Two Iranian Families With a Novel Mutation in GJB2 Causing Autosomal Dominant Nonsyndromic Hearing Loss

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Mutations in GJB2, encoding connexin 26 (Cx26), cause both autosomal dominant and autosomal recessive nonsyndromic hearing loss (ARNSHL) at the DFNA3 and DFNB1 loci, respectively. Most of the over 100 described GJB2 mutations cause ARNSHL. Only a minority has been associated with autosomal dominant hearing loss. In this study, we present two families with autosomal dominant nonsyndromic hearing loss caused by a novel mutation in GJB2 (p.Asp46Asn). Both families were ascertained from the same village in northern Iran consistent with a founder effect. This finding implicates the D46N missense mutation in Cx26 as a common cause of deafness in this part of Iran mandating mutation screening of GJB2 for D46N in all persons with hearing loss who originate from this geographic region. © 2011 Wiley-Liss, Inc.

Key words: connexin 26; D46N; autosomal dominant nonsyndromic hearing loss; DFNA3; Iran

INTRODUCTION

Approximately one in 1,000 newborns has severe-to-profound hearing loss and in more than half of this group of babies the etiology is genetic [Smith et al., 2005]. The pattern of inheritance is most frequently autosomal recessive, while autosomal dominant, X-linked and mitochondrial inheritance occur less frequently. Mutations in one gene, GJB2, have been found to cause up to half of autosomal recessive nonsyndromic hearing loss (ARNSHL) cases in developed countries [Estivill et al., 1998; Kelley et al., 1998; Green et al., 1999].

GJB2 encodes connexin 26 (Cx26), a member of a highly conserved protein family found throughout the animal kingdom [van Steensel et al., 2004a]. Six connexins oligomerize to form pore-like plasma membrane hemichannels called connexons. Connexons
in adjacent cells then align to form gap junction channels, which facilitate electrical and biochemical coupling between cells. Although the function of gap junction channels in the inner ear has not been definitively determined, a role in maintaining cochlear homeostasis by recycling $K_\text{+}$ ions following hair cell depolarization has been postulated [Kikuchi et al., 1995].

The majority of the over 100 deafness-causing mutations described in $GJB2$ causes ARNSHL, however 29 $GJB2$ mutations have

<table>
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<tr>
<th>Mutation</th>
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<th>Number of affecteds</th>
<th>Phenotype</th>
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<td>1</td>
<td>—</td>
</tr>
<tr>
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<td>2 French families</td>
<td>Nonsyndromic</td>
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<td>4</td>
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<td>1 family</td>
<td>Syndromic (palmoplantar keratoderma with deafness)</td>
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</table>
been associated with autosomal dominant hearing loss (ADHL) (Table I). About two-thirds of these mutations cause a syndrome in which hearing loss is associated with distinct skin manifestations. Examples of these syndromes include Keratitis-Ichthyosis-Deafness (KID) syndrome [MIM 148210], Vohwinkel syndrome [MIM 124500], Bart-Pumphrey syndrome [MIM 149200], and palmoplantar keratoderma (PPK) with deafness [MIM 148350]. The remaining one-third of dominant GJB2 mutations causes autosomal dominant nonsyndromic hearing loss (ADNSHL). In this study, we present a novel GJB2 mutation responsible for ADNSHL in two families from a village in northern Iran.

MATERIALS AND METHODS

Subjects

This study was approved by the Institutional Review Boards of the University of Social Welfare and Rehabilitation Sciences in Tehran, Iran and the University of Iowa, Iowa City, IA, United States.

FIG. 1. A: Pedigrees of two families, L-1301 (upper pedigree) and L-3057 (lower pedigree) with hearing loss caused by a novel mutation in GJB2, D46N. The proband of each family is indicated by an arrow. All individuals who did not have audiograms are indicated with an asterisk. Studied branches are shown with capital letters. B: All genotypes are indicated on pedigrees beneath the studied individuals.
Informed consent was obtained from all family members wishing to participate in this research. For each consenting person, clinical assessment and genetic counseling were performed. Audiometric testing was performed by measuring air and bone conduction thresholds.

Haplotyping

Genomic DNA was isolated from peripheral blood using established techniques [Miller et al., 1988] and used to complete haplotype reconstruction for microsatellite markers that map to chromosome 13q12, the genomic region that includes \textit{GJB2}. Markers were PCR amplified and products were resolved on the ABI Prism 3130 Genetic Analyzer. Alleles were assigned using GeneMapper 3.0 software (Applied Biosystems, Inc., Foster city, CA), with haplotypes reconstructed using custom-made software.

\textbf{GJB2 Screening}

Mutation screening was completed on an Applied Biosystems (ABI) model 3700 automated sequencer. Sequence data were compared to published sequence for \textit{GJB2} using the Sequencher 4.1.2 software program package (Gene Codes, MI, Ann Arbor, MI). The putative structural and functional significance of observed mutations was assessed using the ConSeq web server (http://conseq.tau.ac.il/). Conseq scores obtained using this alignment method range from 1 (variable) to 9 (conserved).

