCR product mixed with 3  $\mu$ l 1 $\times$  Buffer 4 (New England Biolabs) and 1  $\mu$ l *BanI* (20 U/ $\mu$ l). Products were resolved on a 3% agarose gel (3:1 New Sieve) in 0.5 $\times$  TBE at 100 V for 90 min. The constitutive *BanI* site at 245 bp in the amplification product results in 245-bp and 44-bp fragments on a gel. If a C is present at 1245, an additional *BanI* site is created at 97 bp in the 245-bp fragment. This yields three bands of 44 bp, 97 bp, and 148 bp, respectively. Heterozygotes exhibit 4 bands at 44 bp, 97 bp, 148 bp, and 245 bp (Fig. 1).

To test for SNP A1285G, 10  $\mu$ l PCR product was digested with 1  $\mu$ l *HaeIII* (20 U/ $\mu$ l; New England Biolabs) and incubated at 37°C for 2 h. Electrophoresis was as described above. There are three constitutive *HaeIII* sites at 225 bp, 143 bp, and 36 bp in the 289-bp



**Fig. 1** PCR analysis of SNP1245. Lane 1 Negative control, lane 2 no digestion, lanes 3, 6, 9, 10 homozygous 1245T/1245T, lanes 4, 11, 12 heterozygous 1245C/1245T, lanes 5, 7, 8 homozygous 1245C/1245C

PCR product, resulting in four fragments of 36 bp, 64 bp, 82 bp, and 107 bp. When an A is present at 1285, the *Hae*III site is eliminated, resulting in only three bands visible at 36 bp, 64 bp, and 189 bp. Heterozygotes exhibit five bands at 36 bp, 64 bp, 82 bp, 107 bp, and 189 bp.

Mutation analysis for 35delG was performed by PCR, as described by Wilcox et al. (2000). Assays for the SNPs hCV1918223 and hCV1813042 (Celera ID number) were carried out by using the Applied Biosystem Assay on Demand reagents (Applied Biosystem, Foster City, Calif.) and were analyzed by using an ABI Prism Sequence Detection System.

## Results

In hearing loss studies of an extended (>3,000 members) American family of German descent, affected family members were found to be homozygous, heterozygous, or negative for the 35delG mutation. To determine whether another Cx26 mutation was segregating in this pedigree, both exons and the promoter region of the GJB2 gene were sequenced in several family members. Two SNPs were found 186 bp and 146 bp upstream from the start of transcription (positions 1245 and 1285 of GenBank clone U43932) (Fig. 2). The frequency of the A allele of SNP1285 was more than 10% in our extended family but was much less common in the general US population (~5%). No 1285A allele was found on a 35delG-containing chromosome.

We found that SNP1245C was also relatively rare in the non-35delG chromosomes of the extended family (10/190) and in the general American population (21/104) but was always present on independent chromosomes carrying the 35delG mutation (5/5; see Table 1). Two Ashkenazi patients who were homozygous for 35delG carried the same rare C allele at SNP1245 and the common G allele at SNP1285 (Table 1.)

