

MUTATIONS IN THE CONNEXIN 26 GENE (*GJB2*) AMONG ASHKENAZI JEWS WITH NONSYNDROMIC RECESSIVE DEAFNESS

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ABSTRACT

Background Mutations in the *GJB2* gene cause one form of nonsyndromic recessive deafness. Among Mediterranean Europeans, more than 80 percent of cases of nonsyndromic recessive deafness result from inheritance of the 30delG mutant allele of *GJB2*. We assessed the contribution of mutations in *GJB2* to the prevalence of the condition among Ashkenazi Jews.

Methods We tested for mutations in *GJB2* in DNA samples from three Ashkenazi Jewish families with nonsyndromic recessive deafness, from Ashkenazi Jewish persons seeking carrier testing for other conditions, and from members of other ethnic groups. The hearing of persons who were heterozygous for mutations in *GJB2* was assessed by means of pure-tone audiometry, measurement of middle-ear immittance, and recording of otoacoustic emissions.

Results Two frame-shift mutations in *GJB2*, 167delT and 30delG, were observed in the families with nonsyndromic recessive deafness. In the Ashkenazi Jewish population the prevalence of heterozygosity for 167delT, which is rare in the general population, was 4.03 percent (95 percent confidence interval, 2.5 to 6.0 percent), and for 30delG the prevalence was 0.73 percent (95 percent confidence interval, 0.2 to 1.8 percent). Genetic-linkage analysis showed conservation of the haplotype for 167delT but the existence of several haplotypes for 30delG. Audiologic examination of carriers of the mutant alleles who had normal hearing revealed subtle differences in their otoacoustic emissions, suggesting that the expression of mutations in *GJB2* may be semidominant.

Conclusions The high frequency of carriers of mutations in *GJB2* (4.76 percent) predicts a prevalence of 1 deaf person among 1765 people, which may account for the majority of cases of nonsyndromic recessive deafness in the Ashkenazi Jewish population. Conservation of the haplotype flanking the 167delT mutation suggests that this allele has a single origin, whereas the multiple haplotypes with the 30delG mutation suggest that this site is a hot spot for recurrent mutations. (N Engl J Med 1998;339:1500-5.)

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CONGENITAL deafness occurs in approximately 1 in 1000 live births, and 50 percent of these cases are hereditary.¹ Nonsyndromic recessive deafness, defined as lack of hearing with no other associated clinical features, accounts for approximately 80 percent of cases of hereditary deafness.¹ The existence of at least 20 genes that, if mutated, result in nonsyndromic recessive deafness has been demonstrated through genetic-linkage studies, although most of these genes have not yet been identified.² Population studies suggest that many more are likely to exist.³⁻⁸ Recently, mutations in the *GJB2* gene were shown to be among the causes of the condition.^{9,10} The *GJB2* gene encodes a gap-junction protein, connexin 26, which is expressed in the inner ear and is thought to be important in maintaining endocochlear potential. Subsequent studies showed that a single mutant allele of *GJB2* accounts for the majority of cases of nonsyndromic recessive deafness among the Mediterranean European population.¹⁰⁻¹² This mutation is referred to as 30delG (or 35delG), since it is the deletion of a single nucleotide in a string of six guanine residues that begins at nucleotide position 30 (and ends at position 35).

We have identified two mutant alleles of *GJB2* in Ashkenazi Jewish families with nonsyndromic recessive deafness. Our findings on the hearing ability of family members who are heterozygous for one of these mutant alleles suggest that the expression of mutations in *GJB2* may be semidominant. We determined the frequencies of these mutant *GJB2* alleles in a representative population of Ashkenazi Jews.

