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

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Abstract

Mutations in *GJB2*, encoding connexin 26 (Cx26), cause both autosomal dominant and autosomal recessive nonsyndromic hearing loss at the DFNA3 and DFNB1 loci, respectively. Most of the over 100 described *GJB2* mutations cause autosomal recessive nonsyndromic hearing loss. 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.

Keywords

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

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mitochondrial inheritances 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 [Kelley et al, 1998; Estivill 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, 2004]. 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^+ 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 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 [OMIM 148210], Vohwinkel syndrome [OMIM 124500], Bart-Pumphrey syndrome [OMIM 149200] and palmoplantar keratoderma (PPK) with deafness [OMIM 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. 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 (Fig 1).

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 *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.), with haplotypes reconstructed using custom-made software.

GJB2 Screening

Mutation screening was completed on an Applied Biosystems (ABI) model 3700 automated sequencer. Sequence data were compared to published sequence for *GJB2* using the Sequencher 4.1.2 software program package (Gene Codes, MI, USA). 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).

Results

Subjects

Family members from two families, L-1301 and L-3057, segregating ADNSHL were recruited for this study (Fig 2). Although most persons consented to participate, some individuals (shown with asterisk in Fig 2) declined to have audiograms. 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 (Figure 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 2). This missense mutation replaces the acidic aspartate residue at position 46 with the neutral asparagine residue (D46N). The mutation was not present in non-affected 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 [Meşe et al, 2007;White et al, 1995;Rubin et al, 1992;Martin and Evans, 2004]. The ConSeq alignment confirmed conservation of this amino acid in multiple species (ConSeq score = 9; Figure 5). Based on these data, D46N is believed to be the disease-causing mutation in both families segregating ADNSHL.

Discussion

In this report we present two families with ADNSHL caused by a novel amino acid change – D46N – in *GJB2*. To date, nine 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 Keratitis-Ichthyosis-Deafness syndrome [OMIM 148210, Janecke et al, 2005;Richard et al, 2002;Sonoda et al, 2004], and W44C and W44S, which cause ADNSHL [OMIM, 601544; Denoyelle et al, 1998;Tekin et al, 2001;Marziano et al, 2003]. The D46N mutation affects the same region of E1 (Fig 6). The recently resolved crystal structure of a connexin 26 gap junction channel has shown that this segment of E1 forms a 3_{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 colleagues, 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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OMIM 124500; Vohwinkel syndrome

