July/August 2002 · Vol. 4 · No. 4

GJB2 (connexin 26) variants and nonsyndromic sensorineural hearing loss: A HuGE review

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Despite the enormous heterogeneity of genetic hearing loss, variants in one locus, *Gap Junction Beta 2* or *GJB2* (connexin 26), account for up to 50% of cases of nonsyndromic sensorineural hearing loss in some populations. This article reviews genetic epidemiology studies of the alleles of *GJB2*, prevalence rates, genotype-phenotype relations, contribution to the incidence of hearing loss, and other issues related to the clinical validity of genetic testing for *GJB2*. This review focuses primarily on three alleles: 167ΔT, 35ΔG, and 235ΔC. These alleles are recessive for nonsyndromic prelingual sensorineural hearing loss, and the evidence suggests complete penetrance but variable expressivity. ***Genet Med* 2002:4(4):258–274.**

Key Words: GJB2, *connexin 26, hearing loss*

Note: The term hearing loss *is used in this article instead of the term* hearing impairment, *which is considered to be pejorative by people who are deaf or hard of hearing.*[1] *We use the term* hearing loss *to include all levels of loss (mild to profound) and any age of onset including congenital losses. The word* deaf *refers to hearing status as determined by an audiogram. The word* Deaf *refers to the cultural community of people who are deaf and hard of hearing.*[2]

GENE

The *Gap Junction Beta 2* or *GJB2* gene (*GenBank* M86849, *OMIM*: *121011) resides at the chromosomal location 13q11 and encodes for the protein connexin 26, a beta class gap junction protein expressed in the cochlea and in the epidermis.[3] Connexin 26 hexamers form channels between cells that, when open, allow cell-to-cell diffusion of small molecules. This function is necessary for recycling potassium in the cochlea that plays a critical role in sensorineural hearing function.[4] The *GJB2* gene is small, with the entire coding region of 680 base pairs falling within exon 2.

GENE VARIANTS

Aided by the relatively small size of the *GJB2* gene, the flourish of activity on the genetics of hearing loss has resulted in rapid identification of *GJB2* variants. Missense, nonsense, frameshift, insertion, and deletion variants have all been reported. To identify published genetic epidemiology studies related to *GJB2*, we searched the MEDLINE database using the keywords *GJB2*, connexin 26, and hearing loss to identify relevant studies. References in related studies were also reviewed.

A list of *GJB2* variants is presented in Table 1. Some variants are benign polymorphisms, with a high prevalence rate in various populations. For example, the V27I, E114G, and I203T variants were found on 54%, 55%, and 94% of Japanese chromosomes, respectively.[5,6]

The 167ΔT, 35ΔG (also known as 30ΔG), 235ΔC, and R143W alleles are the most common hearing loss-associated *GJB2* alleles in the Ashkenazi Jewish, Caucasian, Japanese, and Ghanian populations, respectively. The best-characterized population is Caucasian of northern European descent. Table 2 presents the heterozygote carrier frequencies of the first three alleles either in the general population (hearing status unknown) or in control groups (without hearing loss). Ascertainment details were generally lacking and are listed in Table 2 as described in the publication. Likewise, descriptive information, including sex and age, were generally not provided. Despite these limitations, the studies consistently reported a prevalence of the 35ΔG allele in the range of 1% to 3%. In fact, one population-based study, which genotyped 560 randomly selected neonatal bloodspots in the Midwestern United States, detected a 35ΔG heterozygosity rate of 2.5% in this predominantly Caucasian population.[7]

In addition to Caucasians, published studies have focused on Mediterranean, Japanese, Korean, and Ashkenazi Jewish populations. The 35ΔG allele is common in individuals of Mediterranean descent (1 in 30) and *GJB2* testing has begun to be included in prenatal genetic counseling in Greece on a pilot basis.[8] Although the numbers were small, and ascertainment and demographic details were generally lacking, studies indicated that 10% of Ashkenazi Jews carry the 167ΔT allele, and 1% of Japanese and Korean individuals carry the 235ΔC allele (Table 2).

The 35ΔG, 167ΔT, and 235ΔC alleles are all recessive alleles associated with nonsyndromic prelingual hearing loss. No ho-

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Received: July 13, 2001.

Accepted: April 22, 2002.

DOI: 10.1097/01.GIM.0000020750.60733.CA

Table 1

Published *GJB2* variants with corresponding change in the connexin 26 protein and the putative allele type

Nucleotide change	Protein change	Putative allele type	Ref
−3712 G→A	Splice site		7
−3170 G→A	Splice site	Recessive	20
1 A→G	M1V	Recessive	44
	T8M	Recessive	30
31 Δ14	Frameshift		43
31 Δ 38	Frameshift		35
35 G→T	G12V	Recessive	70
35 ΔG	Frameshift	Recessive	71
35 insG	Frameshift	Recessive	44
51 Δ12 insA	Frameshift	Recessive	24
56 G→C	S19T	Recessive	70
59 T→C	I20T	Recessive	22
71 G→A	W24X		3
79 G→A	V27I	Polymorphism	49
90 T→A/C	I30I	Polymorphism	71
	R32C	Recessive	53
95 G→A	R32H		39
101 T→C	M34T		3
	I35S		41
109 G→A	V37I		49
122 A→G	K41R	Polymorphism	38
125 delAGG	E42Δ	Dominant and Vohwinkel syndrome	72
132 G→A	W44C	Dominant	18
132 G→A	W44X	Recessive	7
134 G→A	G45E	Recessive	5,6
139 G→T	E47X	Recessive	20
	E47K	Recessive	53
	C53R		41
167 ΔT	Frameshift	Recessive	72
169 C→T	Q57X	Recessive	45
176 C→G	G59A	Dominant and Vohwinkel syndrome	55
176 Δ16	Frameshift	Recessive	5,6
195 C→G	Y65X	Recessive	44
196 G→C	D66H	Dominant and Vohwinkel syndrome	54
215 C→G	S72C	Polymorphism	38
223 T→G	R75W	Dominant and Vohwinkel syndrome	56
229 T→C	W77R		73
231 G→A	W77X	Recessive	3
235 ΔC	Frameshift	Recessive	5,6
236 T→C	L79P	Recessive	25
249 C→G	F83L	Polymorphism	74
250 G→C	V84L	Recessive	3
251 T→C	V84A	Polymorphism	38
253 T→C	S85P	Recessive	38
267 C→A	L89L	Polymorphism	71
269 T→C	L90P	Recessive	20,43
269 insT	Frameshift	Recessive	20
283 G→A	V95M	Recessive	49
293 G→A	R98Q	Polymorphism	7
298 C→T	H100Y	Polymorphism	7
299 ΔAT	Frameshift	Recessive	34
304 ΔGAG	E100Δ	Recessive	75
312 Δ14	Frameshift	Recessive	35
316 Δ14	Frameshift	Recessive	49
333 ΔAA	Frameshift	Recessive	49
339 T→G	S113R	Recessive	49
341 A→G	E114G	Polymorphism	5
358 ΔGAG	E120Δ	Recessive	20
365 A→T	K122I	Recessive	7
367 A→G	T123A	Polymorphism	56
	T123N		41
370 C→T	Q124X	Recessive	74
380 G→A	R127H	Recessive	44
	R127C		41
384 C→T	I128I	Polymorphism	71
	E129K		30
408 C→A	Y136X	Recessive	5
416 G→A	S139N		39
427 C→T	R143W	Recessive	76
428 G→A	R143Q		22
445 G→A	A149T		70
457 G→A	V153I		39
465 T→A	Y1555X		22
478 G→A	G160S	Polymorphism	74
487 A→G	M163V		39
504 insAACG	Frameshift	Recessive	52
509 insA	Frameshift	Recessive	20
511 G→A			52
	P173R	Recessive	70
523 C→T	P175T	Recessive	20
533 T→C	V178T	Recessive	25
546 G→C	V182V	Polymorphism	74
551 G→C	R184P	Recessive	35,43
	R184Q	Dominant	74
589 G→T	A197S		25
596 C→T	S199F	Recessive	7
605 G→T	C202F	Dominant	36
608 TC→AA	I203K	Recessive	25
608 T→C	I203T	Polymorphism	5,6
617 A→G	N206S		30,39
631 ΔGT	Frameshift	Recessive	49
641 T→C	L214P	Recessive	25
645 ΔTAGA	Frameshift	Recessive	53
670 A→C	K224Q	Recessive	77