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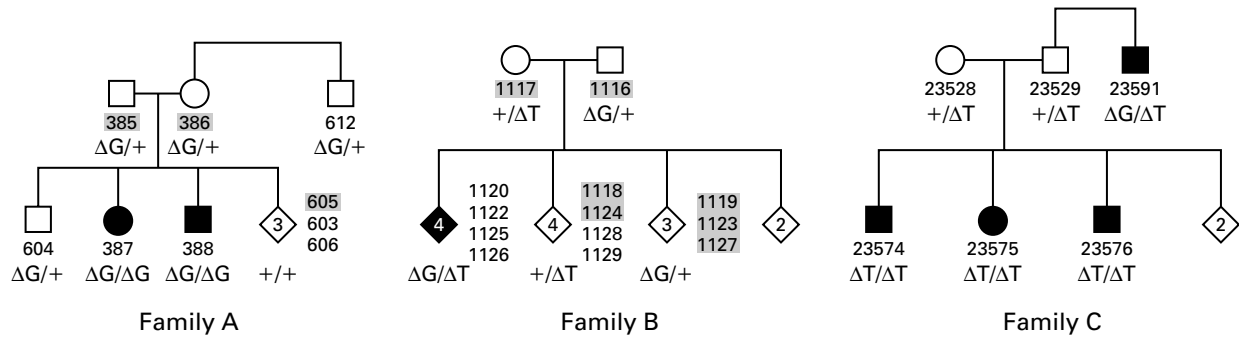


Figure 1. Pedigrees of Families A, B, and C.

The pedigrees have been modified to protect the anonymity of the families. Offspring with the same genotypes and phenotypes are represented by a single symbol; the number inside the symbol indicates the number of subjects represented, if more than one. Birth order is not accurately represented. The identification numbers of each subject are listed below or to the right of the symbols; identification numbers that are shaded indicate subjects who underwent testing of distortion-product otoacoustic emissions. They include the parents in Families A and B, one unaffected noncarrier child in Family A, two unaffected carriers of the 167delT allele in Family B, and three unaffected carriers of the 30delG allele in Family B. Solid symbols represent deaf persons, open symbols unaffected persons, and plus signs the wild-type allele. ΔG denotes the 30delG mutation, and ΔT the 167delT mutation.

METHODS

Families with Nonsyndromic Recessive Deafness

Three families participated in genetic studies of nonsyndromic recessive deafness (protocols 97-DC-0180 and 97-DC-N024 of the National Institute on Deafness and Other Communication Disorders). Affected persons had moderate-to-profound congenital sensory-hearing loss across all frequencies, as assessed by pure-tone audiometry and tests of middle-ear immittance. DNA samples were extracted either from whole blood by standard techniques or from cells obtained by application of a swab to the buccal mucosa.¹³

Detection of Mutations and Genotyping

The *GJB2* gene was amplified from DNA samples from members of the three participating families by means of the polymerase chain reaction (PCR) with primer pair 167F (forward) and 452R (reverse) and primer pair 370F (forward) and 889R (reverse), as described by Kelsell et al.⁹ The numbers refer to the nucleotides at the 5' ends of the oligomers and correspond to nucleotide position numbers in the *GJB2* sequence logged in GenBank (accession number, M86849). These PCR primers produce overlapping fragments that cover the entire *GJB2* coding region and are small enough (285 and 519 base pairs, respectively) to be suitable for single-strand conformation polymorphism analyses. The PCR fragments were labeled by incorporation of [α^{32} P]deoxycytidine triphosphate, denatured, and separated by electrophoresis at 30 W through 0.5× Mutation Detection Enhancement gels (MDE, FMC BioProducts, Rockland, Me.), 0.6× TBE (1× TBE is 89 mM TRIS base, 89 mM boric acid, and 2 mM EDTA) and 10 percent glycerol at 4°C for four hours. Samples that showed conformation polymorphisms were sequenced directly (ThermoSequenase, Amersham Life Sciences, Arlington Heights, Ill.) with the use of the PCR primers.

