Mutations in *CLDN14* are associated with different hearing thresholds

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Abstract

Mutations in *CLDN14*, encoding tight junction protein claudin 14, cause profound deafness in mice and humans. We identified a Pakistani family, in which the affected individuals were homozygous for a known pathogenic mutation c.254 T>A resulting in p.V85D substitution in *CLDN14*; however, in contrast to the previously reported families with mutations in *CLDN14*, most of the affected individuals in this family exhibit only a severe hearing loss. In order to identify the contribution of *CLDN14* to less than profound deafness, we screened for mutations of *CLDN14* in 30 multiplex and 57 sporadic cases with moderately severe to severe hearing loss from Pakistan. We identified one other affected individual homozygous for p.V85D substitution. Comparison of audiometric data from all patients indicates that mutations in *CLND14* cause varying degrees of hearing loss, which may be enhanced at high frequencies. This suggests that a modifier can reduce the severity of hearing loss associated with mutations of *CLDN14*. Our data indicates that mutations in *CLDN14* should be explored when considering the etiology of less severe hearing loss.

Keywords

CLDN14; Claudin; Tight junctions; DFNB29; Hearing loss; Pakistan

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CLDN14 encodes a protein which participates in the formation of tight junctions in different epithelial cells including those of the cochlear sensory epithelia. Patients with mutations in *CLDN14* and *Cldn14* knockout mice are profoundly deaf.[1, 2] In order to determine the etiology of less severe hearing loss in Pakistan, we recruited thirty consanguineous families with multiple affected individuals and fifty seven sporadic cases with moderate to severe hearing loss (50–90dB HL) with help of audiologists and schools for special children. After Institutional Review Board approval and written informed consent was acquired, DNA samples were obtained from blood samples of all participants. Linkage analyses for 45 of the

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known loci of recessively inherited hearing loss were performed for family HLRB5 by genotyping fluorescently labeled microsatellite markers including *D21S1252* and *D21S167* for *DFNB29*. All other families and 57 sporadic cases with hearing loss were similarly checked for linkage or homozygosity for markers flanking *CLDN14*. Only HLRB5 was consistent with linkage to *CLDN14* (Figure 1a) while the single affected individual SA18 was homozygous for the genotyped markers. Sequencing revealed the same mutation c.254 T>A (p.V85D) in *CLDN14* segregating in affected individuals in HLRB5 and SA18 (Figure 1b). Affected individuals in family HLRB5 and individual SA18 were homozygous for the same allele linked to the deafness phenotype for marker *D21S1252* and also shared Single Nucleotide Polymorphisms (SNPs) in *CLDN14* (Table 1), which indicates that this mutation probably arose on a common ancestral chromosome. However, carriers for this mutation are not frequent in the population, since we did not identify this variant in 100 control DNA samples from Sheikhupura (200 chromosomes).

Recently, inheritance of a synonymous SNP in *CLDN14* was reported to be associated with kidney stones and low bone mineral density.[3] Therefore, we examined three members of family HLRB5 for kidney stones. However, Renal ultrasounds failed to detect kidney stones in two affected individuals V:9, V:11 who were homozygous for the mutation and one normal individual V:7, heterozygous for the c.254 T>A mutation.

Pure-tone air conduction averages (PTA) for hearing thresholds at 500, 1000, 2000 and 4000 Hz were calculated to compare the severity of deafness of all affected individuals. Hearing loss thresholds were classified as normal 0–25dB HL, mild 26–40dB HL, moderate 41–55dB HL, moderately severe 56–70dB HL, severe 71–90dB HL and profound >90dB HL.[4] Ten individuals (ages 10–22yrs) in family HLRB5 are hearing impaired. According to parents and self reports, hearing loss was not progressive. The audiometric data for all affected individuals in family HLRB5 gathered two years apart suggests that there is no deterioration of hearing. However, hearing loss may progress slowly and additional time is needed to conclusively establish stability of hearing thresholds.

The best hearing among the affected individuals in family HLRB5 is of a 10 year old child, V:3, PTA 63dB HL (moderately severe hearing loss), while the worst is of a 15 year old child, V:13, PTA 91dB HL (profound deafness) (Figure 2). All other affected individuals in family HLRB5 and SA18 have a severe hearing loss, PTA 74–88dB HL (Figure 2). The previously reported families with p.V85D mutation have profound deafness (PTA 93–100). Thus the hearing loss associated with mutations in *CLDN14* can range from moderately severe to profound deafness.

