

## Performance of Cochlear Implant Recipients With *GJB2*-Related Deafness

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**Congenital profound hearing loss affects 0.05–0.1% of children and has many causes, some of which are associated with cognitive delay. For prelingually-deafened cochlear implant recipients, the etiology of deafness is usually unknown. Mutations in *GJB2* have been established as the most common cause of heritable deafness in the United States. In this report, we identify cochlear implant recipients with *GJB2*-related deafness and examine the performance of these individuals. Cochlear implant recipients received a battery of perceptive, cognitive, and reading tests. Neither subjects nor examiners knew the etiology of deafness in these individuals. The implant recipients were then examined for mutations in *GJB2* using an allele-specific polymerase chain reaction assay, single-strand conformation polymorphism analysis, and direct sequencing. *GJB2* mutations were the leading cause of congenital deafness among the cochlear implant recipients screened. Cochlear implant recipients with *GJB2*-related deafness read within one standard deviation of hearing controls better than other congenitally deaf cochlear implant recipients and non-**

**cochlear implant recipients. Individuals with congenital deafness should be offered *GJB2* screening. Positive results establish an etiologic diagnosis and provide prognostic, genetic, and therapeutic information. Effective rehabilitation for profoundly deaf individuals with *GJB2*-related deafness is possible through cochlear implantation.**

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### INTRODUCTION

Despite outstanding achievements by several deaf individuals, a good outcome is not assured for children with congenital hearing loss. A wide array of prospects may be reasonably expected based on the multiple different etiologies of deafness. Deafness from congenital cytomegalovirus, for example, is associated with particularly poor reading and mathematics performance. On the Stanford Achievement Test, 44% of individuals with congenital cytomegalovirus infection scored in the lowest quartile of deaf individuals [Schildroth, 1994]. Many forms of syndromic deafness are also linked with cognitive delay [Gorlin et al., 1995]. While it is easy to presume that the etiology of non-syndromic deafness may affect rehabilitation potential after cochlear implantation, this area remains largely unexplored.

Recent advances in the genetics of congenital severe-to-profound hearing loss improve our ability to specify the etiology of deafness for many children. Despite the heterogeneity of genetic deafness, mutations in the gene encoding connexin 26 (*GJB2*) are responsible for half of severe-to-profound autosomal-recessive nonsyn-

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dromic deafness in Caucasian families originating from the United States, France, Britain, Tunisia, and New Zealand, suggesting that mutations in *GJB2* are the most common cause of congenital hereditary hearing loss in these countries [Van Camp et al., 1997; Zelante et al., 1997; Kelley et al., 1998; Green et al., 1999]. Unlike several forms of congenital deafness, *GJB2*-related deafness has no known comorbidity. Individuals walk at a normal age and appear to have normal vestibular function. Although connexin 26 is present in the brain, as well as the cochlea, no neurologic problems are associated with this type of deafness [Denoyelle et al., 1997].

We have examined cochlear implant recipients, identifying children with *GJB2*-related deafness, to assess the cognitive abilities and performance of congenitally deaf children in relation to the etiology of deafness. Results suggest that genetic screening for *GJB2* mutations carries diagnostic, prognostic, and counseling importance.

## MATERIALS AND METHODS

### Subject Accrual

The accrual source consisted of 92 prelingually deaf children who underwent cochlear implantation at the University of Iowa. All recipients were younger than 19 years. In 47 recipients, the etiology of deafness was unknown; of this subgroup, 20 individuals participated in this study. The University of Iowa Human Subjects Committee approved all procedures.

### Cochlear Implant Recipients

Cochlear implant recipients received an array of medical, radiologic, cognitive, and audiologic testing. Intelligence and behavior assessments were completed to identify characteristics of children that might provide insight into successful habilitation with a cochlear implant. All children had intelligence and psychosocial behavior profiles within the range of normal. Audiologic assessments indicated that all children were profoundly deaf and unable to benefit from conventional amplification. Speech perception skills were measured a minimum of three years post-operatively using the Word Intelligibility by Picture Identification test (WIPI); the Vowel Perception test (VP); the Phonetically-Balanced Kindergarten Word list (PBK-wd) and phoneme score (PBK-ph); the Monosyllable, Trochee, Spondee test (MTS); and the Four-choice Spondee Test (FST) [Fryauf-Bertschy et al., 1997]. Reading performance was measured using the paragraph comprehension subtest of the revised Woodcock Reading Mastery test and compared to children with normal hearing [Woodcock, 1997].