Table 2
Heterozygote rates of three *GJB2* mutations among control populations

Location	Ref	Description of control group[a]	DNA analysis method	No. of heterozygotes, heterozygote frequency mean, and 95% confidence intervals			N
				35ΔG	167ΔT	235ΔC	
Australia	41	Newborns (dried bloodspots) born in Victoria in May 1986	PCR-based; additional details unspecified	10 (1.0%) (0.48–1.83)			1,000
Belgium	78	Ascertainment not described; unrelated unaffected	PCR with restriction analysis[b]	9 2.50% (1.15–4.69)			360
Brazil	79	Randomly selected neonates	Allele-specific PCR[b]	6 0.97% (0.36–2.09)			620
Egypt	26	Ascertainment not described; unrelated random; 1:1 male:female ratio	PCR with restriction analysis or allele-specific hybridization[b]	0 0% (0–3.81)			95
	56	Individuals without skin disorders from general genetics clinic, 77 Egyptians and 17 Caucasians	PCR with restriction analysis[b]	1 (Egyptian) 1.3% (0.03–7.02)			94
Europe	26	Ascertainment not described; unrelated random; 1:1 male:female ratio	PCR with restriction analysis or allele-specific hybridization[b]	64 1.96% (1.51–2.49)			3,270
France	35	68 unrelated individuals (ascertainment not described) and 51 CEPH individuals	Sequencing	0 0% (0–3.05)	0 0% (0–3.05)	0 0% (0–3.05)	119
	80	Newborns (dried blood spots) born in 1990–1991	Allele-specific PCR[b]	14 2.73% (1.50–4.55)			512
Greece	81	Healthy blood donors aged 18–60 years (mean = 30.8)	Allele-specific PCR or PCR with allele-specific hybridization[b]	14 3.54% (1.95–5.88)			395
Israel—Ashkenazi Jewish	82	Samples from genetics lab	Sequencing	1 0.21% (0.01–1.19)	113 24.2% (20.3–28.1)	0 0% (0–0.79)	467
	33	Individuals undergoing genetic testing for carrier status for Tay-Sachs, etc.	PCR with restriction analysis[b]	1 1.09% (0.03–5.91)			92
					20 7.46% (4.62–11.29)		268
Italy	44	Ascertainment not described; unrelated general population	SSCP followed by sequencing if positive	6 4.0% (1.48–8.50)	0 0% (0–2.43)	0 0% (0–2.43)	150
Japan	34	Ascertainment not described; unrelated individuals known not to have noticeable hearing loss	Sequencing	0 0% (0–3.77)	0 0% (0–3.77)	2 2.08% (0.25–7.32)	96
	5	Ascertainment not described	Sequencing	0 0% (0–7.11)	0 0% (0–7.11)	0 0% (0–7.11)	50
	6	Ascertainment not described; unrelated individuals with normal hearing; 95 males, 108 females	Sequencing	0 0% (0–5.69)	0 0% (0–5.69)		63
						2 0.99% (0.12–3.51)	203
Korea	38	Blood samples from healthy newborns with normal OAE	Sequencing	1 1.00% (0.02–5.45)	0 0% (0–3.62)	1 1.00% (0.02–5.45)	100
Middle East—Jewish	26	Ascertainment not described; unrelated random; 1:1 male:female ratio	PCR with restriction analysis or allele-specific hybridization[b]	5 1.33% (0.43–3.08)			376
Oman	83	Ascertainment not described	PCR with restriction analysis[b]	0 0% (0–1.31)	0 0% (0–1.31)		280

—Continued

Table 2
Continued

Location	Ref	Description of control group[a]	DNA analysis method	No. of heterozygotes, heterozygote frequency mean, and 95% confidence intervals			N
				35ΔG	167ΔT	235ΔC	
Poland	84	Ascertainment not described; unrelated random; 1:1 male:female ratio	PCR with allele-specific hybridization[b]	3 2.00% (0.41–5.73)			150
Russia	85	Ascertainment not described; five ethnic groups: 194 Mari, 51 Komi, 154 Chuvashs, 106 Yakuts, 55 Bashkirs	PCR with gel electrophoresis[b]	12 2.14% (1.11–3.71)			560
Spain	44	Ascertainment not described; unrelated general population	Sequencing	3 2.31% (0.48–6.60)	0 0 (0–2.8)	0 0–2.8)	130
Tunisia	86	Ascertainment not described; unrelated general population	DGGE	3 1.27% (0.26–3.67)			236
Turkey	87	Individuals from unrelated research projects with no known hearing loss	PCR with restriction analysis[b]	12 1.78% (0.92–3.09)	0 0% (0–0.55)		674
U.S.A.—African American	26	Ascertainment not described; unrelated random; 1:1 male:female ratio	PCR with restriction analysis or allele-specific hybridization[b]	0 0% (0–1.92)			190
	28	Samples collected at Clinical Genetics Section of Michigan State University from individuals seeking counseling for disorders other than hearing loss	SSCP followed by sequencing if positive	0 0% (0–2.11)			173
					0 0% (0–2.13)		171
U.S.A.—Ashkenazi Jewish	28	Individuals undergoing genetic testing for carrier status for Tay-Sachs, etc.	SSCP followed by sequencing if positive	4 0.78% (0.21–1.99)			551
					22 4.03% (2.54–6.04)		546
	88		PCR with allele-specific hybridization[b]	7 0.69% (0.28–1.42)	40 3.95% (2.84–5.34)		1,012
U.S.A.—Asian	28	Samples collected at Clinical Genetics Section of Michigan State University from individuals seeking counseling for disorders other than hearing loss	SSCP followed by sequencing if positive	0 0% (0–6.85)	0 0% (0–6.85)		52
U.S.A.—Caucasian	28	Samples collected at Clinical Genetics Section of Michigan State University from individuals seeking counseling for disorders other than hearing loss	SSCP followed by sequencing if positive	1 0.58% (0.01–3.18)	0 0% (0–2.09)		173 175
U.S.A.—Midwest (primarily Caucasian)	7	Randomly selected neonatal bloodspots: 94.8% non-Hispanic white, 1.9% black, 1.7% Hispanic, 1.3% Asian and Pacific Islander, 0.3% American Indian, 0.2% Ashkenazi Jewish	Allele-specific PCR[b]	14 2.5% (1.37–4.16)			560
	49	Unrelated individuals known not to have noticeable hearing loss, self-assessed as primarily Caucasian of Northern and Southern European origin. No Asian, African or Native Americans were available	Heteroduplex analysis followed by sequencing if positive	2 2.08% (0.25–7.32)	0 0% (0–3.77)		96
	74	Ascertainment not described; random individuals	Allele-specific PCR and SSCP followed by sequencing if positive	1 1.00% (0.02–5.45)			100

SSCP, single-strand conformation polymorphism; OAE, otoacoustic emission; DGGE, denaturing gradient gel electrophoresis; CEPH, Centre Etude Polymorphisme Humaine.
[a]Age and sex unspecified unless noted otherwise.
[b]Method does not distinguish between heterozygotes and compound heterozygotes.

mozygotes for any of these three alleles have been reported in control groups. Some of the methods used tested only for specific alleles; therefore, distinguishing between heterozygotes and compound heterozygotes is not possible. This limitation affects the conclusions that can be drawn regarding penetrance in these studies. However, in the studies that did fully characterize the second allele in the control groups, no compound heterozygotes were reported. This finding suggests that for these three common alleles, i.e., 167ΔT, 35ΔG, and 235ΔC, penetrance of hearing loss in homozygotes is complete. However, larger population-based studies are needed to support this model and to characterize the penetrance of the less common alleles.