Population DNA Samples

As population controls for the samples from the Ashkenazi Jewish families, we used blood samples without personal identifiers from a group of 555 Ashkenazim who were undergoing genetic testing for one or more autosomal recessive diseases (including Tay–Sachs disease, Canavan’s disease, Gaucher’s disease, and

cystic fibrosis).¹⁴ No information about a family history of hearing loss was available from these control subjects. Samples from these control subjects have been used previously for population-genetics studies of mutations in the aspartoacylase, breast-cancer susceptibility (*BRCA2*), and adenomatous polyposis coli (*APC*) genes.^{15–17} Anonymous DNA samples representing white, Asian, and black racial and ethnic groups were collected at Michigan State University for comparisons of population frequency.

DNA samples were amplified by PCR for *GJB2* as described above and then sequenced to determine the presence of the 167delT and 30delG alleles. All the samples were also typed for the genetic markers D13S141, D13S175, and D13S250, which flank *GJB2* on chromosome 13, by incorporation of [α^{32} P]deoxycytidine triphosphate and resolved on conventional sequencing gels. Tests of association between the mutations in *GJB2* and nearby genetic markers were performed with the program EH (Estimate Haplotype frequencies).¹⁸ Tests of the significance of differences between carrier rates were calculated with the program 2BY2, which performs Fisher’s exact test.¹⁸ The age of the 167delT mutation was estimated with the following formula: $y = x + (1 - x)(1 - \theta)^g$, where y is the frequency of the ancestral allele among mutation-bearing chromosomes, x is its frequency among normal chromosomes, θ is the recombination fraction between the marker allele and the mutation, and g is the number of generations.¹⁹

Audiologic Analysis

Pure-tone audiometry, testing of middle-ear immittance, and recording of otoacoustic emissions were completed in 10 subjects from Families A and B who had normal hearing and known *GJB2* genotypes. These included four parents and five of their offspring who were heterozygous for mutations in *GJB2* and one noncarrier offspring who had normal hearing (Subject A-605) (Fig. 1). Spontaneous, click-evoked, and distortion-product emissions were recorded, as was efferent suppression of click-evoked otoacoustic emissions.^{20,21} Distortion-product otoacoustic emissions were obtained with pairs of pure tones. In this study, distortion-product otoacoustic emissions were obtained for eight frequency pairs per octave, with the lower frequency at a sound-pressure level of 65 dB, the higher frequency at 55 dB, and a higher-to-lower-frequency ratio of 1.2:1. The mean amplitudes in carriers of a mutation in *GJB2* and in normal control subjects were compared by repeated-measures analysis of variance.

TABLE 1. FREQUENCIES OF CARRIERS OF THE 30delG AND 167delT MUTATIONS OF THE *GJB2* GENE.

POPULATION	30delG			167delT		
	NO. OF SUBJECTS GENOTYPED	NO. OF CARRIERS	FREQUENCY	NO. OF SUBJECTS GENOTYPED	NO. OF CARRIERS	FREQUENCY
			percent			percent
Ashkenazi Jewish, New York City	551	4	0.73	546	22	4.03
White, Michigan State University	173	1	0.58	175	0	0
Centre d'Etude du Polymorphisme Humain*	51	0	0	51	0	0
French*	68	0	0	68	0	0
Spanish or Italian†	280	9	3.21	NA	NA	NA
Asian, Michigan State University	53	0	0	52	0	0
Black, Michigan State University	173	0	0	171	0	0

*These data are from Denoyelle et al.¹² DNA samples from the Centre d'Etude du Polymorphisme Humain are standard reference samples and are primarily from persons of northern European ancestry.

†These data are from Meulenbelt et al.¹³ NA denotes not available.

RESULTS

Genetic Mutations and Frequencies of Alleles

In Families A and B, both from the Ashkenazi Jewish community in North America, and Family C, a European Ashkenazi Jewish family, nonsyndromic recessive deafness was found to be associated with the mutant alleles 30delG and 167delT (Fig. 1). We also sought to determine the frequency of these two alleles among Ashkenazi Jews in the New York City area who have participated in genetic testing for autosomal recessive diseases other than deafness.¹⁴⁻¹⁷ In addition, we tested anonymous DNA samples, supplied by the Clinical Genetics Section of Michigan State University, which were obtained from people seeking counseling for disorders other than deafness. These samples were categorized as from whites, Asians (Indians, Japanese, and Koreans), and blacks.