OMIM 148210; KID syndrome

OMIM 148350; PPK

OMIM 149200; Bart-Pumphrey syndrome

OMIM, 601544; DFNA3

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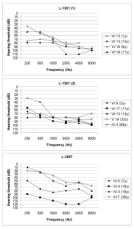
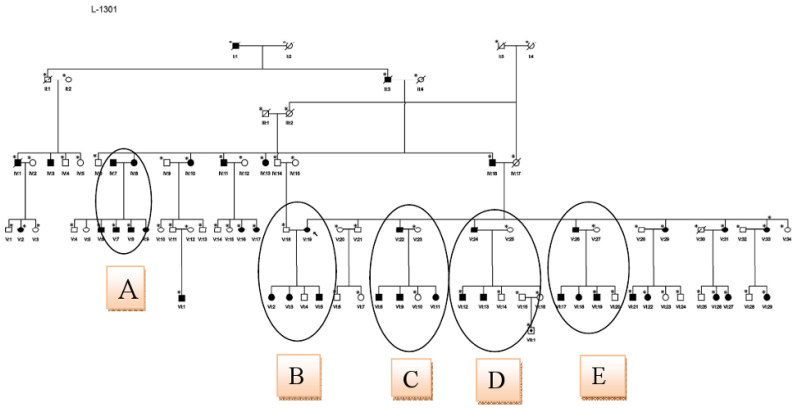
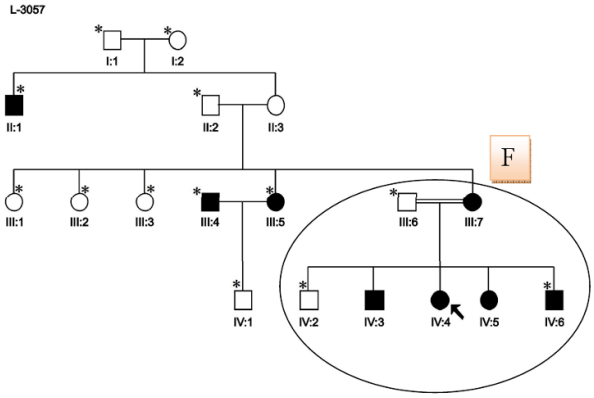
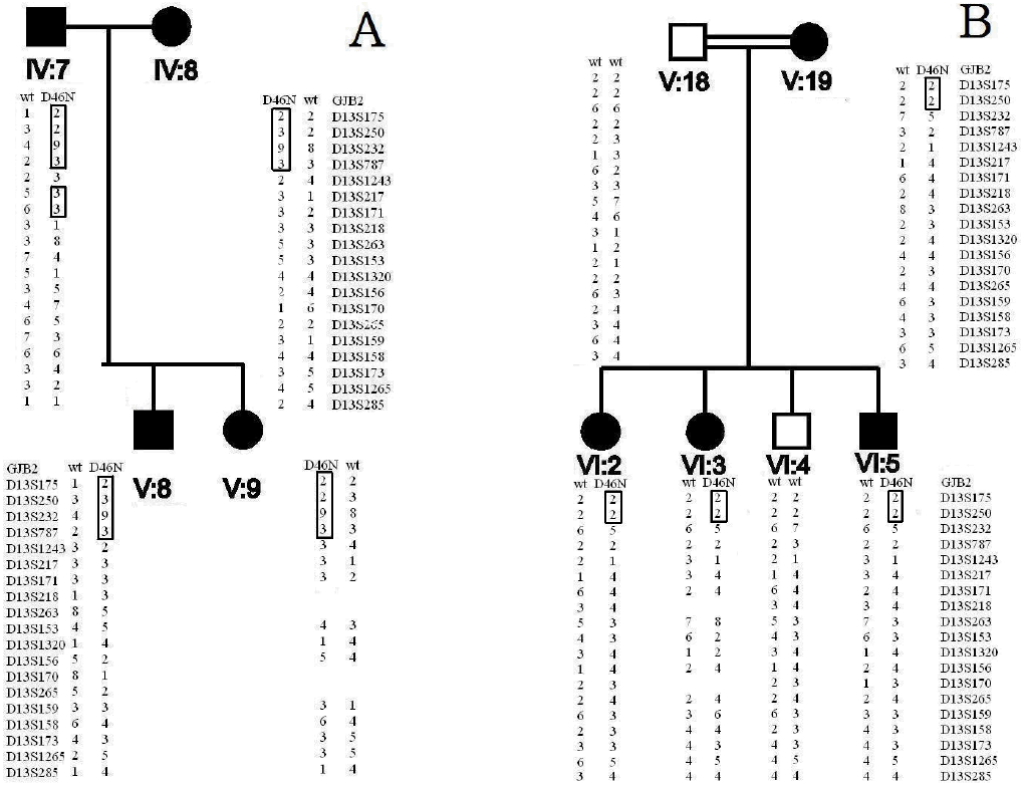


Figure 1.
Audiograms associated with *GJB2* D46N hearing loss (better hearing ear is shown). Top: Sequential audiograms from two persons in family L-1301 showing progression to severe-to-profound hearing loss. Middle: Audiograms from five persons in family L-1301 at different ages showing relatively uniform audioprofiles. Bottom: Audiograms from four persons in family L-3057 at different ages showing variably in hearing thresholds by age.







