Low prevalence of GJB2 mutations in non-syndromic hearing loss in Western India

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Abstract

Objectives To identify the prevalence of GJB2 (Cx 26) and GJB6 (Cx 30) mutations in hearing impaired individuals from Western and South India.

Study design Cross-sectional study.

Methods Families with hearing impaired individuals (prelingual, non-syndromic, sensori-neural hearing loss) were enrolled and genomic DNA was extracted. Primers were designed for amplifying the coding and non-coding exons including flanking splice sites of the Cx 26 gene. Probands heterozygous or negative for Cx 26 mutations were further analyzed for the 342Kb deletion encompassing D13S1830 microsatellite marker on Cx 30.

Results Two hundred and eighty-eight patients were enrolled in the study and 116 (40.3%) were diagnosed to have mutations in the coding exon 2 of Cx 26 gene. Fifty-four (18.8%) probands were found to have mutations in both the alleles while the remaining 62 (21.5%) were heterozygous for Cx 26 mutations. W24X, and W77X were the common mutations identified. The prevalence of familial

deafness was similar in both consanguineous and non-consanguineous families (33% and 34.9% respectively).

Mutations in the non-coding exon 1 and intron 1 as well as the 342 kb deletion involving D13S1830 marker on Cx 30 were ruled out in two hundred and thirty-four deaf individuals carrying none or only one mutation in the exon 2 of Cx 26 gene.

Conclusion Cx 30 mutations do not contribute to the autosomal recessive non-syndromic hearing loss (NSHL) in the Indian population. Homozygous Cx26 mutations account only for about 1/5th (18.8%) of autosomal recessive non-syndromic hearing implying the need to explore other contributory loci.

Keywords Non-syndromic hearing loss · Genetics

Introduction

Prelingual hearing loss is a serious concern because it affects development of language and may interfere with development of social and cognitive skills. Prelingual deafness occurs with a frequency of 1 in 1000 live births, of which about 50% is reported to be genetic in origin [1]. Families with affected individuals are often anxious about recurrence of hearing loss in their future pregnancies as treatment and rehabilitation options are limited and expensive. Prenatal diagnosis for such families is possible only through genetic testing of fetal DNA as this condition cannot be diagnosed by any other non-invasive tests such as ultrasound. Genetic basis of hearing loss is highly complex with evidence of tremendous heterogeneity and variable patterns of inheritance. About 100 loci have been identified for autosomal dominant and recessive deafness (<http://webhost.ua.ac.be/hhh>), apart from sex-linked and mitochondrial deafness. However, mutations in 2 genes Cx 26 and Cx 30

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have been identified as major cause of autosomal recessive deafness in different populations all over the world [1]. In addition, the spectrum of mutations in Cx 26 gene is known to differ in different ethnic populations.

Indian data on the genetics of non-syndromic hearing NSHL is limited and is based on several small studies except a recent larger study by Mani et al. [2]. India is a huge country and hence, it is difficult to extrapolate the results obtained from one part of the country to other regions. We investigated the prevalence of Cx 26 and Cx 30 mutations in hearing impaired individuals from Western and South India. Here we discuss the results of our study as well as the need for further studying the genetic basis of hearing loss in Indians. This study adds to the existing database of mutational spectrum of Cx 26 gene in different regions from India and thus may aid in the genetic testing and prenatal diagnosis in various target families.

Materials and methods

Enrolment of subjects

Families with hearing impaired individuals were contacted through the special schools and social organizations for the deaf and Cochlear Implant centers from various cities of Maharashtra, Western India and from Chennai, Tamil Nadu, Southern India. Preceding the enrolment and sample collection, the research team conducted special sessions to create awareness regarding the hereditary component of deafness.

Inclusion criteria

Individuals with congenital, bilateral, severe to profound sensori-neural deafness without any other dysmorphic features or systemic affection and without any known etiology for deafness were included in the study. The degree of deafness was confirmed by Pure Tone Audiometry (PTA) and/or Brain Stem Evoked Response Audiometry (BERA). A structured questionnaire was used to obtain details of hearing disability including age of diagnosis, type, severity, rehabilitation and educational history etc., along with the three-generation family history, wherever available.