Table 1 summarizes the frequencies of carriers of the 30delG and 167delT mutations in our test populations as well as data on frequencies of carrier status from the literature. The 30delG mutation may account for 85 percent of the alleles causing nonsyndromic recessive deafness in the Mediterranean European population.¹¹ Four carriers of the 30delG mutation were detected among 551 Ashkenazi Jews, and 1 carrier among 173 white persons with DNA samples at Michigan State University. These frequencies are not significantly different from each other or from published frequencies.

We pooled our results from white persons in North America with the results obtained by Denoyelle et al.¹²

for populations of French and northern European ancestry (yielding a total frequency of 1 in 292) and compared that rate with the carrier rate of 9 in 280 for the Italian and Spanish (Mediterranean) population.¹¹ We found a significant difference in carrier rates for 30delG between these two groups ($P=0.01$ by Fisher's exact test). The carrier rates for 30delG in the Ashkenazi Jewish population (0.73 percent; 95 percent confidence interval, 0.2 to 1.8 percent) and the Mediterranean European population were also significantly different ($P=0.04$), but the rates in the Ashkenazim and the pooled non-Mediterranean European population did not differ significantly ($P=0.66$).

We found 22 carriers of the 167delT mutation among 546 Ashkenazi Jewish samples, suggesting a carrier frequency of 4.03 percent (95 percent confidence interval, 2.5 to 6.0 percent). The carrier rate among Ashkenazi Jews was significantly different from that of any other population reported thus far ($P=4.7 \times 10^{-7}$ by Fisher's exact test). The 167delT mutation is rare and has not been detected in surveys of randomly chosen subjects from any of the populations listed in Table 1.

High carrier rates are generally attributed to a founder effect in a population and are usually evidenced by conservation of haplotypes with closely linked markers. We determined the genotypes with respect to markers D13S141, D13S175, and D13S250, which span a 2-centimorgan region with *GJB2* in the center, in the Ashkenazi Jewish samples. Tables 2 and 3 summarize the results of a test of association between alleles at the marker loci D13S141 and

TABLE 2. FREQUENCIES OF MARKER ALLELES ON NORMAL CHROMOSOMES AND CHROMOSOMES WITH THE MUTATION 167delT IN DNA SAMPLES FROM ASHKENAZI JEWS.*

MARKER	NORMAL		167delT	
	NO.	FREQUENCY	NO.	FREQUENCY
D13S141 alleles				
1	3	0.008	0	0
2	221	0.580	1	0.042
3	139	0.365	23	0.958
4	16	0.042	0	0
5	2	0.005	0	0
D13S175 alleles				
1	14	0.036	0	0.000
2	10	0.026	0	0.000
3	89	0.232	1	0.042
4	151	0.393	23	0.958
5	21	0.055	0	0.000
6	41	0.107	0	0.000
7	9	0.023	0	0.000
8	43	0.112	0	0.000
9	5	0.013	0	0.000
10	1	0.003	0	0.000
D13S250 alleles				
1	232	0.601	23	0.958
2	152	0.394	1	0.042
3	2	0.005	0	0

*P=3.1×10⁻⁸ for the association with 167delT by the χ^2 test.

TABLE 3. MARKER GENOTYPES OF CARRIERS OF THE 30delG MUTATION AMONG DNA SAMPLES FROM ASHKENAZI JEWS.*

DNA SAMPLE	D13S141	D13S175	D13S250
78	2/2	4/2	1/1
224	2/3	3/4	1/1
258	1/2	4/4	1/1
414	1/3	3/4	2/2
Family B 1116	2/3	1/4	1/2
Family C†	3/3	3/4	1/1
Family A 385	3/3	4/4	1/1
Family A 386	3/3	4/4	1/1

*Samples 78, 224, 258, and 414 represented random Ashkenazi Jewish DNA samples. P=0.204 for the association with 30delG by the χ^2 test.