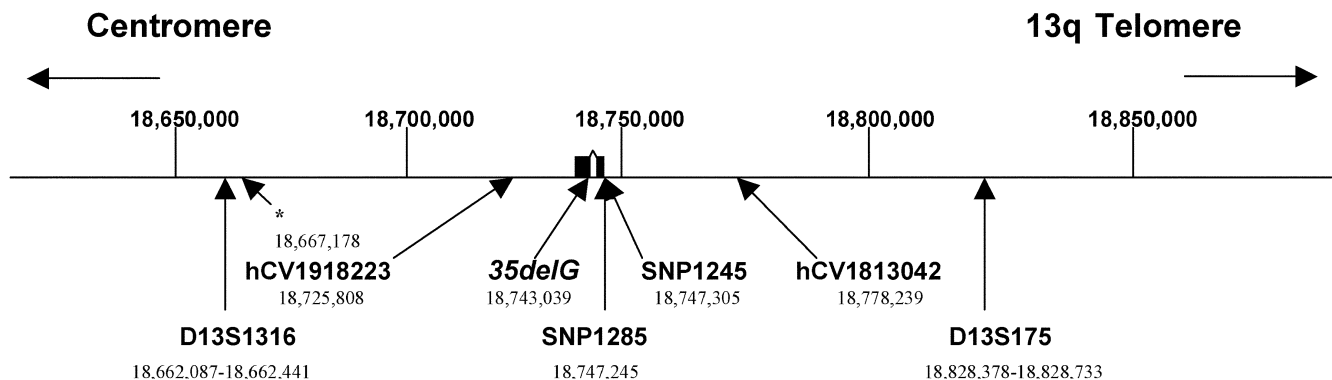
To extend this study, two other populations were sampled. Sixty-nine patients from Italy and homozygous for 35delG were tested. Of the 138 chromosomes in this sample, all but one had the 1245C allele. This SNP is present in the Italian population at only 54/200 chromosomes. None of the 35delG-containing chromosomes had the 1285A SNP, which is present in the Italian population at ~5% (Table 1). Sampling of another population, this one being from South America, indicated that all the 35delG-containing chromosomes also contained this identical 1245C SNP. Thirteen patients from Brazil, of mixed European descent and homozygous for 35delG, were also homozygous for the C allele of SNP1245, whereas in 35delG carriers from the same population, the 1245C allele was present in 15/22.

The finding that all 35delG-containing chromosomes from a variety of populations carried the same rare polymorphism suggests that the mutation probably occurred once on a chromosome carrying the relatively rare 1245C SNP.

## Linkage mapping by using microsatellite markers

To explore reasons for the apparent lack of linkage disequilibrium seen on the 35delG chromosome and found in

**Fig. 2** Chromosome 13 polymorphisms near connexin 26. Marker positions based on the June 2002 version of the UCSC Genome Browser (<http://genome.cse.ucsc.edu/>). This region is represented by sequence AL138688 and AL355984 in contig NT\_009799. Numbers above the line Kilobase pair position from the telomere of the p arm of chromosome 13. Centromere and telomere designations are based on the build30 deCODE genetic map (<http://genome.cse.ucsc.edu/GoldenPath/MapPlots/>); in this version, the centromere is at 17,000 kb. The connexin 26 gene is shown as solid bars above the line (exon 1:18,747,037–18,746,837 bp, exon 2:18,743,741–18,741,608 bp); the direction of transcription is toward the centromere. The base pair positions of the SNPs and microsatellites are given. Asterisk SNP5 (TSC1102325) reported by Van Laer et al. (2001)



**Table 1** Allele frequencies of SNPs on 35delG-containing chromosomes compared with chromosomes that do not carry the 35delG mutation. Individuals from several countries were genotyped for the 35delG mutation and divided into two groups (*Chromosomes with 35delG* homozygous for 35delG, *Non-35delG chromosomes* no 35delG mutation). These same individuals were then genotyped for

SNPs flanking the mutation, and the allele frequencies are shown as a percentage of number of alleles typed. Numbers of individuals were: 35delG; Italy = 69, Brazil = 15, US = 3, Ashkenazi = 2, and Non-35delG; Italy = 100, US = 51. The SNP IDs are according to Celera (*hCV1918223*, *hCV1813042*), dbSNP (*rs877098*), base-pair position in GenBank entry U43932 (*SNP1285*, *SNP1245*)

SNP ID		Chromosomes with 35delG				Non-35delG chromosomes	
		Italy	Brazil	US	Ashkenazi	Italy	US
hCV1918223	C	83.8	95.8	–	–	68.6	–
	T	16.2	4.2	–	–	31.4	–
SNP1285	G	100	100	100	100	94.6	96.2
	A	0	0	0	0	5.5	4.8
SNP1245	C	99.3	100	100	100	26.0	20.2
	T	0.7	0	0	0	74.0	79.8
hCV1813042 (rs877098)	A	95.4	89.2	–	–	43.2	–
	G	4.6	10.7	–	–	56.7	–

**Table 2** Flanking microsatellite frequencies in control and 35delG-containing chromosomes. Simple sequence repeat polymorphism analysis was performed. The number of chromosomes tested in each ethnic group is shown in *parentheses*. For the 35delG chromosomes, only unrelated individuals, homozygous for the mutation, were included. None of the control samples contained 35delG. The US control samples were from our extended American family