\section*{RESULTS}

\subsection*{Subjects}

Family members from two families, L-1301 and L-3057, segregating ADNSHL were recruited for this study (Fig. 1). Although most persons consented to participate, some individuals (shown with asterisk in Fig. 1) declined to have audiograms taken. For those persons with hearing loss, audiometry confirmed the loss to be sensorineural. Figure 2 shows hearing thresholds for the better hearing ear in six persons from family L-1301 and in four persons from family L-3057. In most persons, the hearing loss was postlingual with an onset in the first decade, however VI:13 and VI:9 had prelingual hearing loss that quickly progressed to become severe to profound in VI:13 (Fig. 2: L-1301:1 and L-1301:2). Intrafamilial variation was observed (Fig. 2). Pure tone audiograms by age group are shown in Figure 3. No features of syndromic hearing loss were observed in any person with hearing loss in these families (Fig. 4).
Haplotype Analysis and Mutation Screening

Haplotype reconstruction was consistent with linkage to the DFNA3 locus on chromosome 13q12. Mutation analysis of GJB2 identified a novel mutation, 351G > A, in affected subjects (Fig. 1). This missense mutation replaces the acidic aspartate residue at position 46 with the neutral asparagine residue (D46N). The mutation was not present in nonaffected family members and was similarly absent in 200 Iranian control samples. D46 is located in the first extracellular loop (E1) of Cx26, a region known to be highly conserved among connexin family members and to play an important role in voltage gating and in facilitating docking between opposing connexons [Rubin et al., 1992; White et al., 1995; Martin and Evans, 2004; Mese et al., 2007]. The ConSeq alignment confirmed conservation of this amino acid in multiple species (ConSeq score = 9; Fig. 5). Based on these data, D46N is believed to be the disease-causing mutation in both families segregating ADNSHL.
FIG. 1. (Continued)

FIG. 2. Audiograms associated with GJB2 D46N hearing loss (better hearing ear is shown). Top: Sequential audiograms from two persons in family L-1301 showing progression to severe-to-profound hearing loss. Middle: Audiograms from five persons in family L-1301 at different ages showing relatively uniform audioprofiles. Bottom: Audiograms from four persons in family L-3057 at different ages showing variably in hearing thresholds by age.

FIG. 3. Audioprofiles showing average audiograms by age group for both families.
DISCUSSION

In this report we present two families with ADNSHL caused by a novel amino acid change—D46N—in GJB2. To date, 9 mutations in GJB2 have been linked to ADNSHL whilst 21 mutations cause syndromic hearing loss associated with a variety of skin diseases. Two mutations, R75W and R75Q, cause both syndromic and nonsyndromic hearing loss.

Most of the known dominant mutations, whether syndromic and nonsyndromic, occur in the highly conserved first extracellular loop (E1) of Cx26, which is crucial for voltage gating and connexon–connexon docking (Fig. 6) [Richard et al., 2002; Martin and Evans, 2004]. Prominent among these mutations are G45E, D50N, and D50Y, which cause KID syndrome [Richard et al., 2002; Sonoda et al., 2004; Janecke et al., 2005; OMIM 148210], and W44C and W44S, which cause ADNSHL [Denoyelle et al., 1998; Tekin et al., 2001; Marziano et al., 2003; MIM 601544]. The D46N mutation affects the same region of E1 (Fig. 6). The recently resolved crystal structure of a Cx26 gap junction channel has shown that this segment of E1 forms a $\alpha_10$ helix structure which lines the channel pore [Maeda et al., 2009].

Many mutations in GJB2 that cause ARNSHL cause a simple loss-of-channel activity, however it is less clear how dominant mutations affect gap junction function [Bruzzone et al., 2001]. The broad phenotypic heterogeneity seen with autosomal dominant mutations suggests variability in the molecular defects caused by individual mutations. For example, some dominant GJB2 mutations, like R75W, are known to inhibit formation of functional channels. Expression studies in Xenopus oocytes have shown that R75W, which occurs in a highly conserved region involved in voltage gating, inhibits normal gap junction function of co-expressed wild-type Cx26 in a dominant-negative manner [Richard et al., 1998]. Dye transfer studies using the gap junction permeant tracer Cascade Blue have also demonstrated a disruption of intercellular coupling with W44S, R75W, G59A, and D66H. For G59A and D66H mutants, this change correlates with impaired intracellular trafficking and targeting to the plasma membrane [Marziano et al., 2003]. Functional studies also indicate that the mutation T55N produces a protein that is deeply impaired in its intracellular trafficking and fails to reach the plasma membrane [Melchionda et al., 2005].

Although functional studies of D46N are needed to determine the effect of this mutation on channel activity, useful information can be gleaned by studying the structure of wild-type Cx26 gap junction channels. Based on the structural analysis by Maeda and coworkers, D46 is one of the key residues involved in interactions between adjacent connexins. Additionally, D46 and D50 line the gap junction pore at the level of the cell membrane and face the interior of the channel, creating a negatively charged path between adjacent cells [Maeda et al., 2009]. This region is hypothesized to contribute to charge selectivity and size restriction of the gap junction. While the size of the side chain would not be significantly altered, the D46N mutation would reduce the negative charge, potentially altering the permselectivity of the channel.

The strong evolutionary conservation of D46 between species and among connexins, its location lining the pore of the Cx26 gap junction, the predicted importance of its negative charge, and its absence in 200 Iranian control subjects are convincing evidence for the disease-causing effect of this mutation. The identification of this mutation in two families from the same small village in northern Iran is consistent with a founder effect, which we confirmed by genotyping additional microsatellite markers in the DFNB1 region. On this basis, we recommend screening for this mutation in deaf persons from the Northern provinces in Iran.

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FIG. 5. Multiple sequence alignment of the first extracellular loop (E1) of connexin 26 shows high cross-species conservation (D46 indicated by arrow).
Professor, University of Iowa College of Medicine, who supported the project with National Institutes of Health (NIH)-NIDCD grants RO1 DCOO2842 and RO1 DCO3544.

REFERENCES


A novel M163L mutation in GJB2 gene associated with autosomal dominant hearin...