HEARING LOSS

"With over a million essential moving parts, the auditory receptor organ, or cochlea, is the most complex mechanical apparatus in the human body."[9] Given this complexity, it is not surprising that sequence variation in any one of hundreds of genes can lead to hearing loss. Hearing loss occurs in approximately 1 to 3 of 1,000 children,[10] and is generally attributed to pure genetic factors in approximately 50% of cases.[11] In approximately 30% of cases, a specific syndrome can be identified, with more than 400 syndromes claiming hearing loss as a component. The other 70% of cases are nonsyndromic.[11,12] Nonsyndromic cases may be familial or sporadic. The nature of familial nonsyndromic prelingual hearing loss is usually described as follows: 75% to 80% are autosomal recessive (designated with the prefix DFNB), 20% to 25% are autosomal dominant (DFNA), and 1% to 1.5% are X-linked (DFN).[13] The extraordinary genetic heterogeneity of hearing loss has been demonstrated by linkage studies, which indicated many loci for nonsyndromic hearing loss: 30 autosomal recessive, 29 autosomal dominant, and 8 X-linked.[14] Two mitochondrial variants, A1555G and A7445G, also have been implicated in nonsyndromic hearing loss. Several other mitochondrial mutations are associated with syndromic forms of hearing loss.[15] One of the autosomal recessive loci, DFNB1, was mapped to chromosome 13q12[16] and was attributed recently to the *GJB2* gene.[3] Although the majority of *GJB2* variants are recessive, dominant alleles have been identified that account for the DFNA3 locus mapped to the same region.[17,18]

ASSOCIATIONS

Contribution of *GJB2* to hearing loss

Given the extraordinary genetic heterogeneity of nonsyndromic hearing loss, it was surprising to find that sequence variations at the *GJB2* locus account for up to 50% of cases of nonsyndromic prelingual sensorineural hearing loss in some populations. A recent model to explain this observation is based on the tradition of intermarriage between individuals with hearing loss in some populations. A gradual increase in the proportion of hearing loss due to a hypothetical autosomal recessive mutation would be a consequence of this assortative mating.[19]

More than 90 variants of the *GJB2* gene have been reported, and many are rare. One variant generally predominates in any given population, such as 167ΔT in the Ashkenazi Jewish population, 35ΔG among Caucasians of northern European descent, 235ΔC in the Japanese population, and R143W in Ghana. Table 3 summarizes studies of the prevalence of various *GJB2* genotypes among individuals with prelingual hearing loss. The studies presented in this table vary in their ascertainment methods, case definitions, inclusion of presumably acquired cases, and genotyping methods. Because the genotype information that can be gleaned from the studies depends on the methodology, the data must be considered accordingly.

Twenty-two of the 30 studies in Table 3 used DNA sequence analysis to fully characterize both alleles in each individual. These studies provided the best estimate of the proportion of hearing loss cases associated with *GJB2* variants. The percentages of cases of prelingual hearing loss associated with two variants in *GJB2* (i.e., homozygotes or compound heterozygotes) for these studies were calculated (Table 3). Sequence variations at the *GJB2* locus were detected in approximately 20% of individuals with nonsyndromic prelingual sensorineural hearing loss in populations of Caucasians of northern European descent. *GJB2* variants were detected in approximately 5% of individuals with hearing loss in Korea, 14% in Australia, 17% in Tunisia, 20% in Japan, and 43% in Israel. Thus the contribution of *GJB2* variants to hearing loss varied between populations. Table 3 also demonstrates that the frequencies of the various *GJB2* alleles differed between the populations.

Most of these DNA-sequencing studies analyzed exon 2 of *GJB2*, which contains the entire coding region and 92 of the 94 variants described in Table 1. However, seven of the studies also sequenced exon 1, which contains the 3' untranslated region and the other two known variants.[7,20–25] Only one of these studies detected a sequence variation in exon 1,[20] suggesting that exon 2 analysis will detect most of the mutations in *GJB2* in individuals with hearing loss. However, studies with larger groups are necessary to determine the frequency of the variants found in exon 1.

Other studies used methods that detect only specific alleles, or reported data only on the major alleles, particularly 35ΔG. Because these data did not fully characterize the second allele, heterozygotes could not be distinguished from compound heterozygotes (Table 3). These studies also tended to include small numbers and generally lacked ascertainment and descriptive details. However, despite these limitations, the study results were consistent with those presented above and indicated that the 35ΔG allele accounts for approximately 10% to 20% of cases of hearing loss in Caucasians of northern European descent, but approximately 30% to 40% of cases in Mediterranean regions.

Table 3

Contribution of *GJB2* variants to hearing loss: Summary of *GJB2* genotypes characterized in cases of hearing loss (familial, non-familial,[a] and both)

Location	Ref	Case definition & methods[b]	Genotypes present			% of cases with two GJB2 variant alleles[c] (95% CI)
Australia	40	Nonsyndromic sensorineural mild to profound, unilateral or bilateral, hearing loss patients at a Pediatric Hearing Loss Investigation Clinic, excluding cases of known environmental exposure and cases of inner ear malformations, mean age = 7 years; sequenced exon 2	Mixed $N = 74$	3 35ΔG/35ΔG 1 35ΔG/V371 1 35ΔG/167ΔT 1 M34T/R184W 3 M34T/+	2 35ΔG/L90P 1 35ΔG/M34T 1 V37I/V37I 4 35ΔG/+ 1 V37I/+	13.5% (6.6–23.4)
	41	Children unilateral or bilateral nonsyndromic prelingual hearing loss seen at clinics in Melbourne and Sydney between January 1, 1998, and October 31, 2000; age 4 weeks to 16 years (median = 4 years); sequenced exon 2	Mixed $N = 243$	16 35ΔG/35ΔG 4 V37I/+ 2 35ΔG/L90P 2 35ΔG/M34T 2 35ΔG/W24X 1 35ΔG/W77R 1 35ΔG/R127C 1 M34T/R184W 1 T123N/T123N	10 35ΔG/+ 3 M34T/+ 2 35ΔG/V37I 2 35ΔG/C53R 1 35ΔG/167ΔT 1 35ΔG/R143W 1 V37I/V37I 1 W24X/I35S 1 333ΔAA/+	14.0% (10.0–19.0)
Austria	22	Sequential unrelated patients with nonsyndromic sensorineural hearing loss referred to centers for Hearing, Speech, and Voice Disorders in Austria; sequenced exons 1 & 2	Familial $N = 25$	4 35ΔG/35ΔG 1 L90P/R143Q 2 G160S/+	2 35ΔG/L90P 1 35ΔG/+	28.0% (10.4–45.6)
			Non-familial $N = 44$	3 35ΔG/35ΔG 1 L90P/314Δ14 1 L90P/+	1 35ΔG/V84L 1 L90P/I20T 1 35ΔG/+	13.6% (5.2–27.4)
Belgium & United Kingdom	89	Non-consanguineous nonsyndromic sensorineural hearing cases, excluding acquired cases; allele-specific PCR, followed by sequencing	Mixed $N = 68$	3 35ΔG/35ΔG 3 35ΔG/unknown 62 unknown/unknown		N/A
France	20[d]	Nonsyndromic sensorineural hearing loss patients recruited from consecutive individuals attending the genetic counseling service for deaf people at two Parisian hospitals between April 1997 and September 1998; aged 4 to 20 years; 73 females and 67 males; sequenced exons 1 & 2	Recessive[e] $N = 39$	10 35ΔG/35ΔG 1 35ΔG/−3170G→A	1 35ΔG/333ΔAA 1 35ΔG/509insA	28.6% (34.8–67.6)
			Non-familial $N = 49$	1 35ΔG/312Δ14 5 35ΔG/+	1 35ΔG/P175T 1 L90P/+	
	90[d]	Individuals with sensorineural hearing loss recruited from the genetic counseling service for individuals with hearing loss at the Pasteur Hospital and at the Armaud-Trousseau Children's Hospital, Paris, April 1997–September 1998; aged 4 years or greater; PCR with allele-specific hybridization	Mixed $N = 181$	50 35ΔG/35ΔF 47 35ΔG/unknown 84 unknown/unknown		N/A
France & United Kingdom	35	Prelingual hearing loss; sequenced exon 2	Familial (at least one affected sibling) $N = 47$	8 35ΔG/35ΔG 1 35ΔG/310Δ14	14 35ΔG/+	19.2% (9.2–33.3)
Germany	47	Individuals with symmetric moderate to profound hearing loss of unknown cause; 47 under age 12, 117 over age 12; PCR with allele-specific hybridization	Mixed $N = 164$	4 35ΔG/35ΔG 9 35ΔG/unknown 151 unknown/unknown		N/A
Ghana	25	Unrelated students with nonsyndromic profound sensorineural hearing loss at schools for the deaf in Ghana; individuals with known environmental risk factors were excluded; aged 6 to 20 years; sequenced exons 1 & 2	Unspecified $N = 365$	51 R143W/R143W 1 R143W/35insG 1 R143W/I203K 1 V178A/V178A 1 A197S/+	4 R143W/+ 1 R143W/L90P 1 R143W/L214P 1 R184Q/+	16.7% (13.0–20.9)