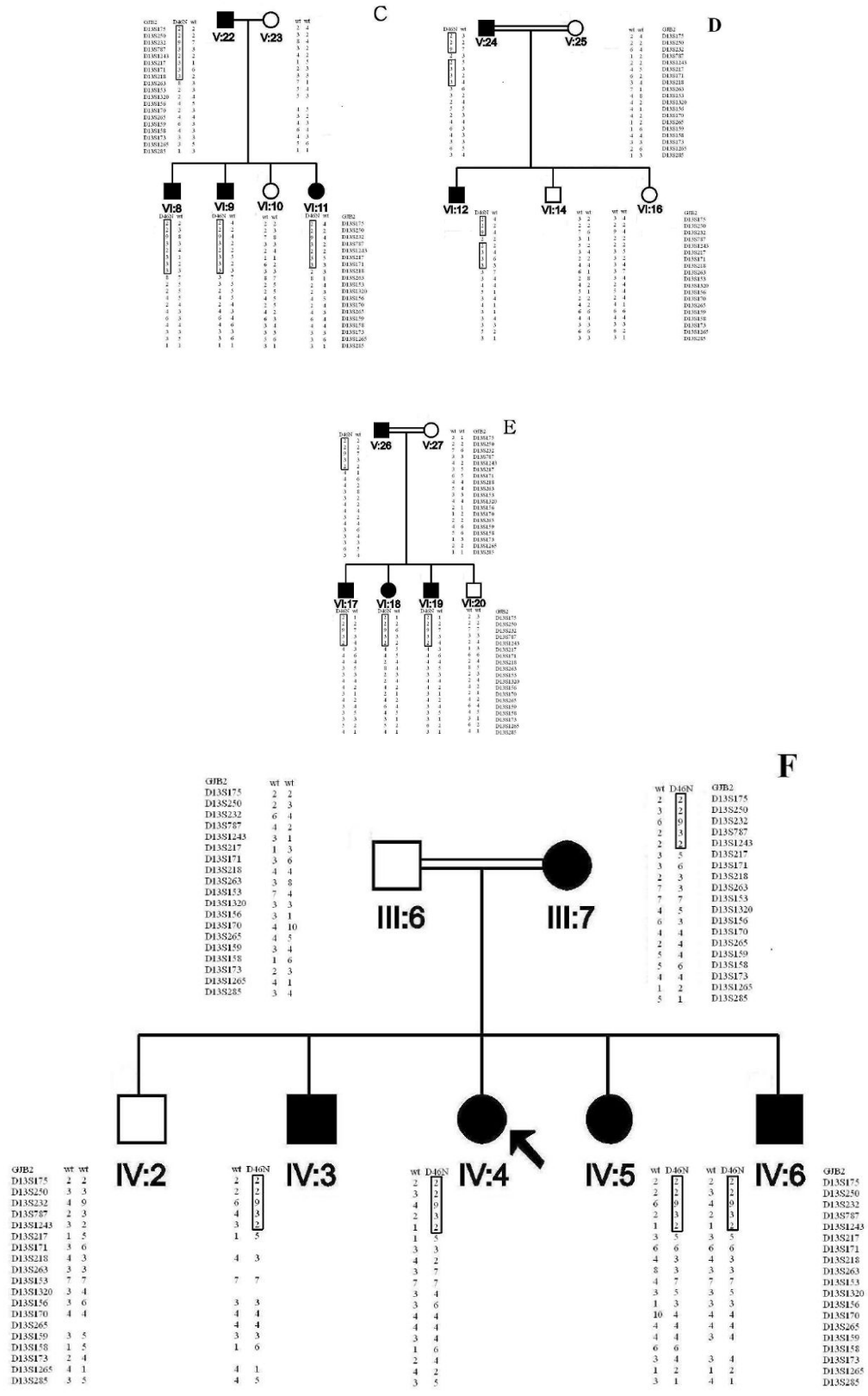


Figure 2.

Figure 2 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. Figure 2 B. All genotypes are indicated on pedigrees beneath the studied individuals.