### *GJB2* Screening

DNA was extracted from 5 ml of whole blood obtained by peripheral venipuncture from each participant. DNA samples were screened for mutations in *GJB2* using a combination of allele-specific polymerase chain reaction

(ASPCR) and single strand conformational polymorphism analyses [Green et al., 1999]. The most common mutation in *GJB2*, 35delG, was screened by ASPCR. Homozygotes for this mutation were diagnosed as having *GJB2*-related hearing loss and no other studies were performed. 35delG heterozygotes were screened by both single strand conformational polymorphism (SSCP) analysis and direct sequencing to determine if a second mutation was present. If no 35delG alleles were identified by ASPCR, the *GJB2* coding sequence was screened by SSCP and sequencing was performed on samples with SSCP shifts. In all cases with only a single coding sequence mutation, the non-coding exon of *GJB2* (exon 1) was sequenced. PCR products were cleaned using a QIAquick PCR purification kit (Qiagen, Chatsworth, CA) and directly sequenced.

### Statistical Comparison

The performance scores of individuals with *GJB2*-related deafness were compared to the scores of individuals with *GJB2*-unrelated deafness. For categorical data, the Fisher exact test was used to compare the groups. For scaled data, the Student's *t*-test was used. Evaluation also was performed, including test age and duration of implant, to exclude these factors as confounding variables.

## RESULTS

Mutations in *GJB2* were the most common identified etiology of congenital deafness in children receiving cochlear implants. Eight of twenty (40%) cochlear implant recipients with an unknown etiology of deafness had deafness-causing mutations in *GJB2*. Of the three cochlear implant recipients with a deaf sib, two had *GJB2*-related deafness. The most common mutation in *GJB2* within the cochlear implant population was 35delG, and all cochlear implant recipients with deafness from *GJB2* mutations carried this mutation (four were 35delG heterozygotes and four were 35delG homozygotes). SSCP screening of the *GJB2* coding sequence (exon 2) was positive in three of four heterozygotes, and in the remaining individual, a mutation was found in exon 1. Sequencing identified the second mutation at the nucleotide level in each person (167delT, 269^270insT, W77R, and IVIS1+1G-A). SSCP also identified two additional individuals with benign polymorphisms (M34T/+, R98Q/+) unrelated to their deafness [Green et al., 1999] (Table I).

Cognitive assessment of cochlear implant recipients was similar in persons with *GJB2*-related deafness when compared to persons with *GJB2*-unrelated deafness (Hiskey-Nebraska test of learning aptitude, 109 vs. 94 [ $P=0.474$ ]). A trend toward slightly poorer performance on speech recognition parameters was noted in the *GJB2*-mutation group, but none of the test differences was significant: WIPI, 61 vs. 73 ( $P=0.36$ ); VP, 84.4 vs. 88.3 ( $P=0.55$ ); PBK-wd, 24.5 vs. 33.1 ( $P=0.53$ ); PBK-ph, 44 vs. 55 ( $P=0.44$ ); MST word, 80 vs. 90 ( $P=0.34$ ); MST pattern, 88 vs. 95 ( $P=0.27$ ); FST, 90 vs. 95 ( $P=0.38$ ). Inclusion of age at testing and

TABLE I. *GJB2* Mutations Identified in 20 Cochlear Implant Recipients

Deafness-causing mutations <sup>a</sup>	Benign polymorphisms <sup>b</sup>
35delG/35delG	R98Q/+
35delG/35delG	M34T/+
35delG/35delG	
35delG/35delG	
35delG/W77R	
35delG/167delT	
35delG/269~270insT	
35delG/TVIS1 + 1G-A	

<sup>a</sup>Two mutations were found in all patients with *GJB2*-related deafness.