—Continued

Table 3
(*Continued*)

Location	Ref	Case definition & methods[b]	Genotypes present			% of cases with two GJB2 variant alleles[c] (95% CI)
Greece	77	Unrelated individuals with prelingual nonsyndromic sensorineural hearing loss who attended major referral centers for prelingual hearing loss in Greece; allele-specific PCR, DGGE, and sequencing	Familial (definition not reported) $N = 8$	2 35ΔG/35ΔG 4 35ΔG/unknown 2 unknown/unknown		N/A
			Non-familial $N = 18$	4 35ΔG/35ΔG 11 35ΔG/unknown 3 unknown/unknown		N/A
Israel	33	Nonsyndromic prelingual mild to profound hearing loss cases attending a Speech and Hearing Clinic, excluding cases of known environmental factors; sequenced exon 2	Non-familial $N = 9$	4 167ΔT/167ΔT 1 167ΔT/+		44.4% (13.7–78.8)
			Recessive[e] $N = 18$	8 167ΔT/167ΔT 1 167ΔT/+ 1 35ΔG/+	5 167ΔT/35ΔG 2 35 G/35 G	83.3% (58.6–96.4)
	24	Nonsyndromic prelingual mild to profound sensorineural hearing loss, excluding cases of infection, trauma, acoustic trauma, ototoxic drug use, rubella, or premature birth; sequenced exons 1 & 2	Non-familial $N = 29$	3 35ΔG/35ΔG 4 167ΔT/167ΔT	2 35ΔG/167ΔT 1 35ΔG/+	31.0% (15.3–50.8)
			Recessive[e] $N = 46$	8 35ΔG/35ΔG 4 167ΔT/167ΔT 1 51ΔTinsA/51ΔTinsA	3 35ΔG/167ΔT 3 35ΔG/+	34.8% (21.4–50.2)
Italy	91	Consecutive unselected children with nonsyndromic sensorineural hearing loss from an audiology service who were aged 1 to 6 years at onset; PCR with allele-specific restriction analysis	Mixed $N = 90$	7 35ΔG/35ΔG 36 35ΔG/unknown 47 unknown/unknown		N/A
	23	Nonsyndromic prelingual hearing loss greater than 40 dB from audiology and phoniatrics clinics, excluding individuals with known risk factors; SSCP and CSGE of exons 1 and 2, followed by sequencing if positive	Mixed $N = 94$	27 35ΔG/35ΔG 3 35ΔG/IVS+1 G→A 1 35ΔG/31Δ14 1 118ΔE/167ΔT 1 R184P/+ 2 M34T/+	5 35ΔG/Q47X 2 35ΔG/R184P 1 35ΔG/L90P 3 35ΔG/+ 1 31Δ14/+	41.5% (31.4–52.1)
	43	Nonsyndromic prelingual sensorineural hearing loss greater than 40 dB; aged 3 to 35 years, mean = 12 years; 21 females and 32 males; sequenced exon 2	Non-familial $N = 25$	8 35ΔG/35ΔG 1 167ΔT/E120Δ 1 R184P/+	2 35ΔG/E47X 3 35ΔG/+	44.0% (24.4–65.1)
			Recessive[e] $N = 17$	3 35ΔG/35ΔG 1 35ΔG/+	1 35ΔG/E47X 1 L90P/+	23.5% (6.8–49.9)
			Familial (at least one affected nonsibling relative) $N = 11$	5 35ΔG/35ΔG 1 314Δ14/+	1 35ΔG/L90P	54.6% (23.4–83.2)
Italy & Spain	44	Nonsyndromic prelingual moderate to profound hearing loss, includes cases of infection, oto-trauma, and ototoxic drug use; sequenced exon 2	Mixed $N = 92$	25 35ΔG/35ΔG 2 35ΔG/+	9 35ΔG/other	37.0% (27.1–47.7)
Japan	35	Nonsyndromic bilateral mild to profound sensorineural hearing loss with no inner ear malformation; sequenced exon 2	Mixed $N = 35$	2 235ΔC/235ΔC 2 235ΔC/R143W 1 G45E/299ΔAT 1 235ΔC/+	3 235ΔC/Y136X 1 R143W/176Δ16 1 R143W/V37I	28.6% (14.6–46.3)
	5	Nonsyndromic hearing loss; sequenced exon 2	Recessive[e] $N = 20$	3 235ΔC/235ΔC 1 235ΔC/+		15.0% (3.2–37.9%)
	6	Prelingual (onset before age 3) hearing loss, excluding cases of viral infections, meningitis, or ototoxic drug use; 16 females and 23 males; sequenced exon 2	Non-familial $N = 24$	1 235ΔC/235ΔC 1 235ΔC/176Δ16		8.3% (1.0–27.0)

—*Continued*

Table 3
(Continued)

Location	Ref	Case definition & methods[b]		Genotypes present		% of cases with two GJB2 variant alleles[c] (95% CI)
			Recessive[e] $N = 15$	2 235ΔC/235ΔC 1 176Δ16/Y136X		20.0% (4.3–48.1)
Korea	38	Nonsyndromic prelingual moderate to profound hearing loss patients from two rehabilitation schools and one out-patient otolaryngology clinic, excluding cases of meningitis, ototoxic drug use, or other known cause; sequenced exon 2	Mixed $N = 147$	5 235ΔC/235ΔC 1 235ΔC/299ΔAT 2 35ΔG/+ 1 T123N/+	1 S85P/S85P 4 235ΔC/+ 1 176Δ16/+ 1 R143W/+	4.8% (1.9–9.6)
Poland	84	Children with nonsyndromic profound sensorineural hearing loss seen at a Department of Audiology in Warsaw or from a High School for Deaf Children in Warsaw, excluding cases of known environmental exposures; sequenced exon 2	Unspecified $N = 102$	32 35ΔG/35ΔG 1 35ΔG/M34T 1 35ΔG/R184P	6 35ΔG/313Δ14 1 35ΔG/Q47X	40.2% (30.6–50.4)
Tunisia	86	Nonsyndromic mild to profound hearing loss; sequenced exon 2	Unspecified $N = 70$	10 35ΔG/35ΔG 1 E47X/47X	1 35ΔG/E47X	17.1% (9.2–28.0)
United Kingdom	21	Nonsyndromic prelingual (onset before age 3) sensorineural hearing loss cases ascertained from a variety of sources, including specialists in ENT, audiological medicine, and clinical Genetics, the Family Fund and the British Deaf Association, excluding cases of known environmental exposure; sequenced exon 1 & 2	Non-familial $N = 138$	9 35ΔG/35ΔG 5 35ΔG/+	2 35ΔG/167ΔT 1 35ΔG/W77R	8.0% (4.0–13.8)
			Recessive $N = 72$	16 35ΔG/35ΔG 1 35ΔG/302Δ13 1 167ΔT/167ΔT 1 469ΔG/+	1 35ΔG/310 14 10 35ΔG/+ 1 310Δ14/+	27.8% (17.9–39.6)
United States	7	Nonsyndromic prelingual sensorineural bilateral moderate to profound hearing loss cases sequentially accrued from hearing loss and cochlear implant referrals to otolaryngology clinic, excluding known acquired cases; sequenced exons 1 & 2	Mixed $N = 52$	11 35ΔG/35ΔG 1 35ΔG/+ 1 R98Q/+	6 35ΔG/other 1 M34T/+ 2 other/other	36.5% (23.6–51.0)
	49,64	Ascertainment not described; nonsyndromic sensorineural hearing loss; sequenced exon 2	Recessive[e] $N = 58$	14 35ΔG/35ΔG 1 35ΔG/V84L 1 35ΔG/S113R 2 167ΔT/+ 1 M34T/V95M	1 35ΔG/314Δ14 1 35ΔG/333ΔAA 3 167ΔT/167ΔT 1 167ΔT/631ΔGT 1 M34T/+	40.0% (27.0–53.4)
	42	Identified infants who were deaf or hard of hearing (definition not reported) through the Rhode Island statewide newborn hearing screening program over a 5-year period excluding syndromic cases and cases associated with known risk factors; PCR with sizing, PCR with allele-specific restriction, and sequencing	Mixed $N = 42$	3 35kG/35ΔG 1 35ΔG/+ 37 unknown/unknown	2 35ΔG//167ΔT	N/A
	53	209 consecutive individuals with congenital sensorineural hearing loss of unknown etiology referred for hearing loss or cochlear implant assessments, excluding syndromic, mild, unilateral, acquired, dominant, or consanguineous cases; allele-specific PCR, followed by SSCP, followed by sequencing	Mixed $N = 209$	24 35ΔG/35ΔG 31 35ΔG/unknown 154 unknown/unknown		N/A
	30	Children with hearing loss of unknown etiology who received clinical services at an outpatient otolaryngology clinic in Boston; age newborn to 18 years; sequenced exon 2	Mixed $N = 99$	4 35ΔG/167ΔT 3 167ΔT/+ 2 35ΔG/35ΔG 1 35ΔG/R143W 1 35ΔG/N206S 1 V84L/V84L 1 T8M/V153I 1 M34T/+ 1 E129K/+ 1 E47X/+	3 35ΔG/M34T 3 35ΔG/+ 2 35ΔG/E47X 1 35ΔG/G12V 1 167ΔT/167ΔT 1 V37I/V37I 1 V27I/+ 1 310Δ14/+ 1 L90P/+	18.2% (11.2–27.2)