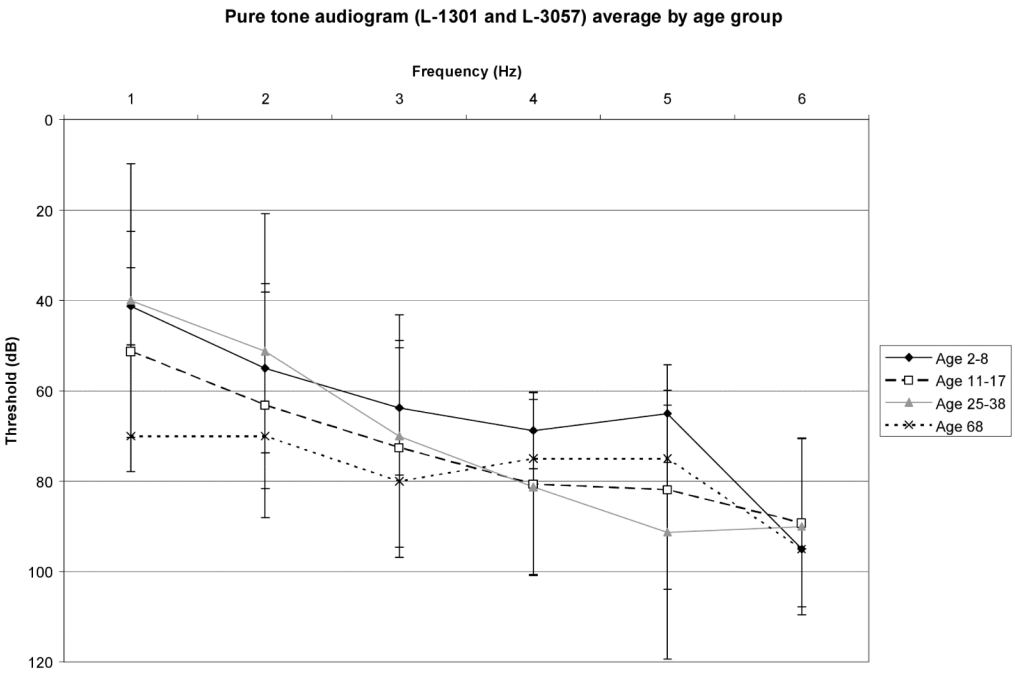


Figure 3.
Audioprofiles showing average audiograms by age group for both families.