Variations can also be seen at different frequencies when comparing audiometric data from all families with *CLDN14* mutations. At low frequencies (250 and 500 Hz) the hearing loss ranges from moderate to severe. At conversational frequencies (500, 1000, 2000 Hz) hearing loss ranges from moderately severe to profound, while at high frequencies it ranges from severe to profound. However, for family HLRB5, the loss at high frequencies is still less than that reported earlier, with 7 out of 10 individuals responding to these tones while none of the patients in the previously published reports responded to the 8000 Hz frequency.[5]

This study shows that individuals with mutations of *CLDN14* may have different degrees of hearing loss and the loss is greater at high frequencies. Some preservation of low frequency hearing with a greater degree of high frequency hearing loss may indicate involvement of *CLDN14* in deafness, a finding similar to the hearing loss associated with mutant alleles of *GJB2*.[6] Although *CLDN14* is not a contributor to profound deafness in Turkey, Tunis and Spain,[7–9] it is possible that some cases of less severe hearing loss in these and other countries could be attributed to *CLDN14* mutations.

p.V85D is probably a null mutation as functional assays show that its ectopic expression in fibroblast cell lines results in mislocalization of this mutant CLDN14 and its absence from plasma membranes in contrast to the wild type protein.[10] These data suggest that in patients with p.V85D mutation, the dysfunction is due to loss of CLDN14 from tight junctions. It is known that CLDN14 is absent from tight junctions in *Cldn14* knockout mice leading to progressive loss of hair cells.[2] Mutations in *CLDN14* resulting in hearing loss may similarly cause hair cell loss in humans. However, the different degree of hearing loss in humans with *CLDN14* mutations indicates that the extent of hair cell loss may vary. In *Cldn14*$^{-/-}$ mice, variation in hair cell loss is seen with the base of the cochlea affected earlier than the apex. This may also be the case in humans. Since the apex of the cochlea responds to low frequency sounds, it may account for the residual hearing at the 4000 and 8000 Hz observed in the individuals reported in this study. Alternately, variations resulting in regulation of another inner ear expressed claudin may partially compensate for the loss of *CLDN14*.

Our research indicates that the same pathogenic mutations may not result in identical phenotypic severity and genetic or epigenetic factors may modify the clinical course of this genetic disorder. The search for modifier genes is difficult in humans[11] though a few modifiers for deafness have been mapped or cloned using linkage analyses or candidate gene approaches[12]. In family HLRB5, one individual is profoundly deaf and other individuals exhibit moderately severe to severe hearing losses. A clear demarcation of phenotype is absent, making linkage analyses for mapping a modifier locus difficult. However, mice are more amenable to search for modifiers of different phenotypes and many genes which modify effects of deafness causing genes have been identified[12]. Therefore transferring *Cldn14* null allele to different genetic backgrounds and isolating the most and least affected strains may help in identification of modifying variants in one or more causative genes.

## Acknowledgments

References

1. Wilcox ER, Burton QL, Naz S, Riazuddin S, Smith TN, Ploplis B, et al. Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. Cell. 2001; 104:165–172. [PubMed: 11163249]

2. Ben-Yosef T, Belyantseva IA, Saunders TL, Hughes ED, Kawamoto K, Van Itallie CM, et al. Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration. Hum Mol Genet. 2003; 12:2049–2061. [PubMed: 12913076]

3. Thorleifsson G, Holm H, Edvardsson V, Walters GB, Styrkarsdottir U, Gudbjartsson DF, et al. Sequence variants in the CLDN14 gene associate with kidney stones and bone mineral density. Nat Gene. 2009; 41:926–930.

4. Katz, J. Handbook of Clinical Audiology. Williams & Wilkins; 1994.

5. Ahmed ZM, Riazuddin S, Friedman TB, Riazuddin S, Wilcox ER, Griffith AJ. Clinical manifestations of DFNB29 deafness. Adv Otorhinolaryngol. 2002; 61:156–160. [PubMed: 12408079]

6. Wilcox SA, Saunders K, Osborn AH, Arnold A, Wunderlich J, Kelly T, et al. High frequency hearing loss correlated with mutations in the GJB2 gene. Hum Genet. 2000; 106:399–405. [PubMed: 10830906]

7. Uyguner O, Emiroglu M, Uzumcu A, Hafiz G, Ghanbari A, Baserer N, et al. Frequencies of gap- and tight-junction mutations in Turkish families with autosomal-recessive non-syndromic hearing loss. Clin Genet. 2003; 64:65–69. [PubMed: 12791041]

