Prevalence of Cx26 (GJB2) Gene Mutations Causing Recessive Nonsyndromic Hearing Impairment in India

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KEYWORDS NSHI; GJB2; W24X; mutation; India

ABSTRACT Autosomal Recessive Nonsyndromic Hearing Impairment (ARNSHI) is caused by mutations in the gap junction gene GJB2 or Connexin26 gene (Cx26) in many of the world populations. In the present study screening of 200 probands with profound Nonsyndromic Hearing Impairment (NSHI) in comparison to 200 normal hearing controls from Andhra Pradesh, India revealed high prevalence of W24X mutation (6.5%) and low frequency of W77X (0.5%) and 235delC (0.5%) mutations. Incidence of 35delG and 167delT were not detected. High incidence of heterozygosity with R127H both in NSHI patients (28.0%) and in controls (36.5%) were observed indicating that the mutation could be a polymorphism and may not be the cause of NSHI. Two of the probands (1.0%) showed homozygosity for the mutation and causative nature of this mutation has to be evaluated. Cx26 mutations causing hearing impairment are found to be specific to certain populations exhibiting ethnic diversity.

INTRODUCTION

Hearing loss is one of the frequent sensory defects affecting 1 in 500-650 children born deaf worldwide (Mehl and Thomson 1998; Mehl and Thomson 2002). The genetic basis of hearing impairment is well established with about 60% of the cases with definite genetic etiology (Cohen and Gorlin 1995). Approximately in 85% of these cases, the hearing impairment is described as non-syndromic exhibiting autosomal dominant, recessive, X-linked or mitochondrial inheritance showing significant genetic heterogeneity. Several genes responsible for NSHI are now cloned (http://www.uia.ac.be/dnalab/ hh). Mutations in the GJB2 gene (13q11-q12) is implicated as a major cause for the development of congenital hearing impairment specially the recessive types (Kelsell et al. 1997; Estivill et al. 1998; Kenneson et al. 2002; MIM #220290; http://www.crg.es/ deafness/).

Connexins are the family of gap junction proteins that play a major role in intracellular communication (Simon et al. 1998). The protein encoded by GJB2 (gap junction beta-2) gene referred as connexin26 (Cx26) is responsible for the causation of prelingual hearing impairment. The mutation 35delG is the most common that causes NSHI among white populations (Denoyelle et al. 1997; Estivill et al. 1998; Kelley et al. 1998; Rabionnet et al. 2000; Roux et al. 2004). In the Ashkenazi Jews population, the 167 del T is considered as a founder mutation occurring with a carrier frequency of about 4% with comparatively low frequency of 35delG mutation (0.7%). These two mutations in the GJB2 gene in general are observed to contribute to about 70-80% of childhood NSHI (Morell et al. 1998; Sobe et al. 1999; Lerer et al. 2000; Sobe et al. 2000). In addition to Cx26, genes encoding other members of the connexin family like Cx30 (GJB6), Cx31 (GJB3), Cx32 (GJB1), and Cx43 (GJA1) are also identified as responsible for hearing loss. Involvement of digenic mutations, large deletions in Cx30 have also been associated with NSHI (del Castillo et al. 2002; Pallares-Ruiz et al. 2002; Roux et al. 2004).

Preliminary studies conducted on Indian samples from other countries revealed absence of the mutation 35delG, which is rather highly prevalent in some of the worlds’ populations (Scott et al. 1998). Instead high frequency of W24X mutation among probands of NSHI is observed with a prevalence of 13.3% (Maheswari et al. 2003) and 18.1% (Ramshanker et al. 2003). Other mutations contributing to NSHI are yet to be identified. The present study was undertaken to identify the mutations in the coding region exon2 of connexin26 gene from Andhra Pradesh, India.

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MATERIALS AND METHODS

We examined 356 probands with NSHI from the Ear Nose and Throat (ENT) Hospital and Schools for Deaf from Hyderabad and near by Districts, Andhra Pradesh, India to identify the mutations in the coding region, exon2 of GJB2 (connexin 26) gene. All the probands were examined by the ENT specialists and the loss of hearing was graded based on the Pure Tone Audiometry (PTA). Cases diagnosed as congenital bilateral profound sensorineural type of deafness alone were included in the study. Cases of acquired hearing impairment and syndromes associated with deafness were eliminated from the study.