—Continued

Table 3
(*Continued*)

Location	Ref	Case definition & methods[b]		Genotypes present		% of cases with two GJB2 variant alleles[c] (95% CI)
	52	Individuals with hereditary nonsyndromic hearing loss recruited from Universities in San Francisco and Baltimore, excluding cases of known environmental exposures; denaturing high-performance liquid chromatography of exon 2, confirmed by sequencing	Unspecified $N = 154$	4 35ΔG/35ΔG 1 167ΔT/167ΔT 1 504insAACC/235ΔC 24 heterozygotes or compound heterozygotes	3 V37I/V37I 1 299ΔAT/299ΔT	N/A
Venezuela	92	Children with prelingual sensorineural hearing loss evaluated for cochlear implants at two institutions in Caracas between November 1998 and May 1999; age less than 10 years; SSCP of exon 2	Unspecified $N = 42$	2 35ΔG/35ΔG 1 35ΔG/unknown		4.8% (0.6–16.2)

SSCP, single-strand conformation polymorphism; N/A, not applicable (i.e., cannot be calculated with data in this review); CSGE, conformation sensitive electrophoresis.
[a]Nonfamilial cases are cases with no family history of hearing loss. They may represent genetic (e.g., autosomal revessive) or nongenetic cases.
[b]Age and sex unspecified unless noted otherwise.
[c]Excludes carriers of putative polymorphisms: V27I, K41R, S72C, V84A, E114G,I203T. CI, confidence interval.
[d]Overlap of samples?
[e]Recessive cases are individuals with hearing loss who have at least one affected sibling and no affected parents.

Population differences in contribution of *GJB2* to hearing loss

As indicated above, there are population differences in the distribution of the various *GJB2* alleles. Despite that different alleles predominate in different populations, there is a relatively high carrier rate of *GJB2* alleles in all described populations. Furthermore, the carrier rate seems to be slightly higher in certain geographical areas, such as the Mediterranean region.[26,27] The cause of this high carrier rate is unknown.

A notable gap in the literature is the lack of assessment of the contribution of *GJB2* variants to hearing loss over a wide range of populations, as illustrated by the African American population as described below. Characterization of these populations is important to determine (1) the prevalence of *GJB2* variants among individuals with nonsyndromic hearing loss, and (2) the prevalence of the different alleles in the control populations. As demonstrated in Table 3 and discussed above, both of these measures appear to be population-specific.

The proportion of individuals with nonsyndromic hearing loss in African Americans who are carriers of *GJB2* variants has not been determined. However, two studies have looked for specific *GJB2* variants among African American control groups. The first group consisted of individuals receiving genetic counseling at the University of Michigan for disorders unrelated to hearing loss. This study tested 173 African Americans for the 35ΔG variant and 171 African Americans for the 167ΔT variant, and found no carriers of either allele.[28] The other study looked for 35ΔG variants among 190 African Americans (ascertainment details not reported) and found none.[26] These two studies indicated that 35ΔG is significantly less common among the African American population than it is among the Caucasian population (as described above and in Table 2). The rate of nonsyndromic hearing loss is not lower in African Americans than in Caucasians.[29] Two possible explanations for these data are (1) the proportion of cases of hearing loss attributed to *GJB2* variants is lower in African American than in Caucasian populations, and/or (2) *GJB2* alleles other than 35ΔG play a significant role in the African American population.

In support of the latter model, no individuals with the 35ΔG variant were found among 365 students with profound sensorineural hearing loss in Ghana. Likewise, the 167ΔT and 235ΔC variants were not found in this population. Of the 63 individuals in this study who carried *GJB2* variants, 51 (81.0%) were homozygous for the R143W allele and 8 (12.7%) were compound heterozygotes for R143W and a second variant allele.[25] Assessment of *GJB2* variants among non-Caucasian hearing loss and control populations are necessary to address these issues so that the clinical validity can be defined in these populations.

Type of hearing loss

Connexin 26 is expressed in the stria vascularis, spiral ligament, spiral limbus, and between the supporting cells in the cochlea,[3] and appears to function in the recycling of potassium that is used by the hair cells to generate an action potential in response to sound waves.[4] Consequently, it has generally been presumed that hearing loss associated with mutations in the *GJB2* gene will be sensorineural in nature. The nature of *GJB2*-related hearing loss has not been formally assessed by genetic epidemiologic methods. With one exception, the studies presented in this review either excluded conductive and mixed cases of hearing loss or did not distinguish between the different types of hearing loss. In the study of 99 unrelated children with hearing loss of unknown etiology who were attending an outpatient otolaryngology clinic in Boston, 30 were found to carry one or two *GJB2* mutations. Temporal bone abnormalities were identified in four of these individuals (35ΔG/167ΔT, 35ΔG/G12 V, L90P/+, and 35ΔG/+), and conductive or

mixed hearing loss was reported for one (E47X/+) and two (both 35ΔG/M34T) cases, respectively.[30] These associations may be coincidental, but additional studies are needed to describe the type of hearing loss associated with of *GJB2* variants.

Age of onset of hearing loss

GJB2 variants are generally described as causing prelingual hearing loss. However, in most published studies, it is not possible to distinguish between congenital (present at birth) and noncongenital prelingual hearing loss. Only one published study has examined the contribution of *GJB2* variants to congenital hearing loss. The prevalence of the 35ΔG genotypes in a Rhode Island newborn population with hearing loss did not differ from other American populations with hearing loss who were ascertained in childhood and who were of similar race/ethnicity (Table 3). More studies of this type, as well as studies including documented noncongenital prelingual hearing loss, are needed to assess the relationship between *GJB2* variants and congenital hearing loss. In this regard, the reports of newborns who passed the newborn hearing screen but in whom *GJB2*-related hearing loss was diagnosed later in infancy are notable.[31,32] Whether these cases represented false-negative results of the newborn hearing screening programs or indicated a late-onset and/or progressive nature of some *GJB2*-related cases of hearing loss is not clear. Likewise, Orzan et al. reported three Italian children with biallelic *GJB2* genotypes who had a sudden onset of hearing loss between 18 and 24 months of age, although it is not clear whether prior hearing status was formally documented or based on parental report.[23]