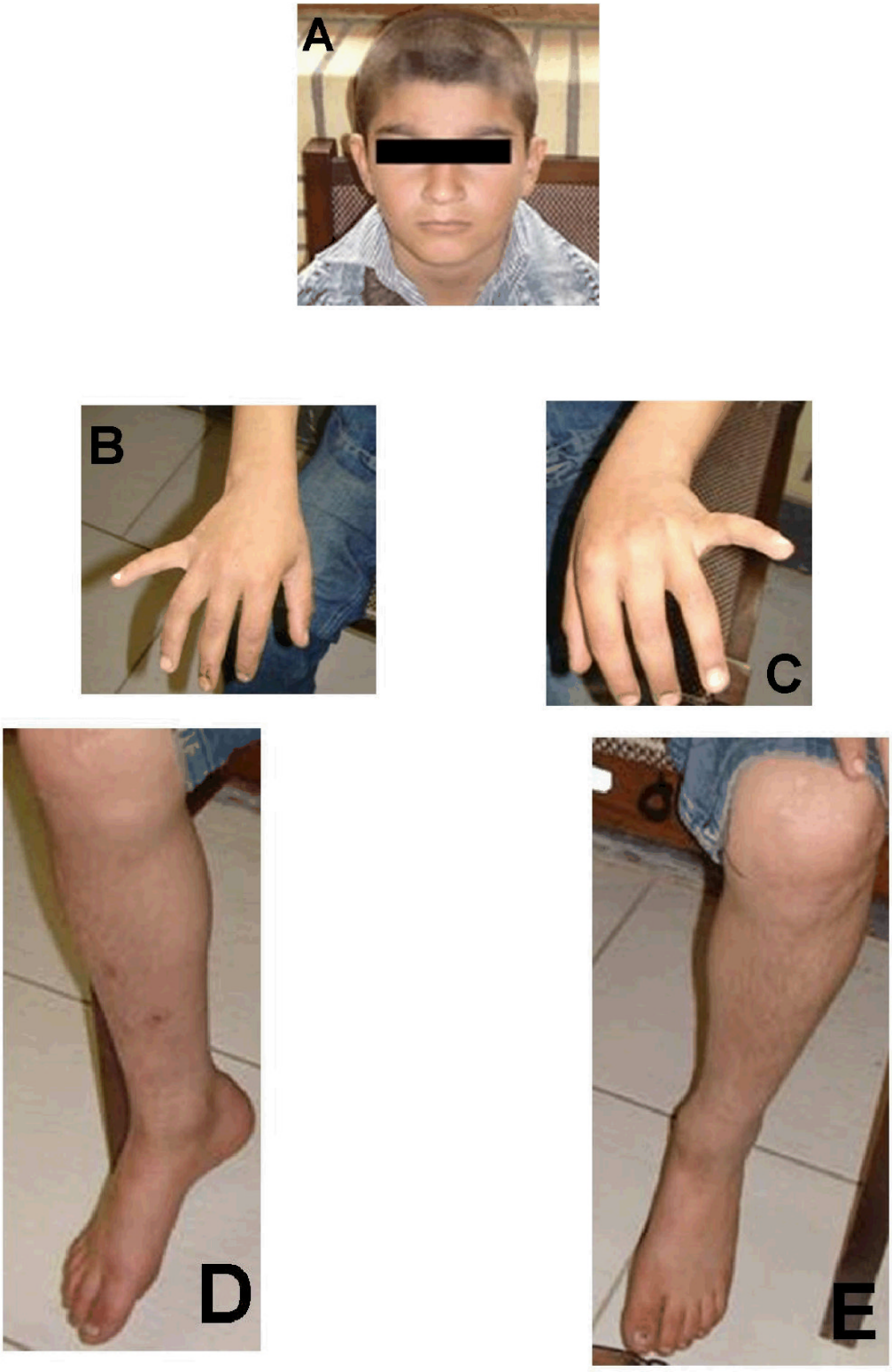


Figure 4.
Photos of one affected individual showing absence of skin symptoms.

