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Mutations of *GJB2* Encoding Connexin 26 Contribute to Nonsyndromic Moderate and Severe Hearing Loss in Pakistan

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Abstract

Mutations of *GJB2* which encodes connexin 26, contribute to 6–7% of profound deafness in Pakistan. We investigated the involvement of *GJB2* mutations in a cohort of 84 pedigrees and 86 sporadic individuals with moderate or severe hearing loss. Individuals in eight consanguineous families and four sporadic cases (9.52% and 4.65%, respectively) were homozygous or compound heterozygous for p.W24X or p. W77X mutations in *GJB2*. These two variants are also among the most common mutations known to cause profound deafness in South Asia. The association of identical mutations with both profound and less severe phenotype of hearing loss suggests that alleles of other genes modify the phenotype due to these *GJB2* nonsense mutations. Our study demonstrates that *GJB2* mutations are an important contributor to aetiology of moderate to severe hearing loss in Pakistan.

Keywords

Connexin 26; deafness; hearing loss; GJB2; Pakistan

Introduction

Genetics contributes to hearing loss in more than 50% of individuals affected with prelingual deafness. Mutations in gap junction beta-2, *(GJB2*) are a major cause of non-syndromic hereditary hearing loss in European, American Caucasians and a few other populations. *GJB2* encodes a gap junction protein, connexin 26, which is involved in the movement of small metabolites and ions between the neighboring cells [1]. Some mutations in *GJB2* are quite common, but their frequency is variable worldwide [2]. One of the most prevalent mutations in Caucasians as a cause of recessive inherited deafness is c.35delG. This mutation may account for 86% (95 percent confidence interval, 79–91%) of all *GJB2* related deafness in the French [3] and 85% (95% confidence interval, 78–93%) in some

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

other European populations [4]. The carrier frequency of the mutation in these populations can be as high as 1.96%, (95%, confidence interval, 1.4–2.5%) [5]. Similarly, c.167delT is the most frequent *GJB2* mutation in the Ashkenazi Jews with a carrier frequency of 4.3%, (95% confidence interval, 2.5 to 6.0%), [6].

The contribution of mutations in *GJB2* in Pakistan to severe to profound deafness has been reported to be 6.1% (95 percent confidence interval, 3.2–10.4%) in a study involving 196 large pedigrees with multiple affected individuals with hearing loss [7]. Mutations in *GJB2* account for 6.9% (95%confidence interval, 3.2–10.4%), of moderate to profound deafness in Pakistanis living in the UK, as inferred from analyses of samples from 123 sib pairs [8]. However, the contribution of *GJB2* mutations to deafness in Pakistan was reported to be as high as 53%, (95% confidence interval, 35–71%), in a study involving 30 families with severe to profound deafness [9]. The large discrepancy between the results of this research on 30 families and the estimates of frequency of mutations in *GJB2* obtained from previous studies is not clear, although small sample size of these studies may have contributed to the difference. We sought to focus on the identification of *GJB2* mutations and assessment of hearing function in a series of families in Pakistan, which were characterized by a high rate of consanguineous marriages. The probands were affected by essentially moderate and severe hearing loss.

Methods

Institutional review board approval was obtained for the study from School of Biological Sciences, University of the Punjab, Lahore. Probands were identified from schools catering to children with different disabilities and with help of audiologists in the Punjab province. Detailed clinical histories were obtained to minimize inclusion of individuals with hearing loss due to syndromes and environmental factors. Audiometry was performed in ambient noise conditions at participants' homes since sound-proof rooms were not available. Hearing loss was classified as moderate to severe (41 to 70 dB HL), severe (71 to 95 dB HL) and profound (above 95 dB HL) [10]. Blood samples were collected from 84 large families and 86 sporadic participants segregating non-syndromic, recessive, moderate to severe hearing loss. Written informed consent was obtained from the participants or the parents in case of minor children. The single coding exon of *GJB2* was sequenced from DNA of all affected individuals and 100 ethnically matched controls.

Results

The affected participants of the study were born in consanguineous unions to unaffected parents. Hearing loss in affected individuals in 80% of the participating families could be classified as moderate to severe. Intrafamilial phenotypic variability was observed in 20% of the families in which 1–3 individuals were affected with severe to profound deafness, while the majority of the individuals had moderate to severe hearing loss. All affected individuals were born in consanguineous unions to unaffected parents. Sequence analysis revealed two disease causing mutations in *GJB2* for some of the participants (Table 1). Eight out of 84 families had nonsense mutations in *GJB2* (c.71G>A, p.W24X or C.231G>A, p.W77X). Among the non-familial cases of hearing loss, homozygous mutations in *GJB2* were

identified in 4 out of 86 individuals. The p.W24X mutation was most frequently observed among the participants (Table 1). Samples from all other participating familial and non-familial participants were negative for homozygous, or compound heterozygous, mutations in *GJB2*.