Recent research has not focused on rigorous analysis of the possible contribution of *GJB2* to postlingual hearing loss. Four published studies have included individuals with postlingual hearing loss. The first consisted of genetic analysis of *GJB2* among individuals recruited from consecutive patients at the genetic counseling service for deaf individuals at two hospitals in Paris. Of the participants, 43 of the 88 individuals with prelingual sensorineural hearing loss carried variations in the *GJB2* gene, but no changes were found among the 16 individuals with postlingual (before age 20) sensorineural hearing loss.[20] Likewise, a study in Israel ascertained individuals with nonsyndromic hearing loss (ascertainment details not reported) and tested them for *GJB2* variants. Of the 66 individuals with prelingual hearing loss, 25 were homozygous or compound heterozygous for *GJB2* variants, and 4 were heterozygous. No *GJB2* variants were found among the 11 cases of postlingual (definition not provided) hearing loss.[24] In Japan, 5 of 39 individuals with prelingual hearing loss were homozygous or heterozygous *GJB2* variant carriers, but no changes were found among the 39 individuals with postlingual (onset between 3 and 30 years) sensorineural hearing loss (ascertainment details not reported).[6]

The fourth study, taking place in Austria, found four carriers of *GJB2* variants among 16 individuals with postlingual (undefined) hearing loss.[22] The genotypes were L90P/I20T (onset in first decade), L90P/35ΔG (onset in first decade), 35ΔG/+ (onset in first decade), and G160S/+ (onset in fourth decade). The L90P allele is of interest in this population because it is seen in 2 of 16 postlingual (undefined) cases, and 3 of 53 prelingual cases of hearing loss. Thus this allele may be a significant contributor to postlingual hearing loss. The failure to detect *GJB2* variants in the other three studies may be due to a higher prevalence of the L90P allele in the Austrian population, as this allele was detected only rarely in individuals with hearing loss in France (2 of 135)[20,35] but not at all in Israel (0 of 102)[24,33] or Japan (0 of 94).[5,6,34]

Two dominant alleles have been specifically implicated in noncongenital hearing loss. The C202F allele was observed to cosegregate with postlingual (age of onset at 10 to 20 years) and progressive hearing loss in a 5-generation French family.[36] Likewise, the W44C allele cosegregated with progressive hearing loss in an American family of mixed Northern European descent, with age of onset ranging from infancy to age 18 years.[37] These alleles were not detected in studies that provided sequence data on controls, including 100 Korean newborns,[38] 209 Japanese individuals,[5,6,34] and 204 French individuals.[36,35]

These studies suggest that hearing loss associated with the more common *GJB2* sequence variations is likely to be prelingual. However, additional population-based studies involving individuals with congenital, noncongenital prelingual, postlingual, and late-onset hearing loss will be needed to fully assess the relationship between *GJB2* variants and age of onset, particularly in reference to the less common alleles.

Severity of hearing loss

Hearing loss associated with *GJB2* variations generally fall into the moderate to profound range. Three European studies have looked at the severity of hearing loss among children with and without *GJB2* sequence changes. In one of these studies, in France (ascertainment described above), *GJB2* homozygotes or compound heterozygotes accounted for 31 (55%) of 56 profound (≥ 90 dB) cases, 14 (48%) of 29 severe (70–89 dB) cases, 8 (42%) of 19 moderate (40–69 dB) cases, and 1 (14%) of 7 mild (20–39 dB) cases.[20] Of the 47 individuals who carried the 35ΔG/35ΔG genotype, the hearing loss was profound in 29 (62%), severe in 10 (21%), moderate in 7 (15%), and mild in 1 (2%).[39] The profile for individuals with one 35ΔG and one other allele was two profound (22%), three moderate (33%), three severe (33%), and one mild (11%) Although the latter group is small in size, the results are suggestive of variability in degree of hearing loss between alleles.

In 1999, a United Kingdom (U.K.) group ascertained a group of 284 individuals with nonsyndromic prelingual sensorineural hearing loss from several sources, including otolaryngologists, audiologists, clinical geneticists, and the British Deaf Associations.[21] They found biallelic *GJB2* carriers among 0 (0%) of 19 mild (20–39 dB) cases, 9 (10%) of 92 moderate (40–69 dB) cases, 11 (17%) of 64 severe (70–94 dB) cases, and 30 (30%) of 100 profound (≥95 dB) cases. The 35ΔG/35ΔG genotype was present in 6 individuals with moderate, 10 with severe, and 24 with profound hearing loss. Only two 167ΔT/167ΔT individuals were found in this study, and both had

moderate hearing loss. Two 35ΔG/167ΔT individuals were found: one with moderate and one with severe hearing loss.

Also in 1999, 94 individuals with nonsyndromic prelingual hearing loss were recruited from Italian audiology and phoniatrics services. Of these individuals with profound hearing loss (≥95 dB), 63% carried *GJB2* variant alleles as did 43% of individuals with severe (70–94 dB) and 33% of those with moderate (40–69 dB) hearing loss. Of the individuals with *GJB2* variant genotypes, 27 were homozygous for 35ΔG and 13 were compound heterozygotes. As in the French study discussed above, the 35ΔG homozygotes fell into the moderate or profound range, whereas the compound heterozygotes were dispersed among the three categories (moderate, severe, and profound), suggesting allelic difference in expressivity.[23]

Seven additional studies presented data regarding the severity of hearing loss among individuals with *GJB2* variants. Because the number of alleles and, therefore, the number of possible genotypes was large, the absolute numbers of cases for each genotype in the studies combined were small. Therefore, the data presented here focus on the 35ΔG/35ΔG and 167ΔT/167ΔT genotypes. Two Israeli studies,[24,33] two Australian studies,[40,41] one Austrian study,[22] and two American studies[30,42] described the level of hearing loss among individuals with *GJB2* mutations. In the seven studies combined, information was presented on 50 people with the 35ΔG/35ΔG genotype: 8 moderate (16%), 12 severe (24%), and 30 profound (60%). Likewise, of the 30 people with the 167ΔT/167ΔT genotype, one had mild hearing loss (3.3%), 5 moderate (16.7%), 8 severe (26.7%), and 16 profound (53.3%). These data are consistent with the above reports, and indicate that the hearing loss associated with the 35ΔG/35ΔG and 167ΔT/167ΔT genotypes is generally in the moderate to profound range, with profound hearing loss being the most common manifestation.

Although these data suggest that *GJB2* variants tend to be associated with moderate to profound hearing loss, the numbers were small, dB ranges of degrees of hearing loss varied among the studies, and the specific relationship between various *GJB2* alleles and severity of hearing loss were not addressed. In addition, the nonpopulation-based approach may have resulted in underascertainment of mild hearing loss. However, the low prevalence of *GJB2* biallelic genotypes among the individuals with mild hearing loss in the British[21] and French[20] studies described above suggested that *GJB2*-associated hearing loss, particularly with the 35ΔG and 167ΔT alleles, tends to be moderate to profound. Likewise, *GJB2* biallelic individuals have not been described in the general hearing population. On the other hand, the Australian group described three individuals with mild hearing loss (25–40 dB) with less common *GJB2* genotypes: M34T/R184W, 35ΔG/M34T, and 35ΔG/V37I.[40] Likewise, the Austrian study reported three individuals with mild hearing loss: L90P/314Δ14, Y155X/+, and G160S/+.[22] It is possible that carriers of these alleles do not always have hearing loss, but because these alleles are less common than the 35ΔG and 167ΔT alleles, larger population-based studies are needed to address this issue.

Laterality of hearing loss

The inter-ear difference in severity of hearing loss was described for 54 French children with biallelic *GJB2* genotypes. In 48 (89%) of the children, the severity did not differ between the ears. In the other six (11%), the ears differed by one degree of severity (dB ranges described above).[20] These children were ascertained through genetic counseling services for deaf individuals at two hospitals in Paris. Therefore, individuals with unilateral hearing loss may have been underascertained. The study included two children with two degrees, and one child with three degrees of difference in severity, none of whom carried *GJB2* variants. However, the number of children in these groups was clearly small.

In an analysis of children with nonsyndromic prelingual hearing loss ascertained through Italian audiology services, more than 90% of the 46 children with *GJB2* variants demonstrated a symmetrical hearing loss (inter-ear difference of <15 dB at two frequencies or 10 dB at four frequencies). In this study, *GJB2* variants were detected in 43 of 75 (66%) individuals with symmetrical hearing loss but in only four of the 19 (21%) of those with asymmetrical hearing loss.[23]

In the U.K. study,[21] individuals were ascertained through a variety of sources, including otolaryngologists, clinical geneticists, and Deaf associations. The data were presented as average dB difference in hearing loss between the ears: 6.33 (N = 45, SD = 8.09) for *GJB2* homozygotes and compound heterozygotes, 6.66 (N = 26, SD = 8.05) for heterozygotes, and 7.86 (N = 175, SD = 11.19) for individuals without *GJB2* variations. There was no significant difference between any of these groups.