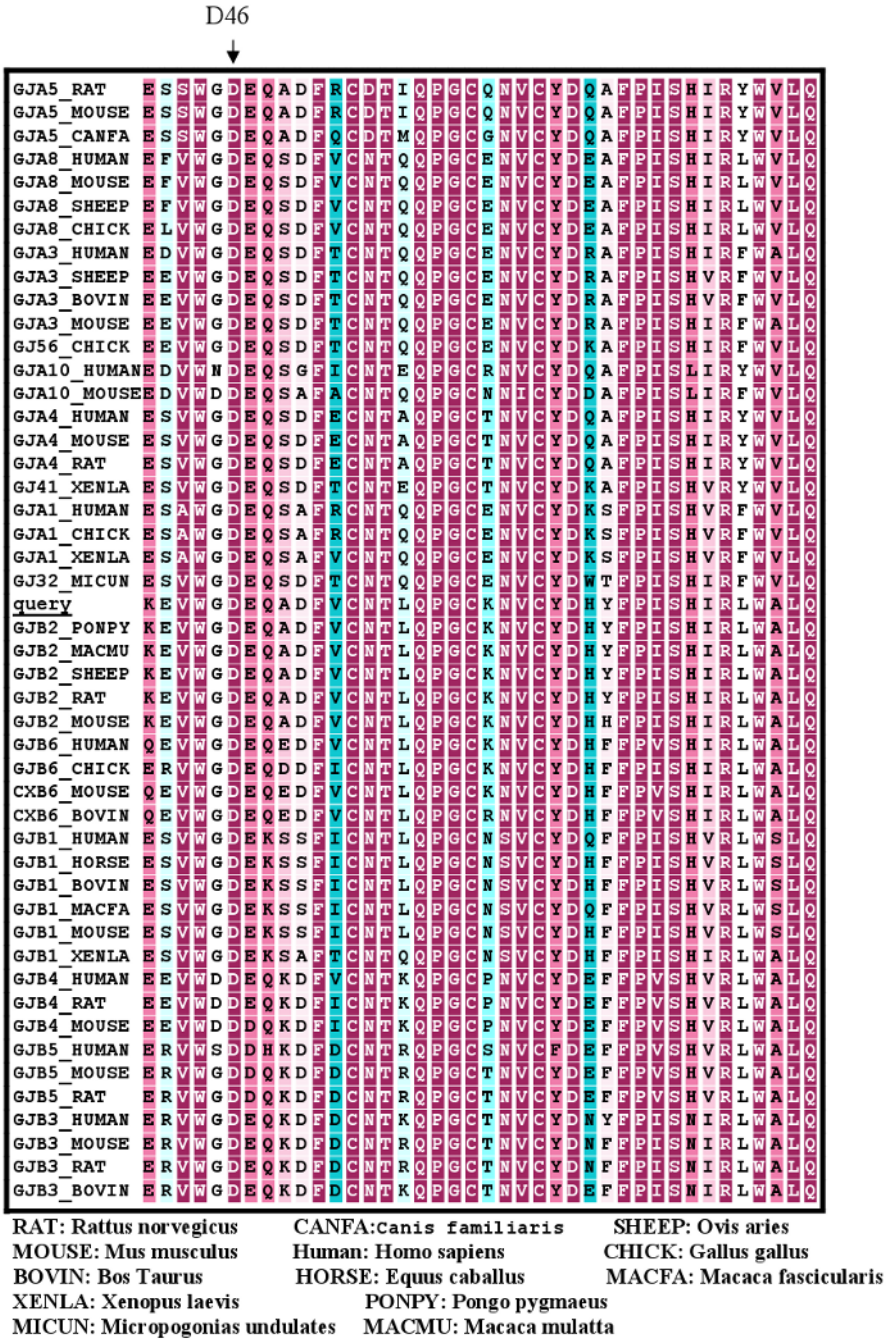


Figure 5.
Multiple sequence alignment of the first extracellular loop (E1) of connexin 26 shows high cross-species conservation (D46 indicated by arrow).

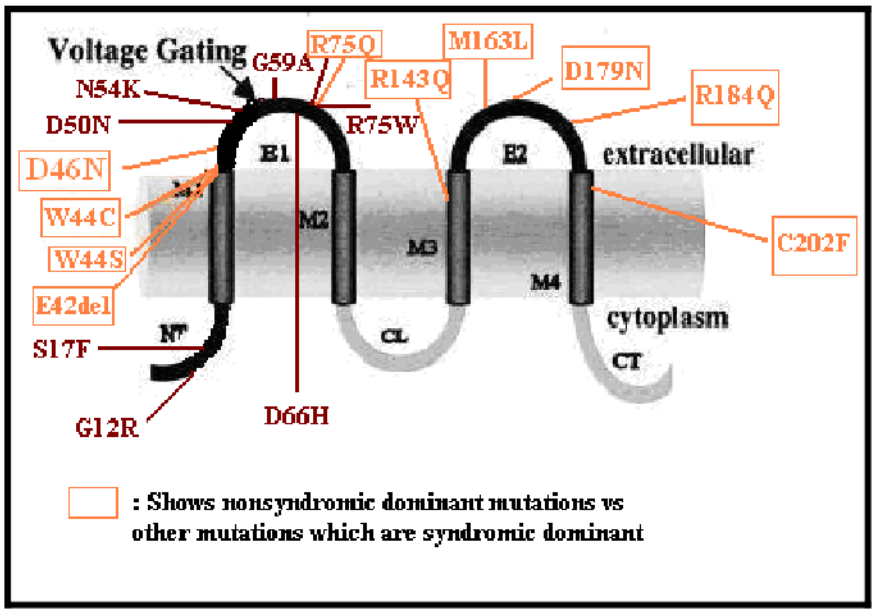


Figure 6.
Syndromic and nonsyndromic mutations in *GJB2* involved in autosomal dominant hearing loss.