8. Arican ST, Incesulu A, Inceoglu B, Tekin M. Alterations in the GJB3 and CLDN14 genes in families with nonsyndromic sensorineural hearing loss. Genet Couns. 2005; 16:309–311. [PubMed: 16259330]

9. Belguith H, Tlili A, Dhouib H, Ben Rebeh I, Lahmar I, Charfeddine I, et al. Mutation in gap and tight junctions in patients with non-syndromic hearing loss. Biochem Biophys Res Commun. 2009; 385:1–5. [PubMed: 19254696]

10. Wattenhofer M, Reymond A, Falciola V, Charollais A, Caille D, Borel C, et al. Different mechanisms preclude mutant CLDN14 proteins from forming tight junctions in vitro. Hum Mutat. 2005; 25:543–549. [PubMed: 15880785]

11. Génin E, Feingold J, Clerget-Darpoux F. Identifying modifier genes of monogenic disease: strategies and difficulties. Hum Genet. 2008; 124:357–368. [PubMed: 18784943]

12. Johnson KR, Zheng QY, Noben-Trauth K. Strain background effects and genetic modifiers of hearing in mice. Brain Res. 2006; 1091:79–88. [PubMed: 16579977]
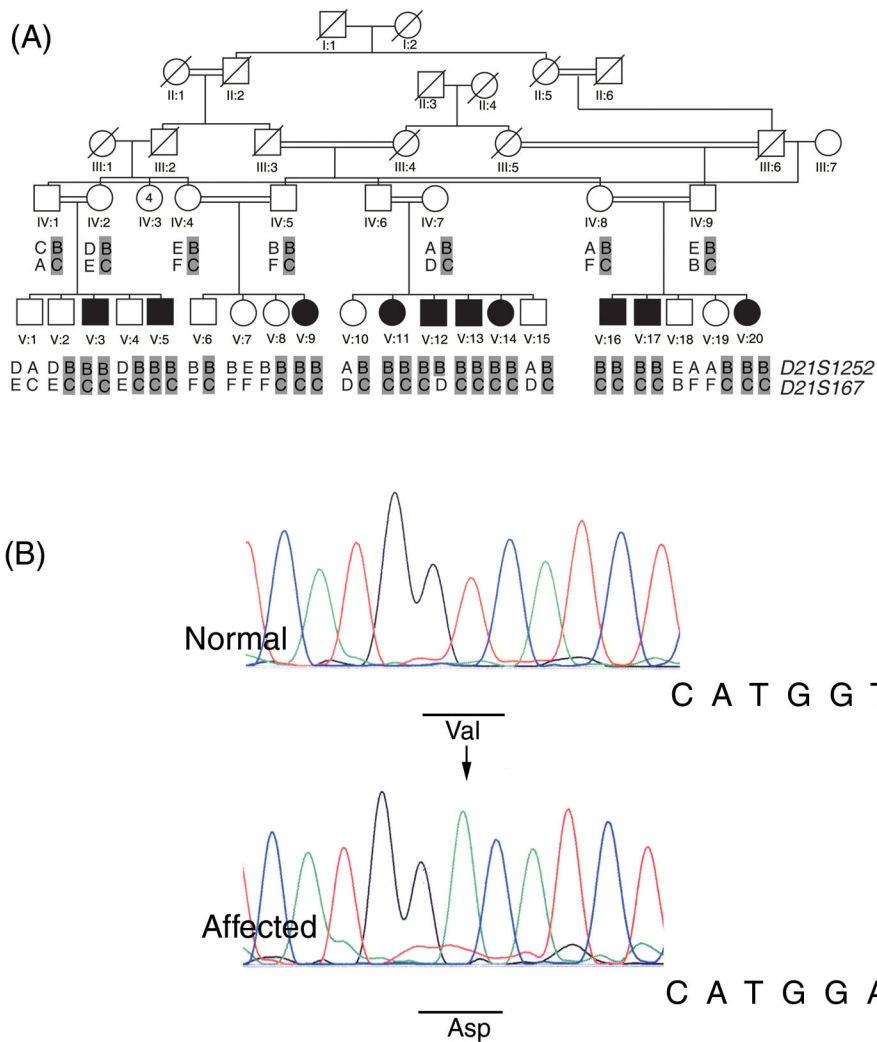
Figure 1.