In a group of consecutive individuals with sensorineural hearing loss seen at a center for Hearing, Speech, and Voice Disorders in Austria, 24 individuals with *GJB2* variant genotypes were identified. Of these, five displayed asymmetry of hearing loss: three by two degrees and two by one degree of severity.[22]

In all of these studies, the ascertainment of individuals with unilateral hearing loss is unclear. Therefore, although the described *GJB2* variants tend to be associated with bilateral hearing loss, population-based data on all individuals with any type of hearing loss are needed to clarify the issue.

Progression of hearing loss

Longitudinal data are lacking on individuals with *GJB2*-related hearing loss. Follow-up on individuals with *GJB2*-associated hearing loss over 1 to 20 years indicated no changes in the threshold of hearing loss.[43] However, details about the number of such cases and the timing of repeated testing were not published.

The French group studied children ascertained through genetic counseling services for the deaf in Paris, and described 16 children with biallelic *GJB2* genotypes for whom test results were available over a 10-year span. In 11 children, no change (≤5 dB) in the threshold was noted. Three children (one with severe and two with profound hearing loss) showed slight pro-

gression (5–10 dB). Two children (one with moderate to severe, and one with profound hearing loss) had a progression >10 dB.[20]

Likewise, a retrospective analysis of audiograms (over 2 to 15 years) in Italian children ascertained through audiology services detected a progression of hearing loss in only 1 child of 47 who had a *GJB2* variant genotype. Progression was defined as a >15 dB change in two or more frequencies, or a >10 dB change over an average of four frequencies.[23]

Among 24 Austrian individuals with *GJB2*-related sensorineural hearing loss, 3 were described as progressive in nature, although the definition was not provided.[22] Thus the limited data suggest the *GJB2*-related hearing loss is primarily nonprogressive in nature.

High-frequency hearing loss

Audiograms of individuals with biallelic *GJB2* genotypes tend to be flat or slightly descending, indicating equal loss across all frequencies.[20–24,38,40,41,43–47] Two individuals with the 35ΔG/L90P genotype have been described with high-frequency (2,000–8,000 Hz) hearing loss.[40] This finding suggested that certain alleles, other than the more common and well-studied 35ΔG and 167ΔT, may be associated with high-frequency hearing loss. Most individuals with *GJB2* variants have been ascertained through services for individuals with hearing loss, and many of the studies did not assess the high-frequency (2,000–8,000 Hz) range. Thus the contribution of *GJB2* variants to high-frequency–only hearing loss has not been well-studied.

M34T allele and hearing loss

In 1997, the M34T variant was reported to cosegregate with three generations of hearing loss in one family in an apparently dominant manner, implicating *GJB2* in nonsyndromic hearing loss.[3] Several years later, a second variant was characterized in this family, found *in trans* with the M34T allele in the individuals with hearing loss. This finding suggested that the M34T allele is recessive.[48] On the other hand, the M34T allele failed to cosegregate with hearing loss in several families, raising the possibility that M34T is a benign polymorphism.[36,49–51]

The prevalence of M34T heterozygote carriers and compound heterozygotes among individuals with hearing loss and control groups is summarized in Table 4. The M34T allele is present in approximately 2% to 3% of the general Caucasian population, but has not been reported in Japan or Korea. Data on other populations are limited.

A similar prevalence of the M34T alleles was seen among Caucasian individuals with hearing loss, supporting the model that the M34T allele is a benign polymorphism. However, the M34T allele was sometimes found as a compound heterozygote among individuals with hearing loss,[7,30,34,39,46,49–52] and more rarely, in the homozygous form in individuals with hearing loss.[39,51,53] No changes have been reported in the other allele among the M34T carriers in the control groups. Thus, the evidence appears to support the hypothesis that M34T is a recessive allele, although the lack of compound heterozygotes in the control groups may be due to their smaller sample sizes. This example demonstrates the importance of looking at data on genotypes rather than allele frequency. In addition, a recent report of linkage between M34T and an upstream 10-base pair deletion raised the possibility that the association between M34T and hearing loss may be due to linkage disequilibrium.[51] Additional studies are needed to clarify the involvement of the M34T allele in hearing loss.

GJB2 variants and Vohwinkel syndrome

The vast majority of *GJB2* variants are associated with nonsyndromic hearing loss (see Table 1). Ironically, the original report of a *GJB2* allele associated with hearing loss occurred in a family that also displayed palmoplantar keratoderma (PPK). PPK is a form of hyperkeratosis in which the overgrowth is limited to the palms of the hands and the soles of the feet. The M34T allele cosegregated with hearing loss but not PPK in this family.[3] Subsequently, it was shown that the PPK in this family was due to a D66H variant in the *GJB2* gene, a variant not seen in the 122 unrelated controls.[48] The combination of dominant sensorineural hearing loss and hyperkeratosis is also known as Vohwinkel syndrome (OMIM: 124500), and hearing loss with PPK appears to be a mild variant. The D66H allele has been implicated in this syndrome in three additional families.[54] Neither group detected the D66H allele among the control groups of 122 and 145 unrelated individuals.

Likewise, the G59A variant cosegregated with Vohwinkel syndrome in a three-generation family. The G59A allele was not detected among 50 hearing controls or among 55 individuals with nonsyndromic hearing loss.[55]

The R75W variant was described in an Egyptian family with autosomal PPK and congenital deafness. It was also detected in one individual in the control group of 77 Egyptian individuals attending a clinic for reasons unrelated to skin disorders; however, the hearing status of this control individual is unknown. R75W was not found in the 17 Caucasian controls.[56]

Several other studies included sequence information about control individuals, and none of them detected any carriers of D66H, G59A, or R75W. The studies included 100 Korean newborns,[38] 209 Japanese individuals,[5,6,34] and 119 French individuals[35] (ascertainment details provided in Table 3). The numbers of controls examined in these studies were small and did not necessarily come from the same population as the cases; therefore, we cannot rule out the possibility that these alleles may present in the general population at a low frequency due to incomplete penetrance.

INTERACTIONS

Many study groups reported variations in the degree of hearing loss in individuals with the same genotype (see the Severity of Hearing Loss section above), even within sibships.[20,22,24,33,41,43] For example, one Israeli family consisted of five siblings with the 167ΔT/167ΔT genotype; three had profound (≥90 dB), one had severe (70–89 dB), and one had mild (20–39 dB) hearing loss.[33] Likewise, in the French report of 16

Table 4

Prevalence of M34T heterozygote carriers and compound heterozygotes in cases and controls in various geographical areas (raw numbers, frequencies, and 95% confidence intervals)

		General population		People with hearing loss	
Location	Reference	M34T/+	M34T/other	M34T/+	M34T/other
France	20			0/88 0% (0–4.1)	0/88 0% (0–4.1)
	7	3/128 2.3% (0.5–6.7)	0/128 0% (0–2.8)		
	39	1/116 0.9% (0.0–4.7)	0/116 0% (0–3.1)	3/96 3.1% (0.6–8.9)	1/96 1.0% (0.0–5.7)
Japan	34	0/96 0% (0–3.8)	0/96 0% (0–3.8)	0/35 0% (0–10.0)	0/35 0% (0–10.0)
	6	0/63 0% (0–5.7)	0/63 0% (0–5.7)	0/39 0% (0–9.0)	0/39 0% (0–9.0)
Korea	38	0/100 0% (0–3.6)	0/100 0% (0–3.6)	0/147 0% (0–2.5)	0/147 0% (0–2.5)
United Kingdom & Ireland	51	25/630 4.0% (2.6–5.8)	0/630 0% (0–0.6)	7/173 4.0% (1.6–8.2)	3/173 1.7% (0.4–5.0)
	48	0/40 0% (0–8.8)	0/40 0% (0–8.8)		
	21			0/210 0% (0–1.7)	0/210 0% (0–1.7)
United States	7			1/52 1.9% (0.1–10.3)	1/52 1.9% (0.1–10.3)
	49	3/96 3.1% (0.6–8.9)	0/96 0% (0.3–8)	2/58[a] 3.4% (0.4–11.9)	
	30			1/30 3.3% (0.1–17.2)	3/30 10.0% (2.1–26.5)
	52			3/154[a] 3/154[a] 2.0% (0.4–5.6)	
	53			0/209 0% (0–1.8)	2/209 1.0% (0.1–3.4)
	74	1/100 1.0% (0.0–5.4)	0/100 0% (0–3.6)		

[a]Publication did not distinguish between heterozygotes and compound heterozygotes.

children with biallelic *GJB2* genotypes, the degree of hearing loss differed between the siblings in 50% of the families.[20] This finding suggests that other factors, genetic and/or environmental, may be modifying the phenotypic outcome.