Table I

AD nonsyndromic and syndromic *GJB2* mutations

Mutation		Protein domain	Number of affecteds	Phenotype	Reference
cDNA level	Protein level				
c.131G>C	W44S	E1	-	Nonsyndromic	[http://davinci.crg.es/deafness/]
c.132G>C	W44C	E1	2 French families	Nonsyndromic	[Denoyelle et al. 1998]
c.223C>T	R75W	M2	-	Nonsyndromic	[Janecke et al. 2001; Mani et al. 2008]
c.224G>A	R75Q	M2	4	Nonsyndromic	[http://davinci.crg.es/deafness/]
c.428G>A	R143Q	M3	1 Austrian family	Nonsyndromic	[Löffler et al. 2001]
c.487A>C	M163L	E2		Nonsyndromic	[Matos et al, 2008]
c.535G>A	D179N	E2	1 Italian family	Nonsyndromic	[http://davinci.crg.es/deafness/]
c.551G>A	R184Q	E2	-	Nonsyndromic	[Hamelmann et al, 2001]
c.605G>T	C202F	M4	A large French family	Nonsyndromic	[Morlé et al. 2000]
c.34G>C	G12R	NT	1	Syndromic (Keratitis-Ichthyosis-Deafness)	[Richard et al. 2002]
c.42C>G	N14K	NT	1	Hypotrichosis-deafness	[van Steensel et al. 2004]
c.40A>C	N14Y	NT	1	Syndromic (Keratitis-Ichthyosis-Deafness)	[Arita et al. 2006]
c.50C>T	S17F	NT	1	Syndromic (Keratitis-Ichthyosis-Deafness)	[Richard et al. 2002]
c.119C>T	A40V	M1	1	Syndromic (Keratitis-Ichthyosis-Deafness and the follicular occlusion triad)	[Montgomery JR et al. 2004]
125_127delAGG	E42del	M1	1 family	Syndromic (Palmoplantar keratoderma with deafness)	[Rouan et al. 2001]
c.134G>A	G45E	E1	-	Syndromic (Keratitis-Ichthyosis-Deafness)	[Janeck et al. 2005; Jonard et al. 2008]
c.148G>A	D50N	E1	7	Syndromic (Keratitis-Ichthyosis-Deafness; Hystrix-like Ichthyosis-Deafness)	[van Steensel et al. 2002]
c.148G>T	D50Y	E1	1	Syndromic (Keratitis-Ichthyosis-Deafness)	[Yotsumoto et al. 2003]
c.160A>C	N54H	E1	1	Syndromic (Bart-Pumphrey)	[Akiyama et al. 2007]
not described	N54K	E1	1 family	Syndromic (Bart-Pumphrey)	[Richard et al. 2000]
c.175G>C	G59R	E1	1	Syndromic (Palmoplantar keratoderma with deafness)	[Leonard et al. 2005]
c.175G>A	G59S	E1	1	Syndromic (Palmoplantar keratoderma with deafness)	[Alexandrino et al. 2005]
c.176C>G	G59A	E1	1 family	Syndromic (Palmoplantar keratoderma with deafness)	[Heathcote et al. 2000]
c.196G>C	D66H	E1	10	Syndromic (Vohwinkel)	[Maestrini et al. 1999]
c.219A>G	H73R	M2	1 family	Syndromic (Palmoplantar keratoderma with deafness)	[de Zwart-Storm et al. Mar. 2008]

Mutation		Protein domain	Number of affecteds	Phenotype	Reference
cDNA level	Protein level				
c.223C>T	R75W	M2	-	Syndromic (Palmoplantar keratoderma with deafness)	[Richard et al. 1998; Yuan et al. 2008]
c.224G>A	R75Q	E1	1 Turkish family	Syndromic (Palmoplantar keratoderma with deafness)	[Uyguner et al. 2002]
c.389G>T	G130V	CL	1 family	Syndromic (Vohwinkel)	[Snoeckx et al. 2005]
c.424T>C	F142L	M3	1	Syndromic (Mucositis-deafness)	[Brown et al. 2003]
c.548C>T	S183F	E2	1 family	Syndromic (Palmoplantar keratoderma with deafness)	[de Zwart-Storm et al. 2008]