We observed compound heterozygous mutations (p.W24X, p.W77X) in samples of affected individuals in two large families, HLRB8 and HLAM09, although the subjects were born in consanguineous marriages. This may be due to the high carrier frequencies of these founder mutations in the population. However, we did not detect these two mutations in 200 chromosomes from ethnically matched controls.

In 4 families (HLRB1, HLAI-02, HLAI-12 and HLGM25), there was marked intrafamilial variability of the phenotype (Table 1 and Fig. 1). The majority of the affected subjects in these pedigrees had moderate to severe hearing loss, while 1 to 3 individuals in each family had profound deafness. There was no correlation with age or environment which could account for the worse hearing loss of these individuals as compared to the other affected individuals with relatively better hearing in their families.

Discussion

The present study revealed that *GJB2* mutations contribute to 9.52% (95% confidence interval, 3.2 to 15.7%) of moderate to severe hearing loss among familial participants and 4.65% (95% confidence interval, 0.2 to 9.1%) among sporadically affected individuals. The lower contribution of 4.65% in the sporadic cases as compared to that with a clearly demonstrated recessive mode of inherited deafness, may reflect the inclusion of some individuals in this cohort who are either affected due to non-genetic causes, or have hearing loss as a result of *de novo* mutations in other deafness causing genes.

Previously, truncating mutations in *GJB2*, present together with non-truncating mutations (i.e. compound heterozygote) have been reported to result in a less severe hearing loss [11,12]. To date, c.35delG is the only known deletion mutation in *GJB2* which has been widely reported to lead to less severe hearing loss in individuals who are homozygous for the allele. However, we found that the two nonsense mutations in the Pakistani population, p.W24X and p.W77X, present either in the homozygous state or in compound heterozygosity, may similarly result in moderate to severe hearing loss in a large number of affected individuals. Interestingly, p.W24X and p.W77X are also the most common mutations involved in profound deafness in Pakistan [7]. The variability in hearing thresholds of individuals with p.W24X and p.W77X mutations is similar to the phenotypic difference exhibited by the individuals homozygous for the c.35delG allele.

The association of p.W24X and p.W77X mutations with both profound and less severe phenotype of hearing loss suggests the effect of modifier genes that lead to variation in hearing thresholds [13] and is similar to that suspected for c.35delG related deafness. So far, for lack of a sufficiently large family, it has not been possible to map a genetic modifier, which reduces severity of hearing loss caused by *GJB2* mutations [14]. Whole exome or genome sequencing and a sufficiently large number of individuals in nuclear

consanguineous families with *GJB2* pathogenic alleles showing carefully documented variation in hearing thresholds, as well as matched controls, might allow for the identification of enhancers and suppressors of *GJB2* hearing loss. This will broaden our understanding of audition and may help in devising eventual treatments or cures.

