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The c.42_52del11 mutation in *TPRN* and progressive hearing loss in a family from Pakistan

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Abstract

The *DFNB79* locus harbors *TPRN* mutations that have been reported in a few families with deafness. Four frameshift mutations in *TPRN* have been described to cause severe or severe-to-profound hearing loss in Moroccan and Pakistani families, and a single frameshift mutation was associated with progressive hearing loss in deaf individuals in a Dutch family. We identified a Pakistani family in which the affected individuals were homozygous for a pathogenic mutation, c. 42_52del11, in *TPRN* (p.G15Afs150X). In contrast to the previously reported individuals affected by the same mutation, hearing loss is likely to be progressive in this family. Thus the same mutation of *TPRN* can be associated with different thresholds of hearing as well as differences in the stability of the phenotype.

Keywords

Deafness; Pakistan; progressive hearing loss; *TPRN*

INTRODUCTION

Recessively inherited deafness is typically associated with prelingual and nonprogressive hearing loss. In contrast, dominantly inherited deafness is usually characterized by postlingual onset of hearing loss that is mostly progressive in nature. Findings of progressive hearing loss are less commonly reported in patients with autosomal recessive nonsyndromic hearing loss (Hildebrand et al. 2008). More than 65 loci have been localized for nonsyndromic recessively inherited deafness; however, only a few particular recessively inherited mutant alleles of some genes (*SLC26A4*, *TMCI*, *TMPRSS3*, *CDH23*, *GRXCR1*, *MYO3A*, *PJVK*, *LOXHDI*, *SERPINB6*, and *GIPC3*) cause progressive hearing loss in affected individuals in a small number of families from various world populations (Astuto et al. 2002; Charizopoulou et al. 2011; Elbracht et al. 2007; Grillet et al. 2009; de Heer et al. 2011; Schraders et al. 2010; Schwander et al. 2007; Scott et al. 2001; Sirmaci et al. 2010; Walsh et al. 2002). The *DFNB13* locus is also reported to harbor a gene causing progressive deafness (Mustapha et al. 1998).

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Mutations in only four genes have been implicated in progressive deafness in the Pakistani population. A mutation in *TMPRSS3*, described in a large extended family, results in postlingual progressive hearing loss in the affected individuals (Scott et al. 2001). Recently, a missense mutation in *PJVK* was associated with progressive hearing loss in a family from Pakistan (Mujtaba et al. 2012). Although not documented, the hearing loss in families with mutations in *GIPC3* (Ain et al. 2007; Rehman et al. 2011) and *BSND* (Riazuddin et al. 2009) could be progressive, as the older affected individuals in these families have a more severe hearing loss than that observed in the younger patients in their pedigrees.

TPRN encodes taperin, which is expressed in the organ of Corti in the mouse inner ear (Rehman et al. 2010). *TPRN* is localized within the supporting cells and inner ear hair cell stereocilia, where it is present at the taper region of each stereocilium (Rehman et al. 2010). The four exons of *TPRN* encode a deduced protein of 711 amino acids. Exon 1 of *TPRN* is predicted to be a hotspot for mutations because of the presence of repetitive elements high in GC-rich repeats. All the *TPRN* mutations reported so far are located in exon 1. Four of the five known mutations have been reported in the Pakistani population.

Currently, a single *TPRN* mutation (c.1347delG) is known to cause rapidly progressive hearing loss in a Dutch family (Li et al. 2010). Another deletion mutation, c.42_52del11 located in *TPRN* has been previously reported to cause severe to profound hearing loss in a multiplex Pakistani family (Rehman et al. 2010). The same mutation was identified in members of a large Moroccan family suffering a severe degree of hearing loss, which is stable in the third decade of life (Li et al. 2010). We have identified that the c.42_52del11 mutation of *TPRN* may also be associated with progressive hearing loss.

MATERIALS AND METHODS

We recruited a small Pakistani nuclear family, HLRB6, from a small village near Sialkot. Three affected children were born in a single consanguineous union (Fig. 1). Complete medical records of the family were collected, and the family was questioned to exclude syndromic and environmental cases. Romberg and tandem gait tests were conducted to evaluate the vestibular system. Audiological profiling of the affected individuals was done using previous records, interrogations, and fresh audiometric examinations. Current audiological examinations were performed on two affected individuals, V:1 (17 years old) and V:2 (13 years old), with measurements of both bone and air-conduction by pure tone audiometry in ambient noise conditions. Audiometry was repeated for V:2 at the age of 14 years. Unfortunately, a soundproof room was not available for conducting these tests, and facilities for recording ABR did not exist in the vicinity.