Procedure

Five milliliter whole blood was collected in EDTA from the affected individuals and their family members after obtaining appropriate informed consent. Genomic DNA was extracted from peripheral blood leucocytes according to standard protocols. Primers were designed for amplifying the coding and non-coding exons of the Cx 26 gene

(Cx 26) including flanking splice sites. Polymerase chain reaction (PCR) products were purified, sequenced individually on both strands using ABI Genetic Analyzer 3730 and analyzed using Autoassembler software. Mutations identified in the probands were analysed in their parents to aid in future prenatal diagnosis and genetic counseling. Probands heterozygous or negative for Cx 26 mutations were further analyzed for the 342Kb deletion encompassing D13S1830 microsatellite marker on Cx 30 (Cx 30). The junction fragment caused by the 342 Kb deletion in Cx 30 gene was analyzed using a multiplex PCR approach. The study protocol was approved by ethics committees of both collaborating institutes.

Results

A total of 288 unrelated families comprising of 183 male and 105 female probands were enrolled in the study. Nearly 1/3rd of the families reported consanguineous marriage (106/288; 36.8%). However, the prevalence of familial deafness (more than one individual affected), was similar in both consanguineous and non-consanguineous families (33% and 34.9% respectively) suggesting possible heterogeneity in pattern of inheritance as well as possibility of high carrier frequency of Cx 26 mutations in this study population.

We identified mutations in the coding exon 2 of Cx 26 in 116 out of 288 families (40.3%) screened. Of these, 54 (18.8%) probands were found to have mutations in both the alleles while the remaining 62 (21.5%) were heterozygous for Cx 26 mutations. Amongst the 54 individuals with bi-allelic Cx 26 mutations, 48 were homozygous while six were compound heterozygotes for two different mutations in the Cx 26 gene. W24X and W77X were the common mutations identified with W24X accounting for 19.4% (56/288) of deaf individuals, followed by W77X in 17/288 (5.9%) patients. R127H variant was present in 41 probands that included 3 homozygotes, 36 heterozygotes and 2 compound heterozygotes with W24X mutation. Other mutations including M1V, V27I, and 35insG were found in one proband each. Nearly half of the probands (26/54; 48.1%) with bi-allelic Cx 26 mutations were born to non-consanguineous parents and an equal proportion had consanguineous parents.

Two hundred and thirty four deaf individuals carrying none or only one mutation in the exon two of Cx 26 gene were analyzed for mutations in the non-coding exon one and intron 1 and no mutation was identified. These individuals were then screened for the 342 kb deletion involving D13S180 at a second locus Cx 30 (Δ Cx 30-D13S1830 deletion) followed by sequencing of the complete gene. None of the reported pathological mutations were identified in Cx 30 gene in any of these families including those heterozygous for a Cx 26 mutation.

Discussion

Congenital NSHL accounts for about 50% of childhood prelingual deafness of which close to half is of genetic origin. This may further be segregated into autosomal recessive (75–80%), autosomal dominant (20–22%) and X-linked and mitochondrial deafness in about 2% each [3]. Mutations in Cx 26 gene, encoding gap junction protein connexin 26 account for about 75–80% of autosomal recessive NSHL in the western world. A large deletion involving D13S180 at another locus Cx 30, encoding another gap junction protein Connexin 30 has been reported in 10–50% of patients heterozygous for Cx 26 mutations, suggesting a digenic inheritance [4, 5]. Cx26 and Cx30 are both expressed in the cochlea, leading to the potential formation of heteromeric hemichannels and heterotypic gap junction channels [6]. Cx 26 and Cx 30 are expressed in the supportive cells of the cochlea, suggesting a potential role in endolymph potassium recycling [7].

Our results, in agreement with previous Indian studies confirm that Cx 26 mutations contribute to about 1/5th of NSHL in Indian population and W24X and W77X are predominant mutations rather than the most common Caucasian 35delG mutation in the Cx 26 gene. In fact, none of the 288 probands in our study carried 35delG mutation in contrast to a recent study by Bhalla et al., which reported a 10.9% prevalence of this mutation in North Indian patients [8]. Presence of 35delG in NSHL patients has also been reported by Ghosh et al. [3] and Mani et al. [2] but with low prevalence of 2.5% and 0.75% respectively. A significant ethnic and geographic variation in Cx 26 mutations across the continents has been reported earlier, for example, 35delG mutation is predominant in Europeans [9], 167delT in Ashkenazi Jews [10] and 235delC in Japanese [11] etc.