Genetic marker	Allele	% Total for each allele					
		35delG chromosomes				Non-35delG	
		Ashkenazi (4)	US (5)	Italy (138)	Brazil (30)	Italy (200)	US (190)
D13S1316	1	–	–	5.1	–	4.0	1.0
	2	–	–	1.4	–	1.0	1.0
	3	–	–	5.8	–	5.5	1.5
	4	–	–	4.3	3.8	3.5	6.0
	5	100	20	68.1	80.8	55.7	58.7
	6	–	–	3.6	3.8	4.0	1.0
	7	–	80	11.6	11.5	25.6	27.9
	8	–	–	–	–	0.5	–
D13S175	1	–	–	1.4	–	1.0	3.5
	2	–	–	3.6	–	8.5	12.1
	3	–	–	–	–	0.5	–
	4	–	–	3.6	6.7	8.0	15.1
	5	–	–	0.7	–	5.5	–
	6	100	100	82.0	73.3	53.8	36.4
	7	–	–	7.1	20.0	19.1	29.8
	8	–	–	1.4	–	3.5	6.6

previous studies, genetic markers near the GJB2 gene were also examined. This gene is very near the centromere on the long arm of chromosome 13 (13q11-12). Two microsatellite markers are located especially close to the 35delG mutation site: D13S1316 is 81,247 bp centromeric and D13S175 is 84,820 bp telomeric to the mutation. The Marshfield and Genethon genetic linkage maps place D13S175 at 6.03 cM and 7.4 cM, respectively, and both place D13S1316 at 0 cM.

Genotyping of our samples yielded the distribution shown in Table 2. For the centromeric marker D13S1316, the distribution of alleles is somewhat skewed in the chromosomes containing 35delG, so that a larger proportion contain allele 5. Analysis of the allele distribution for marker D13S175 showed that, whereas allele 6 is the most common allele for this marker in all the populations that

we studied, it is found on a higher proportion of 35delG-containing chromosomes than in the control population.

Overall the haplotype “C at SNP1245, G at 1285, allele 5 at D13S1316, and allele 6 at D13S175” was present as homozygous in 27% of patients from Italy (19/70 individuals) who were also homozygous for the 35delG mutation. Only one individual from the control population of 100 subjects without the 35delG mutation was homozygous for this haplotype.

#### Haplotype with additional SNPs

The implication from the microsatellite and initial SNP data is that there is unusually intense recombination in the region of the Cx26 gene. To examine this issue more thor-

oughly, additional SNPs spaced intermediately between the 35delG mutation and the microsatellite markers were tested (Table 1, Fig. 2). These are common SNPs, with reported minor allele frequencies of more than 40%, as supplied by Applied Biosystems. As predicted, the allele frequencies in the chromosomes containing the 35delG mutation were highly skewed toward one of the two possible alleles. The skewing was more dramatic between D13S175 and the mutation (SNP hCV1813042) than between D13S1316 and the mutation (SNP hCV1918223). A single four marker SNP haplotype was predominant in the individuals whose haplotypes could be obtained. The haplotypes described by marker order hCV1918223, SNP1285, SNP1245, hCV1813042 are as follows for the Italian 35delG homozygotes; 67% were homozygous CGCA/CGCA, 23% were CGCA/TGCA, 5% were CGCA/CGCC, 3% were TGCA/TGCA, and less than 2% were TGCA/TCGG. In the Italian control population, no one was homozygous for the CGCA haplotype, which represents the vast majority of the 35delG-containing chromosomes.