Individuals who are carriers of a single variation in *GJB2* display evidence of reduced hair cell function[57]; therefore, it is possible that these individuals are more likely to develop hearing loss in the presence of additional genetic or environmental factors than are noncarriers. This possibility is supported by recent reports of mutations in other genes found at increased incidence among *GJB2* carriers with hearing loss: *GJB6* (connexin 30)[58] and the mitochondrial 12S rRNA.[59] Additional studies of this nature are expected to continue to characterize the gene-gene interactions involved in the etiology of *GJB2*-associated hearing loss.

Many of the studies in this review excluded cases of suspected environmental causes from the genetic analysis. These factors included infections (e.g., meningitis, rubella), low birth weight, ventilator use, ototoxic medications (e.g., aminoglycosides), and hyperbilirubinemia. However, two individuals with hearing loss attributed to rubella infection were later found to be homozygous for the 167ΔT variant.[32] Thus, the presence of known environmental factors does not necessarily preclude genetic analysis. Indeed, the proportion of *GJB2* cases that have been attributed to other causes has not been elucidated; therefore, the possibility of gene-environment interactions has not been examined.

Likewise, published studies have generally excluded cases of syndromic hearing loss from *GJB2* analysis. Thus the possibility that *GJB2* variants may be involved in the penetrance and expressivity of hearing loss due to syndromic causes has not been examined.

LABORATORY TESTS

Many DNA-based methods are available for detecting the various alleles reported for the *GJB2* gene. Assays have been developed to rapidly test for specific common variants, including allele-specific polymerase chain reaction (PCR), PCR followed by restriction enzyme digestion, and PCR with allele-specific hybridization. These technologies, once analytically validated in the performing laboratory, are both highly sensitive and specific. However, they will only detect the allele for which they were designed. As some common alleles account for the majority of variants in some populations (e.g., 35ΔG in Greece), these methods offer rapid and economical approaches. They also provide simple and reliable methods for carrier testing in families with known alleles.

Scanning methodologies are often used for allele detection, including denaturing gradient gel electrophoresis, single-strand conformation polymorphism detection, heteroduplex analysis, and denaturing high-performance liquid chromatography. Although scanning technologies have the advantage of screening for many variants at once, they tend to be less reliable than the allele-specific PCR-based techniques in that they are more sensitive to laboratory conditions. They also will miss some alleles, the specific alleles being detected dependent on the method and conditions.

Sequencing of PCR products of the *GJB2* gene is a common approach that has the advantage of detecting most alleles, including novel changes. Of the 94 known variants described in Table 1, all but 2 are in exon 2. Both exons 1 and 2 are small and amenable to PCR amplification. Sequencing of exon 1 will pick up these remaining three alleles. As described in the Contribution of *GJB2* to Hearing Loss section, only a few published studies have used the method of sequencing both exons 1 and 2. Therefore, information is lacking to accurately determine the relative clinical validity of these two methods.

Laboratories offering clinical testing for *GJB2* vary in their methodologies of choice (Kenneson et al., unpublished). Clinical validity thus varies accordingly.

POPULATION TESTING

Consistent with the recommendations of the Joint Committee on Infant Hearing,[60] a growing number of states are screening newborns for audiologic function so that infants with hearing loss are identified and referred for intervention services very early in life. Some newborn hearing screening programs may in the near future refer individuals for genetic testing for *GJB2* variants as part of follow-up services. The role that *GJB2* testing will play in conjunction with universal newborn hearing screening programs has not yet been defined. Population-based studies are needed to determine the contribution of *GJB2* variants to congenital hearing loss, as well as the association between *GJB2* variants and progressive hearing loss.

A continuing challenge for laboratories has been the interpretation of novel sequence variants that may have clinical relevance. In recognition of the need, the American College Medical Genetics (ACMG) has published recommendations for interpreting sequence variants of questionable clinical relevance.[61] The report cautions laboratorians to develop any interpretation made based on what is known not only about the sequence variant but also the individual's chance of having the condition, family history, other test results, and the sensitivity and specificity of the test being performed. As *GJB2* testing is used more often in the evaluation of children with hearing loss, interpretation of uncommon and novel mutations will be necessary.

Genetic tests are often offered for clinical use before the clinical validity and utility are fully understood.[62,63] Because this is the case for *GJB2* testing, research participants need to understand the distinction between genetic research, testing, and screening. The identification of *GJB2* variants in infants with hearing loss may prove to have many clinical purposes, including (1) ruling-out risk of syndromic complications, (2) predicting moderate to profound hearing loss requiring aggressive language intervention, (3) indicating sensorineural hearing loss for which cochlear implants may be an intervention option for consideration, and (4) allowing genetic counseling regarding recurrence rates.[42,64,65] The current literature

is not sufficient for a careful review of all of these potential uses of *GJB2* testing. Consequently, the child's course of intervention may not be significantly altered by the knowledge of *GJB2* genotype at the present time. Although the genetic information may be useful to the family, genetic testing of minors is generally not accepted in the absence of direct intervention benefits for the child.[66] However, as more information is collected about *GJB2*-related hearing loss, and the above-mentioned potential uses are evaluated, *GJB2* testing may find a place in medical services that goes beyond reproductive counseling issues.

Genetic testing related to hearing loss is particularly ridden with complex ethical issues. For example, although the ACMG recommends providing genetic services to individuals with hearing loss "to establish the etiology whenever possible,"[67] individuals with hearing loss often argue that genetic testing will devalue individuals with hearing loss.[68] Furthermore, people with hearing loss often have different attitudes and beliefs about genetic testing for hearing loss which in most cases is reflective of different perspectives. One study surveyed parents with normal hearing who have one or more deaf children and demonstrated an overwhelmingly positive attitude toward genetic testing for hearing loss (96%).[69] On the contrary, a survey administered to a group of delegates attending a conference on the "Deaf Nation" reported that 55% thought that genetic testing would do more harm than good and 46% responded that its potential use devalued people with hearing loss.[68] The issues raised by the Deaf community provide a unique opportunity by challenging scientists and society to find culturally sensitive methods for genetic research and testing that are acceptable to all cultural groups.

Acknowledgment

This project was supported in part under a cooperative agreement from the Centers for Disease Control and Prevention through the Association of Teachers of Preventive Medicine.

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APPENDIX

Statements

- National Institute on Deafness and Other Communication Disorders (NIDCD) Working Group Considerations for Developing and Implementing Genetic Diagnostic Tests for Hereditary Hearing Impairment and Other Communication Disorders (December 1998): www.nidcd.nih.gov/textonly/funding/hb/genetic.htm
- Statement of the American College of Medical Genetics on Universal Newborn Hearing Screening (January 2000): www.faseb.org/genetics/acmg/pol-35.htm

Links

Hearing and hearing loss resources

- Connexin 26 Homepage: www.iro.es/deafness/
- GeneClinics: Deafness Overview: www.geneclinics.org/profiles/deafness-overview/details.html
- Hereditary Hearing Loss Homepage: dnalab-www.uia.ac.be/dnalab/hhh/index.html
- National Institute on Deafness and Other Communication Disorders: www.nidcd.nih.gov/health/hb.htm
- Promenade 'round the Cochlea: www.iurc.montp.inserm.fr/cric/audition/english/index.htm
- The Genetics of Infant Hearing Loss: www.cdc.gov/ncbddd/ehdi/genetics.htm

General resources

- Online Mendelian Inheritance in Man: www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
- GenBank: www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html
- Human Gene Mutation Database: archive.uwcm.ac.uk/uwcm/mg/hgmd0.html