<sup>b</sup>In those patients with benign polymorphisms, the second allele was the standard wild type (+).

duration of implant as potentially confounding variables did not substantively alter these results (Table II). All cochlear implant users demonstrated improved speech perception skills compared to pre-operative conditions, with a trend for continued improvement with time.

Reading performance was more consistent in the *GJB2*-mutation group. Seven of the eight patients with *GJB2*-related deafness with sufficient follow-up to obtain reading scores read within one standard deviation of age level or better (compared with 78% of cochlear implant recipients with *GJB2*-unrelated congenital deafness,  $P=0.12$ ) (Fig. 1). If we arbitrarily define a delayed reader as someone reading at more than one grade level below expectation for age, the number of delayed readers with *GJB2*-related deafness was 14%. By comparison, 44% of individuals with *GJB2*-unrelated deafness were delayed readers.

## DISCUSSION

Mutations in *GJB2* are one of the most common causes of congenital deafness in the United States, affecting over one-third of children evaluated for congenital severe-to-profound sensorineural hearing loss, and half of children with heritable hearing loss. Numerous deafness-causing mutations of this gene have been reported, although a single mutation, 35delG, predominates. Overall carrier rates for deafness-causing *GJB2* mutations in the general Caucasian population in the United States approximate 3.0%

TABLE II. Age-at-Implantation (years.months)

<i>GJB2</i> -related deafness	<i>GJB2</i> -unrelated deafness
2.8	1.5
2.9	3.10
2.9	4.2
3.3	5.1
3.4	5.5
5.6	5.8
6.2	6.8
11.11	9.1
	9.6
	9.8
	11.9
	14.0

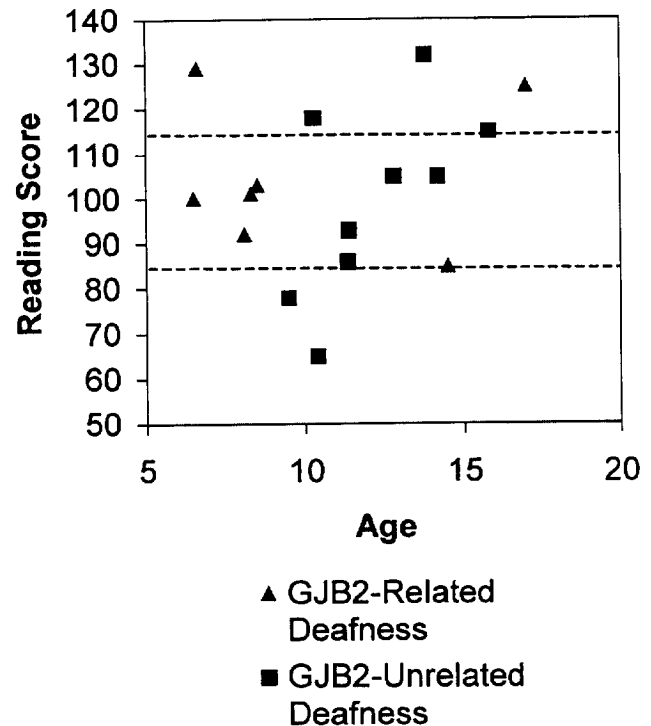


Fig. 1. Reading scores of cochlear implant recipients with sufficient follow-up data. Dashed lines mark one standard deviation above and below average for hearing children. Note that the lowest performers have deafness unrelated to *GJB2*-mutations. There is a trend for children with deafness unrelated to *GJB2* mutations to be more likely to read greater than one standard deviation below average ( $P=0.117$ ).

[Green et al., 1999]. Over two-thirds of children identified as carrying two mutant alleles have profound or severe-to-profound hearing loss, although there can be wide phenotypic variability in the degree of loss even within the same family [Denoyelle et al., 1997].