## Discussion

The 35delG mutation in Cx26 appears to have occurred only once in the Caucasian population. Data from this study thus extend the finding of Van Laer et al. (2001) that, for Northern European populations, the 35delG mutation probably arose only once. Their conclusion is based on data from the SNP analysis of the chromosomal region surrounding the GJB2 gene. They also suggest that the mutation is very old, since an SNP at a distance of 76 kb no longer shows significant linkage disequilibrium with the 35delG mutation. This speculation is based on the predicted recombination rate for this short physical distance. Recent information from the Human Genome Project however suggests that this region of chromosome 13 must harbor a recombination island, since the microsatellite markers flanking the GJB2 gene also show considerable recombination with the 35delG mutation and have been located on both Genethon and Marshfield genetic maps as being 6–7 cM apart. D13S1316 and D13S175 are separated by a genetic distance of 6–7 cM but are only 166,000 bp apart on the physical map. Examination of the Mediterranean population in which the 35delG mutation occurs at the highest frequency indicates that the mutation occurred on a single chromosome carrying a rare SNP allele but common microsatellite alleles. The estimation of the age of the mutation is unlikely to be valid because of the steep recombination gradient that appears to exist across this region. It should be noted that the telomeric marker (D13S175) shows less decay to population norms than the equally distant centromeric marker (D13S1316). This observation is supported by the similarly skewed allele frequencies of SNPs that are located midway between the mutation and either of the microsatellite markers. This high recombination rate within a small genomic distance is an unexpected finding for a region so near to the centromere where recombination is expected to be reduced (International Human

Genome Sequencing Consortium, 2001). Comparison of genetic maps with the working draft of the Human Genome (<http://genome.ucsc.edu/goldenPath/mapPlots>) shows that acrocentric chromosomes 13, 14, and 15 as recently described do not appear to follow the general pattern of suppression of recombination at the centromere. Alternatively, the current centromere location on these maps may be incorrect.

Although we cannot accurately estimate the age of this mutation, it is clear that it has existed in the entire Caucasian population for some time and has reached a high carrier level. Cx26 is a small gene, but over 50 mutations have been described for it. In addition, some populations in which the 35delG mutation is not found have other common Cx26 mutations, such as 235delC in Japanese and Koreans (Abe et al. 2000; Kudo et al. 2000) or R143 W in Ghana (Hamelmann et al. 2001). The high level of Cx26 mutations and the finding that separate populations carry other common mutations suggest that heterozygote advantage for 35delG exists or existed under certain circumstances. Given the widespread expression of the gene in many tissues, reduced expression may confer a selective advantage via a multitude of pathways, making it difficult to predict a selection mechanism.

It is clear that the 35delG mutation arose from a single mutational event in the Caucasian population and that some remnants of linkage disequilibrium still exist.

**Acknowledgements** The authors thank Dr. Robert Morell, Laboratory of Molecular Genetics, NIDCD for patient DNA samples and for helpful discussion. This work was supported by funds from the Hearing Research Center, MSU Foundation (R.F., K.F.) and by NIH grant DC04568 (K.F.).

## References

- Abe S, Usami S-I, Shinkawa H, Kelley PM, Kimberling WJ (2000) Prevalent connexin 26 gene (*GJB2*) mutations in Japanese. *J Med Genet* 37:41–43
- Carrasquillo MM, Zlotogora J, Barges S, Chakravarti A (1997) Two different connexin 26 mutations in an inbred kindred segregating non-syndromic recessive deafness: implications for genetic studies in isolated populations. *Hum Mol Genet* 6:2163–2172
- Cohn ES, Kelley PM (1999) Clinical phenotype and mutations in connexin 26 (*DFNB1/GJB2*), the most common cause of childhood hearing loss. *Am J Med Genet* 89:130–136
- Denoyelle F, Weil D, Maw MA, Wilcox SA, Lench NJ, Allen-Powell DR, Osborn AH, Dahl H-HM, Middleton A, Houseman MJ, Dode C, Marlin S, Boulila-ElGaied A, Grati M, Ayadi H, BenArab S, Bitoun P, Lina-Granade G, Godet J, Mustapha M, Loiselet J, El-Zir E, Aubeis A, Joannard A, Leveilliers J, Garabedian E-N, Mueller RF, Gardner RJM, Petit C (1997) Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene. *Hum Mol Genet* 6:2173–2177
- Estivill X, Fortina P, Surrey S, Rabionet R, Melchionda S, D'Agruma L, Mansfield E, Rappaport E, Govea N, Mila M, Zelante L, Gasparini P (1998) Connexin-26 mutations in sporadic and inherited sensorineural deafness *Lancet* 351:394–398
- Fuse Y, Doi K, Hasegawa T, Sugii A, Hibina H, Kubo T (1999) Three novel connexin26 gene mutations in autosomal recessive non-syndromic deafness *Neuroreport* 10:1853–1857