Blood samples were collected after obtaining approval of the institutional review board at the School of Biological Sciences, University of the Punjab, Lahore, and written informed consent of the participants. Genomic DNA was extracted from blood samples (Grimberg et al. 1989). *DFNB1* was excluded by direct sequencing of *GJB2*. The other 40 known deafness genes including *TPRN* were screened by homozygosity mapping with fluorescently labeled microsatellite markers as described previously (Imtiaz and Naz 2012). Fifty consanguineous families with at least 3 affected individuals and 57 affected sporadic cases, born in consanguineous unions with moderate to severe hearing loss, were also screened for linkage to or homozygosity for three STR markers specific for *DFNB79* (*D9S1826*, *D9S158*, and *D9S1838*). Among these, affected individuals in 10% of the families presented with deafness inferred to be progressive in nature. The phenotypic and genotypic details of all the families and sporadic cases in this cohort will be described elsewhere (Naz et al. manuscript in preparation).

The four exons of *TPRN* were PCR amplified and sequenced in order to identify the underlying mutation. To identify carriers for c.42_52del11 in the normal Pakistani population, primers 5'-FAM-GCGGGCTTTCCCAATGGTC and 5'-CCGGG CACCGTCTCGATGAT were used for amplification of the product spanning the deletion and analyzed as previously described (Naz and Fatima 2012). In brief, the c.42_52del11 mutation was screened in 100 ethnically matched controls (200 chromosomes), using size fractionation of the PCR products spanning this region by capillary electrophoresis. Similarly, DNA from all unaffected members of family HLRB6 was screened to determine carrier status for the deletion.

A DNA sample of an affected individual from a previously described family, PKDF1129 (Rehman et al. 2010), was genotyped with five STR markers spanning *TPRN* to determine if the mutation detected for family HLRB6 had a common origin in the two families.

RESULTS

Detailed medical histories and physical examinations revealed normal development and absence of environmental and syndromic causes of hearing loss in affected individuals of family HLRB6. Tandem walking was normal, and the Romberg test was also negative among affected children, suggesting normal vestibular function. According to the parents, the onset of hearing loss was noticed by 2 years of age. Audiometry was performed both at the time of admission to school and as part of this research. Initial audiometric details provided by the school for the two older affected children of family HLRB6 stated that at the time of admission, individuals V:1 (9 years old) and V:2 (7 years old) had a moderate degree of hearing loss. Unfortunately, the school did not maintain the data in the form of audiograms with hearing thresholds recorded at various frequencies. The current audiograms of the two affected individuals revealed moderate to profound hearing loss (Fig. 1). The third child (V:4) was too young (5 years) for audiometric examination but had significantly better hearing than the older affected siblings, as ascertained by sound perception and presence of good oral communication skills. None of the affected individuals wears hearing aids. Recently, the ears of all affected individuals were re-examined by an ear-nose-throat doctor. The examination revealed that the tympanic membranes of the eldest (V:1) and youngest (V:4) affected individuals were intact but slightly hyperemic, which precluded reassessment by audiometry. The tympanic membrane of individual V:2 was normal. There was no significant change in the audiological profile of V:2 from that recorded one year ago (Fig. 1).

Residual hearing was observed in the affected children. The oldest affected individual (V:1), at 17 years of age, had the most severe hearing loss, with greater involvement of high frequencies compared with his 13-year-old sibling (V:2). Although there is no audiometric documentation of the progression of deafness, the records pertaining to the hearing thresholds of the affected individuals maintained at the school, comparisons of degree of hearing loss among the siblings, and self-reports by the patients strongly support that deafness is progressive. It is interesting to note that the loss in hearing is quite similar at low frequencies, and dramatic shifts of 20-30 dB are observed between the siblings at frequencies of 2-4 KHz. This suggests that hearing at high frequencies worsens rapidly, while loss of hearing at lower frequencies may be more gradual.

Genotyping excluded all loci of hearing loss except for *DFNB79* for this family. Alleles of four markers (*D9S1826*, *D9S158*, *D9S1838*, and *C9S2168*) on chromosome 9 were homozygous for all affected individuals (Fig. 1). A maximum two-point LOD score of 2.0 was obtained at recombination fraction $\theta = 0$ for markers *D9S1826* and *D9S1838*. A homozygous 11 bp deletion, c.42_52del11 (p.G15Afs150X), was identified in the first exon

of *TPRN* in the DNA samples of all affected individuals of family HLRB6 (Fig. 1). The mutation deletes one of the two 11 bp GC-rich tandemly repeated sequences from the *TPRN* gene. All other unaffected family members of HLRB6, except for V:3, were heterozygous carriers of the mutation (Fig. 1). A previously described family, PKDF1129 from Pakistan, with the same c.42_52del11 mutation in *TPRN* (Rehman et al. 2010) did not share the same haplotype for markers spanning *TPRN* as that observed for family HLRB6 (Fig. 1).