†The genotype of this sample was 30delG/167delT.

D13S175 and the mutations in *GJB2* (marker D13S250 was less informative since only two alleles were associated with it, and therefore those data were excluded from statistical analyses). There was strong evidence of an association between 167delT and the 3/4 haplotype (D13S141/D13S175) ($\chi^2=45.92$,

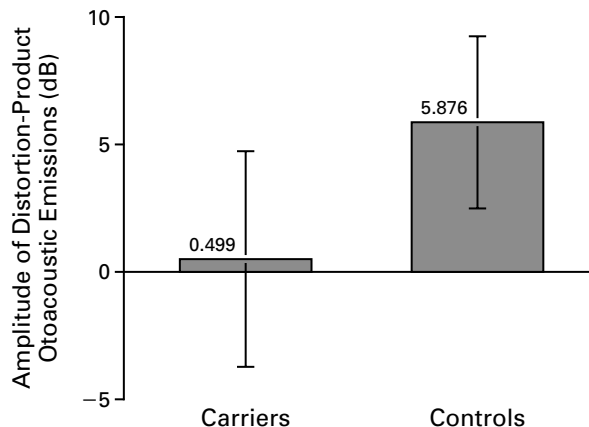


Figure 2. Mean Amplitudes of Distortion-Product Otoacoustic Emissions in the Frequency Range of 1 to 4 kHz.

The mean (\pm SD) values for carriers (parents plus carrier offspring) and control subjects are significantly different ($F_{1,16}=11.439$, $P=0.0038$).

6 df; $P=3.1\times 10^{-8}$) (Table 2). There was, however, no evidence of an association between the 30delG allele and any haplotype in the region ($\chi^2=8.49$, 6 df; $P=0.204$). This result did not merely reflect lack of power to detect an association in a small set of 30delG chromosomes; there are in fact several different 30delG haplotypes (Table 3).

Audiologic Findings

All the tested offspring had normal pure-tone thresholds (defined as a 15-dB hearing level or better) from 250 to 8000 Hz. Parents' thresholds were normal, with the exception of a loss of low-frequency hearing (<500 Hz) in one ear of one parent and a mild, age-appropriate loss of high-frequency hearing (>4 kHz) in five ears of three parents. Middle-ear immittance and reflexes of the middle-ear muscles were normal in all subjects; no history of middle-ear problems was reported by any of the subjects.

The integrity of the outer hair cells of the organ of Corti can be inferred indirectly from otoacoustic emissions. Otoacoustic emissions are low-amplitude acoustic signals that are generated by the cochlea and that can be measured in the external auditory canal by placing a low-noise probe microphone in the canal.²² These emissions are thought to be associated with normal function of the outer hair cells.²³

The results of measurement of distortion-product otoacoustic emissions were informative. In our group of nine control subjects with normal hearing ability, the average sound-pressure level of emissions was 5.88 dB. In contrast, the mean amplitude in the range of 1 to 4 kHz for emissions recorded from the right and left ears was a 0.499-dB sound-pressure level in the nine carrier subjects (four parents and five off-

spring) (Fig. 2). One noncarrier offspring was evaluated, and the sound-pressure level had an emission amplitude of 10.54 dB. Comparison of the amplitudes of distortion-product otoacoustic emissions in carriers and control subjects by repeated-measures analysis of variance showed that the carriers had a significantly lower amplitude ($F_{1,16}=11.439$, $P=0.0038$). The amplitude of emissions showed considerable variability across the frequency range, with sharp amplitude notches (shifts of more than 10 dB from one frequency to the next higher frequency) noted in four ears of three of the four parents and in six ears of four of the five carrier offspring. The noncarrier offspring showed no such amplitude notches.