- Gasparini P, Rabionet R, Barbuji G, Melchionda S, Petersen M, Brondum-Nielsen K, Metspalu A, Oitmaa E, Pisano M, Fortina P, Zelante L, Estivill X (2000) High carrier frequency of the 35delG deafness mutation in European populations. Genetic Analysis Consortium of GJB2 35delG. *Eur J Hum Genet* 8: 19–23
- Green GE, Scott DA, McDonald JM, Woodworth GG, Sheffield VC, Smith RJH (1999) Carrier rates in the Midwestern United States for GJB2 mutations causing inherited deafness *JAMA* 281:2211–2216
- Hamelmann C, Amedofu GK, Albrecht K, Muntau B, Gelhaus A, Brobby GW, Horstmann RD (2001) Pattern of connexin 26 (GJB2) mutations causing sensorineural hearing impairment in Ghana. *Hum Mut* 18:84–85
- International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. *Nature* 409: 860–921
- Kiang DT, Jin N, Tu ZJ, Lin HH (1997) Upstream genomic sequence of the human connexin26 gene. *Gene* 199:165–171
- Kikuchi T, Kimura RS, Paul DL, Adams JC (1995) Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis *Anat Embryol* 191:101–118
- Krawczak M, Cooper DN (1991) Gene deletions causing human genetic disease: mechanisms of mutagenesis and the role of the local DNA sequence environment. *Hum Genet* 86:425–441
- Kudo T, Ikeda K, Kure S, Matsubara Y, Oshima T, Watanabe K, Kawase T, Narisawa K, Takasaka T (2000) Novel mutations in the connexin 26 gene (GJB2) responsible for childhood deafness in the Japanese population. *Am J Med Genet* 90:141–145
- Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fisher R, Van Camp G, Berlin CI, Oddoux C, Ostrer H, Keats B, Friedman TB (1998) Mutations in the connexin 26 gene (*GJB2*) among Ashkenazi Jews with nonsyndromic recessive deafness. *N Engl JMed* 339:1500–1505
- Nance WE, Liu XZ, Pandya A (2000) Relation between choice of partner and high frequency of connexin-26 deafness *Lancet* 356:500–501
- Shahin H, Walsh T, Sobe T, Lynch E, King M-C, Avraham KB, Kanaan M (2002) Genetics of congenital deafness in the Palestinian population: multiple connexin 26 alleles with shared origins in the Middle East. *Hum Genet* 110:284–289
- Tekin M, Akar N, Cin S, Blanton SH, Xia XJ, Liu X-Z, Nance WE, Pandya A (2001) Connexin 26 (GJB2) mutations in the Turkish population: implications for the origin and high frequency of the 35delG mutation in Caucasians. *Hum Genet* 108: 385–389
- Van Laer L, Coucke P, Mueller RF, Caethoven G, Flothmann K, Prasad SD, Chamberlin GP, Houseman M, Taylor GR, Van de Heyning CM, Franssen E, Rowland J, Cucci RA, Smith RJH, Van Camp G (2001) A common founder for the 35delG GJB2 gene mutation in connexin 26 hearing impairment *J Med Genet* 38:515–518
- Wilcox SA, Osborn AH, Dahl H-HM (2000) A simple PCR test to detect the common 35delG mutation in the connexin 26 gene *Mol Diagn* 5:75–78
- Zelante L, Gasparini P, Estivill X, Melchionda S, D'Agruma L, Govea N, Mila M, Della Monica M, Lutfi J, Shohat M, Mansfield E, Delgrosso K, Rappaport E, Surrey S, Fortina P (1997) Connexin 26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. *Hum Mol Genet* 6:1605–1609
- Zhang J-T, Nicholson BJ (1989) Sequence and tissue distribution of a second protein of hepatic gap junctions, Cx26, as deduced from its cDNA. *J Cell Biol* 109:3391–3401