Based on the data in this report, the etiology of non-syndromic hearing loss in prelingual cochlear implant recipients can be defined more precisely. Before genetic testing, we found that 51% of patients had congenital deafness of unknown etiology (10% with familial recurrence), 21% had hearing loss from meningitis, 5% had an inner ear anomaly, 9% had exposure to an in utero infection, 8% had syndromic hearing loss, and 6% had a known anoxic or ototoxic cochlear insult (Fig. 2). These data are consistent with studies from Texas and nationally, and correspond to a relatively higher percentage of meningitis-related deafness and a lower percentage of known hereditary deafness than are seen in the general deaf population [Allen et al., 1993; Fryauf-Bertschy et al., 1997; Woodcock, 1997].

By offering genetic testing, we were able to determine that *GJB2*-related deafness accounted for 40% of congenital hearing loss in the unknown etiology group (Fig. 2). On this basis, in the entire group of prelingually deaf cochlear implant recipients, we estimate that approximately 20% have deafness from *GJB2* mutations. Due to the large number of individuals with *GJB2*-related deafness, we were able to examine and

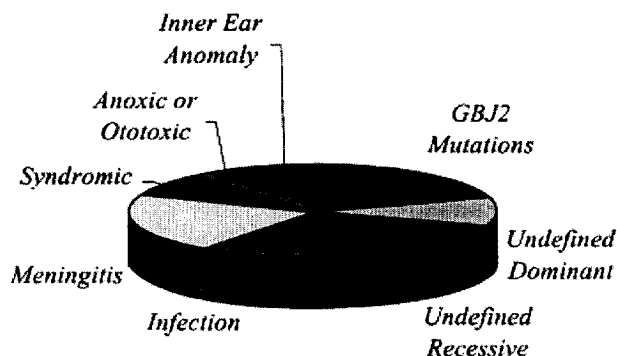


Fig. 2. Deafness from *GJB2*-mutations is estimated to account for 20% of prelingual deafness in cochlear implant recipients at the University of Iowa. In general, *GJB2*-mutations accounted for ~40% of deafness of previously undefined etiology, and ~50% of recessive deafness of undefined etiology.

compare reading performance—86% of cochlear implant recipients with *GJB2*-related deafness read within one year of grade level compared to only 56% of cochlear implant recipients with *GJB2*-unrelated hearing loss. Although the first group cannot be shown to be superior in reading performance to the second group due to a limited sample size, persons with *GJB2*-related deafness can be ascribed as reading at least as well as those without *GJB2*-related deafness. Cochlear implant recipients in both groups outperformed children with profound hearing loss not receiving implantation, a group in which typically less than 10% read within one year of grade level [Holden-Pitt, 2000].

Because approximately 25% of congenitally deaf persons have additional disabilities, the identification of *GJB2*-related deafness carries prognostic significance [Cremers et al., 1991]. Persons with *GJB2*-related deafness are healthy—*GJB2* mutations are not associated with mental retardation, vision difficulties, or sudden death, all of which can affect specific segments of the deaf community [Schildroth, 1994]. Vestibular function appears to be normal, and walking and sitting occur at age-appropriate times. We have been unable to identify any cognitive deficiencies or health risks associated with *GJB2*-related deafness in comparison with the deaf community as a whole.

Establishing a diagnosis of *GJB2*-related deafness also impacts genetic counseling. The recurrence chance for a hearing couple having a child with *GJB2*-related deafness is approximately 25%, consistent with an autosomal recessive inheritance pattern. In comparison, the recurrence chance for a hearing couple having a child with *GJB2*-unrelated deafness is approximately 14% [Green et al., 1999].

The data presented here have additional implications on the effect of in utero sound deprivation, the effect of

childhood sound deprivation, and the effect of deafness comorbidity on development. Since several reports have documented benefits associated with early cochlear implantation, genetic screening may be useful to identify a subset of patients for whom very early implantation can be an option [Rizer and Burkey, 1999; O'Donoghue et al., 2000]. However, a larger number of cochlear implant recipients must be studied to achieve stronger statistical significance before more definitive statements can be made in this regard.

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