Hearing loss in no other family was linked to *DFNB79*, and none of the sporadic individuals were homozygous for markers spanning *TPRN*. This suggests that *TPRN* is not a significant contributor to moderate to severe hearing loss in the Pakistani population, although its contribution to progressive hearing loss remains to be determined.

DISCUSSION

Since the initial reports of mutations in *TPRN*, this is the first independent finding of a *TPRN* mutation in recessive hearing loss. The mutation c.42_52del11 (p.G15Afs150X) has been previously reported in families from Pakistan and Morocco (Rehman et al. 2010; Li et al. 2010). Since the sizes of the linked alleles of the closest STR markers spanning *TPRN* in affected individuals in the two Pakistani families differ considerably, the mutation c.42_52del11 most likely arose independently in the two pedigrees. It was previously hypothesized that this region of *TPRN* is a hotspot for mutations because of the presence of GC-rich repetitive elements (Rehman et al. 2010). This is further supported by our data. Thus, the c.42_52del11 mutation may be a significant contributor to hearing loss, at least in the Pakistani population, due to this mutational hotspot.

Two different mutations in one gene, or the presence of identical mutations in one gene, have been described to cause inter- or intrafamilial variability of hearing loss previously (Astuto et al. 2002; Bashir et al. 2010). This variability of hearing loss can be explained on the basis of severity of the mutations or the presence of modifiers. The c.42_52del11 is most likely to be a null mutation. Therefore, its involvement in progressive hearing loss in a family from Pakistan and in stable hearing loss phenotype in another Pakistani and a Moroccan family strongly suggests the presence of a genetic modifier. To date, all reported mutations of *TPRN* are located in the first exon (Table 1). Sequencing of this exon may be considered in recessively inherited cases of nonsyndromic hearing loss, especially if signs of progression are present since two of the known mutations can be associated with progressive hearing loss (Li et al. 2010 and current work).

Age-dependent hair cell damage seems to be a common mechanism for progressive autosomal recessive hearing loss (Grillet et al. 2009). *Taperin* is localized to the hair cells, as are other gene products reported to cause progressive hearing loss. As *TPRN* function is still unknown, the mechanism of progression of hearing loss cannot be predicted, but its involvement in the progressive phenotype suggests its additional secondary role in either maintenance or protection of hair cells. Our study further supports that *TPRN* mutations must be considered when studying the etiology of progressive hearing loss. Understanding the genetic basis of progressive deafness can lead to development of therapies to stop or delay further progression of hearing loss and can also lead to early diagnosis.

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Fig. 1.

Characteristics of Family HLRB6. **(A)** Pedigree of HLRB6 with genotype data at chromosome 9 demonstrating linkage of the phenotype to *DFNB79* locus. The location of *TPRN* relative to the STR markers is also indicated. *Solid circles* and *squares* denote affected individuals; *dots in open circles* and *squares* denote carriers of the mutation as determined by sequencing and genotyping. Alleles: + denotes wild-type allele of *TPRN* with two complete repetitive units in exon 1; – shows the deletion of one of these two repeats (i.e., the mutant allele); *gray shading* denotes the deafness-linked haplotype. On the far left, the haplotype of a previously described individual from family PKDF1129 with the same *TPRN* mutation is provided for comparison with that observed for HLRB6. Marker distances (cM) according to the Rutgers combined genetic map. *C9S2168* is a self-designed STR marker located upstream of *D9S2168*, at physical position chr9:141001671-141001719 (Feb 2009 GRCh37/hg19). **(B)** Pure-tone audiograms for two affected individuals of family HLRB6, showing moderate to profound hearing loss for 13-year-old V:2 (*dotted line*) and 17-year-old V:3 (*solid line*). Hearing thresholds of right ear are marked by circles (×), left ear by crosses (×). **(C)** Electropherograms revealed an 11-nucleotide deletion (c. 42_52del11) in *TPRN* in the DNA of all affected individuals. *Triangle* indicates the start of deletion. *R1* and *R2* indicate the first and second consecutive GC-rich repeats in the sequence

Table 1All known mutations in *TPRN* and associated phenotypes

Exon	Mutation	Nature of hearing loss	Population	Source
1	c.42_52del11	Severe to profound	Pakistani	Rehman et al. 2010
1	c.42_52del11	Severe	Moroccan	Li et al. 2010
1	c.42_52del11	Moderate to profound, progressive inferred	Pakistani	Present study
1	c.44_54dup11	Severe to profound	Pakistani	Rehman et al. 2010
1	c.1056 G>A	Severe to profound	Pakistani	Rehman et al. 2010
1	c.1244delC	Severe to profound	Pakistani	Rehman et al. 2010
1	c.1347delG	Severe to profound, progressive ^a	Dutch	Li et al. 2010

^aNote that all other previously known mutations cause stable severe or profound deafness