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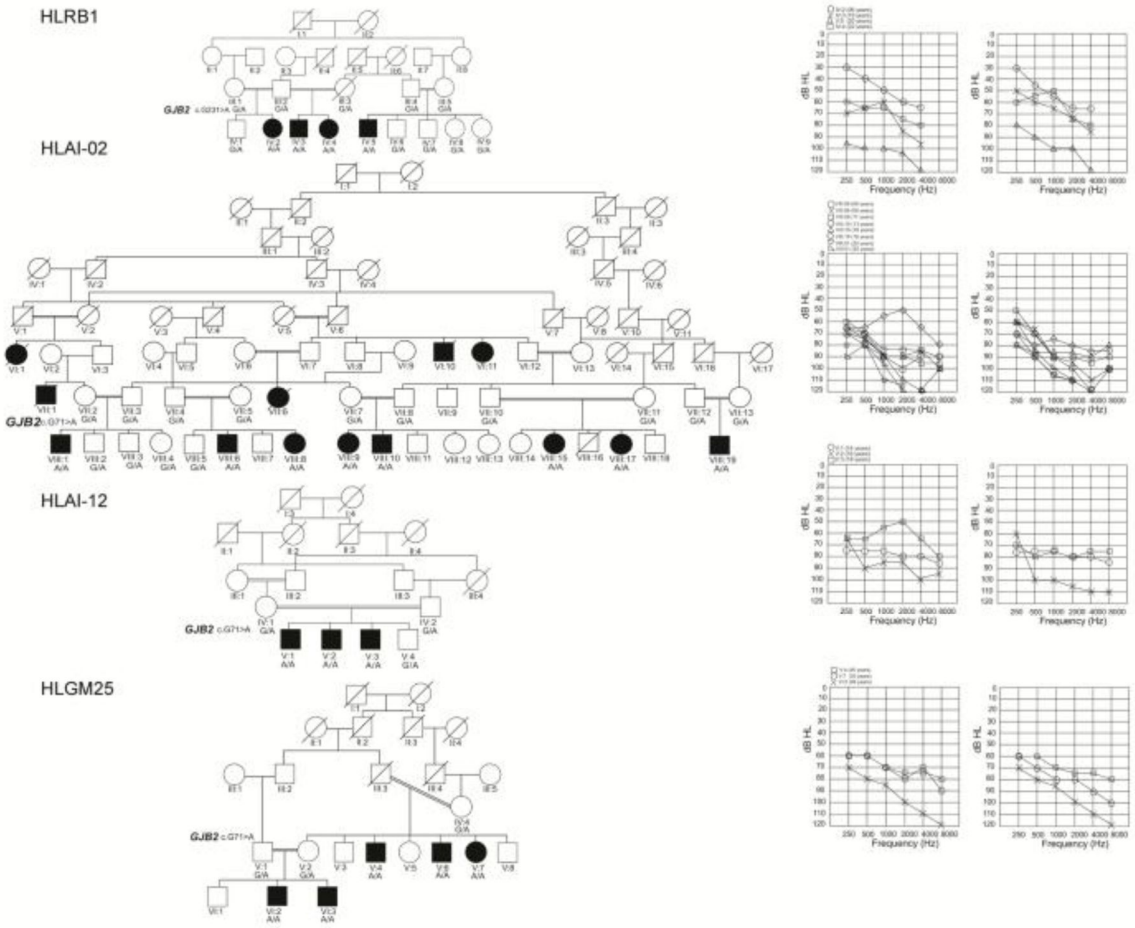

Figure 1. Pedigrees of four families in which the affected individuals exhibit intrafamilial differences in hearing thresholds

Black circle's and squares represent affected female and male participants, respectively. The double horizontal lines denote consanguineous marriages. The genotype for the mutation in *GJB2* is provided below each symbol representing the participant. The audiograms on the right side of each pedigree represent the hearing thresholds of the left and the right ears of the affected individuals, respectively.

Table 1
Description of families, phenotype and *GJB2* mutations

Results are tabulated for 8 of 84 families and 4 of 86 sporadic individuals positive for mutations in *GJB2*.

ID	Hearing Loss, $PTA_{500-4000}$*, dB HL	Mutation	Comment
Familial Cases			
HLRB1	Moderate to severe 54,65, 71,103	p.W77X/p.W77X	4 affected individuals in 3 loops (1 affected individual has a profound HL)
HLRB8	Moderate to severe 70, 78, 78	p.W24X/p.W77X	6 affected individuals in 2 loops
HLAI-02	Severe and profound 81, 86, 88, 98, 103, 104	p.W24X/p.W24X	8 affected individuals in 5 loops (3 individuals have profound deafness)
HLAI-12	Moderate to severe 59, 71, 90	p.W24X/p.W24X	3 affected individuals
HLAM09	Moderate to severe 61	p.W24X/p.W77X	3 affected individuals
HLGM25	Moderate to severe 70, 70, 94	p.W24X/p.W24X	5 affected individuals in 2 loops (1 affected individual has profound HL)
HLMS16**	Severe 74,79	p.W24X/p.W24X	2 affected individuals
HLMS34**	Severe 75, 78	p.W24X/p.W24X	2 affected individuals in 2 loops
Sporadic cases			
HLMS11	Severe 84	p.W77X/p.W77X	NA
HLMS25	Severe 84	p.W24X/p.W24X	NA
SA15	Severe 89	p.W24X/p.W24X	NA
HLRB15#	Severe 82	p.W24X/p.W24X	NA

*Pure-tone air conduction averages, db HL, for hearing thresholds at 500, 1000, 2000 and 4000 Hz ($PTA^{500-4000}$) of the better hearing ear is provided for all affected participants, except for those who refused the evaluation.

**The only two families in the entire collection of 84 large pedigrees with inherited hearing loss which had 2 affected individuals in one or two consanguineous marriages. The other 82 families had multiple (3 or more) affected individuals in a single, or several marriage loops.

#Although family HLRB15 had four affected individuals, sequencing revealed that only one participant had a homozygous mutation in *GJB2*. Therefore, the sample from this family is considered as a sporadic case. HL, Hearing Loss, NA, Not applicable.