a) Pedigree with haplotype data for family HLRB5. Solid symbols denote affected individuals. Results of genotyping are shown for two microsatellite markers. The deafness associated haplotype is shaded in gray. Alleles for each marker are denoted by letters. Allele sizes in base pairs are: *D21S1252*; A, 249; B, 247; C, 245; D, 239; E, 237 *D21S167*; A, 162; B, 154; C, 152; D,146; E, 138; F, 132.

b) Sequence trace files for the c.254 T>A mutation observed in family HLRB5 and SA18 from a normal and affected sample. The mutation is indicated by an arrow in the trace from the affected individual. The normal and mutated codons are underlined in the respective traces.
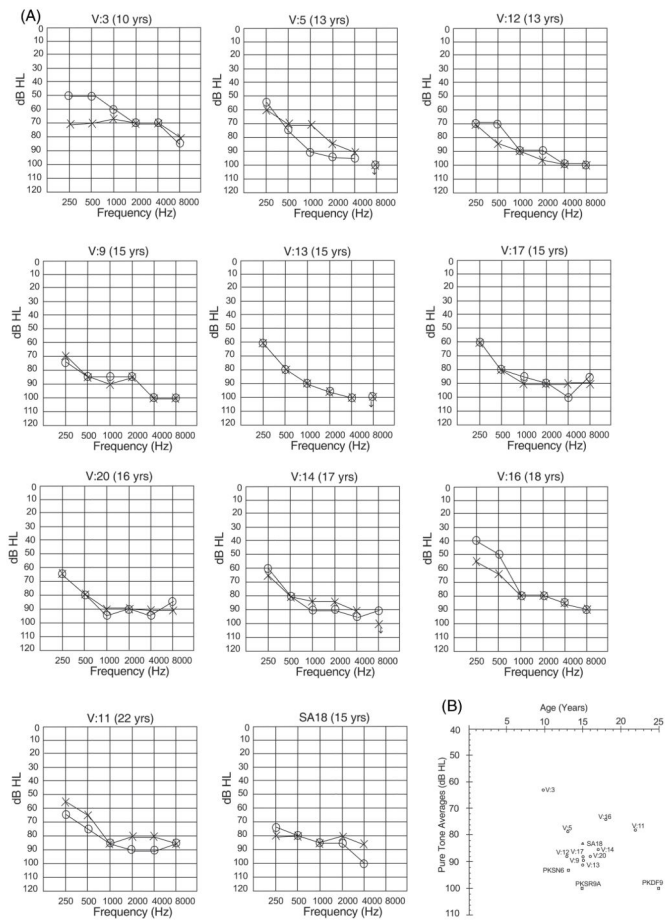
Figure 2.
a) Audiograms for all affected family members of family HLRB5 and SA18. Age at time of first audiometry is indicated on top of each audiogram. "o" indicates air conduction for right ear, while "x" indicates air conduction for left ear

b) Average thresholds at pure tones (500, 1000, 2000 and 4000 Hz) for the better hearing ears in family HLRB5 (circles), individual SA18 (triangle) and previously published families (squares) with mutations in *CLDN14*. The degree of hearing loss is different across and within families with mutations in *CLDN14*. All families have the same mutation, except for family PKSN6.

Table 1

Genotypic data for DFNB29 linked haplotypes

Genotypic data for short tandem repeat markers and single nucleotide polymorphisms in families HLRB5 and SA18 for DFNB29-linked haplotypes with c.254 T>A mutation in *CLDN14*.

Position[1]	Marker	Variation	Allele HLRB5	Allele SA18
37826859	*D21S1252*	$(AC)_n$	247	247
37826859		c.254 T>A	A	A
37834641	rs219778	T>C	T	T
37834704	rs2068750	G>A	G	G
37834721	rs219777	C>T	C	C
37834835	rs219776	G>A	A	A
37834914	rs2835363	T>A	A	A
37834944	rs219775	C>T	T	T
37835043	rs219774	G>A	G	G
37835164	rs60419768	C>T	C	C
37835185	rs34627708	InsC	-	-
37835241	rs72477120	InsAA	-	-
37835333	rs219773	C>T	C	C
37835347	rs219772	T>A	T	T
37835501	rs219771	G>A	G	G
37835648	rs219770	T>C	T	T
37835675	rs219769	G>T	G	G
38195766	*D21S167*	$(GT)_n$	152	158

[1]Nucleotide positions on chromosome 21 from UCSC February 2009, NCBI 37/hg19 assembly.

"Ins"=Insertion "-" = Deletion