W24X is the predominant allele in Spanish gypsy Romani subpopulation accounting for 79% of Cx 26 alleles with possibility of founder effect in this population [12]. The authors suggest that the Romani people trace their origins to the Indian subcontinent, from where they moved in successive migrations, arriving in Europe in the 11th century. The finding of a high prevalence of the W24X mutation in Slovak and Spanish gypsies and in India traces the origins of the mutation to the Indian subcontinent. R127H is mainly considered as a polymorphism rather than pathogenic mutation and its phenotypic expression is thought to be modulated by environmental factors [8].

Our study also confirms the absence of Cx 30 deletion in 234 families of Indian origin as recently reported by Bhalla et al. [8]. Although digenic inheritance with Cx 26-Cx 30 compound heterozygosity has been reported by del Castillo et al., studies from Morocco, Czech Republic and other countries show low prevalence or absence of Cx 30 deletion [13, 14]. This probably supports the hypothesis of evolutionary origins of del (Cx 30-Δ13S1830) [15–19] mutation with possible founder effect in Ashkenazi Jews [5]. Table 1 compares prevalence of Cx 26 and Cx 30 mutations in our study with Indian as well as other studies.

Cx26 mutations have been occasionally shown to be associated with autosomal dominant deafness; however family history of subjects in this study was consistent with an autosomal recessive transmission pattern. One third of these individuals were born to unaffected consanguineous parents and had more than one affected members in the family. This leads to several possibilities including 1) bi-allelic mutations in a different gene with coincidental carrier status for a Cx 26 mutation, 2) another Cx 26 mutation in the promoter or unexplored regulatory region of the gene and 3) a possibility that there could be mutations in some other

Table 1 Worldwide distribution of Cx 26 and Cx 30 mutations

Study	Sample size (Probands)	Country/population	Cx 26 bi-allelic (%)	Cx 26 heterozygote (%)	Cx 30 342 kb deletion/ other mutations (%)
Present study	288	India	18.3	18.3	0
Maheshwari, 2003	45	India	13.3	–	–
Ramshankar, 2003	215	India	17.7	–	–
Mani, 2009	530	India	21.0	–	–
Bhalla, 2009	200	India	3.5	25.5	0
Lerer, 2000	27	Ashkenazi Jews	77.4	–	–
Liu, 2002	210	China	20	1.42	–
del Castillo, 2002	422	Spain, Cuba	30.6	10.4	about 5.5
Wu, 2003	108	USA	26.85	37.03	1.8
Frei, 2004	76	Austria	25.5	9.8	0
Santos, 2005	196	Pakistan	6.1	–	–
Najmabadi, 2005	664	Iran	16.7	–	–
Alvarez, 2005	34	Spain	44.11	8.82	3.5

gene (digenic inheritance), which might disturb the inner ear functioning when associated with Cx 26 heterozygous state. However, no other gene apart from Cx 30 has so far been reported accounting for digenic inheritance pattern.

Conclusions

Our study suggests that Cx 30 mutations do not contribute to the autosomal recessive NSHL in the Indian population. It also suggests that although homozygous Cx 26 mutations account for about 1/5th (18.8%) of autosomal recessive NSHL, there is a need to explore other loci, which might contribute significantly to this condition. Although about 42 genes have been identified to cause NSHL (<http://webhost.ua.ac.be/hhh>), only few of them have been explored in the Indian population and a variable spectrum of mutations in various candidate genes is evident even from the smaller studies [20]. In a country like India where consanguineous marriages and marriages within the same communities is not uncommon, the incidence of recessive hearing loss could be proportionately higher and is likely to be even more heterogeneous in view of ethnic diversity. Studies to dissect the spectrum of mutations in known candidate genes in ethnically homogeneous Indian population and subsequently, extending them towards the identification of new candidate genes using currently available genomic tools are needed. This will ultimately help to plan and offer selected genetic tests to the patients with hearing loss as well as provide carrier screening and in redefining the genetics of NSHL in India.

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