The finding of amplitude notches across the frequency range is consistent with reports of "audiometric dips" in obligate and possible carriers of hearing-loss genes, including nonsyndromic recessive deafness²⁴ and type 2 Usher's syndrome.²⁵ More recently, abnormalities in distortion-product otoacoustic emissions have been observed in obligate carriers of type 1 Usher's syndrome with normal hearing,²⁶ and in patients with Waardenburg's syndrome and normal hearing.²⁷ The distortion-product otoacoustic emissions of carriers suggest that mutant alleles of *GJB2* may be semidominant but that the defects may be subclinical.

DISCUSSION

Analysis of DNA from deaf members of three Ashkenazi Jewish families revealed two frame-shift mutations in the *GJB2* gene, deletion of a guanine residue in a stretch of six guanines beginning at position 30 (30delG) and deletion of a thymine residue at position 167 (167delT).¹⁰ Mutations in *GJB2* are rare in the general population, but we observed a carrier rate of 4.76 percent (95 percent confidence interval, 3.1 to 6.9 percent) among Ashkenazi Jews. Moreover, in the general population, the 30delG allele has been determined to be the most common mutation causing nonsyndromic recessive deafness, whereas the 167delT mutation is rare, but in the Ashkenazi Jewish population, we observed the opposite, with the 167delT mutation having a carrier rate of 4.03 percent and the 30delG mutation a rate of 0.73 percent.¹⁰⁻¹²

High carrier rates for recessive genes are caused by population "bottlenecks" (large reductions in the size of a population) and high rates of endogamy (the tendency for marriages to be limited to partners within the same community). When this happens, the haplotype of the region surrounding the mutation is highly conserved because of the small number of mutant chromosomes present in the population at the time of the bottleneck. A population bottleneck in Ashkenazi Jewish history has been posited on the basis of the existence of conserved haplotypes in the regions of other disease-associated genes.²⁸ For example, Risch et al.²⁹ found a high de-

gree of conservation among markers surrounding the gene governing the inheritance of idiopathic torsion dystonia, and on the basis of the degree of conservation, estimated that there have been 12 or 13 generations since the bottleneck. We found a similar degree of haplotype conservation in the region of the 167delT mutant allele. Using the degree of conservation between *GJB2* alleles and marker D13S141, and assuming a distance of 1 centimorgan between the two loci, we estimate that there have been seven generations since a population bottleneck.

The conservation of haplotypes surrounding the 167delT mutation suggests that this allele has a single origin. In contrast, the 30delG mutation is associated with several different haplotypes, suggesting that it has multiple origins. The likely explanation is that the 30delG allele has arisen spontaneously several times in Ashkenazi Jewish history. The 30delG mutation has been observed on different haplotype backgrounds in other populations as well, suggesting that it is recurrent and arises at a mutation hot spot associated with the string of six guanine residues at this site.^{12,19}

Identification of these two common mutations in the *GJB2* gene should improve genetic counseling in the Ashkenazi Jewish population. The combined carrier rate of 4.76 percent suggests an incidence rate of 0.6 per 1000 for recessive deafness due to mutant alleles of *GJB2* in the Ashkenazi Jewish population (with no consideration of possible assortative or consanguineous marriages). The most recent estimates of the incidence of all congenital deafness among Ashkenazi Jews is 1.2 per 1000, of which 38.1 percent is due to nonsyndromic recessive deafness.^{30,31} Thus, it appears that mutations in *GJB2* account for nearly all cases of nonsyndromic recessive deafness in this population. This conclusion is consistent with analyses of the distribution of deaf children among consanguineous Ashkenazi marriages, which show that as few as two to six genetic loci associated with the inheritance of nonsyndromic recessive deafness are present in the Ashkenazi population.^{30,32}

The 4.76 percent carrier rate is similar to that of other genes governing recessive diseases in the Ashkenazi Jewish population, such as Tay-Sachs disease (4 percent), Gaucher's disease (4 to 6 percent), and familial dysautonomia (3 percent).²⁸ For this reason, Ashkenazi Jews may elect to have their carrier status for mutations in the *GJB2* gene determined as part of genetic screening. An appropriate test would be one designed to detect both the 167delT and the 30delG mutant alleles.

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