From all the patients and controls, information was collected after obtaining the informed consent on the clinical history, maternal reproductive and clinical history, family history for the incidence of hearing impairment and consanguinity by personal interviews. The information was recorded using a proforma specifically prepared for the purpose. Blood samples were collected in vacutainer tubes containing EDTA from all the subjects. DNA was isolated by salting out method (Lahiri and Nurnberger 1991). 200 samples of DNA from NSHI probands and 200 samples from normal hearing controls without family history of NSHI were analyzed for the mutations in the coding region, exon 2 of Cx26 gene.

Allele specific PCR amplification of genomic DNA was performed for mutations 35delG, and W77X as described by Scott et al. (1998). PCR products were analyzed by electrophoresis in 1.5 % agarose gel containing ethidium bromide. Restriction digestion was performed for the detection of W24X (Ramshanker et al. 2003), 167delT (Lerer et al. 2000) and 235delC (Kudo et al. 2000) as described earlier. Single strand conformation polymorphism analysis of PCR amplified products of genomic DNA was performed for all the samples as described previously (Scott et al. 1998) followed by electrophoresis on a fan cooled 12% mini slab gel (49:1) for ~14 hr at 100V. The resulting gels were visualized by silver staining. Sequence analysis for the samples was done by ABI 3100 DNA sequence analyzer using the primers as suggested by Kelsell et al. (1997).

RESULTS

In the present study, analysis of 200 DNA samples from probands (males-112; females-88) in comparison to 200 normal hearing controls (males-107; females-93) yielded the results as specified in table 1. All the probands had profound NSHI with >90dB.

Analysis of DNA samples revealed high prevalence of W24X mutation (6.5%) and rare incidence of W77X (0.5%) and 235delC (0.5%). R127H, which is considered as polymorphism in some of the studies, is found in probands as

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Effect</th>
<th>Protein Domain</th>
<th>Genotype</th>
<th>Probands</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>35delG</td>
<td>Frameshift</td>
<td>IC1</td>
<td>+/-</td>
<td>0/200 (0%)</td>
<td>+/+ 0/200 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/-</td>
<td>0/200 (0%)</td>
<td>+/- 0/200 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-/-</td>
<td>200/200 (100%)</td>
<td>-/- 200/200 (100%)</td>
<td></td>
</tr>
<tr>
<td>167delT</td>
<td>Frameshift</td>
<td>EC1</td>
<td>+/-</td>
<td>0/200 (0%)</td>
<td>+/+ 0/200 (0%)</td>
</tr>
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<td></td>
<td></td>
<td>+/-</td>
<td>0/200 (0%)</td>
<td>+/- 0/200 (0%)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>-/-</td>
<td>200/200 (100%)</td>
<td>-/- 200/200 (100%)</td>
<td></td>
</tr>
<tr>
<td>G to A 71bp</td>
<td>W24X</td>
<td>TM1</td>
<td>+/-</td>
<td>13/200 (6.5%)</td>
<td>+/+ 0/200 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/-</td>
<td>1/200 (0.5%)</td>
<td>+/- 0/200 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-/-</td>
<td>186/200 (93%)</td>
<td>-/- 200/200 (100%)</td>
<td></td>
</tr>
<tr>
<td>G to A 231bp</td>
<td>W77X</td>
<td>TM2</td>
<td>+/-</td>
<td>1/200 (0.5%)</td>
<td>+/+ 0/200 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/-</td>
<td>0/200 (0%)</td>
<td>+/- 0/200 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-/-</td>
<td>199/200 (99.5%)</td>
<td>-/- 200/200 (100%)</td>
<td></td>
</tr>
<tr>
<td>235delC</td>
<td>Frameshift</td>
<td>TM2</td>
<td>+/-</td>
<td>1/200 (0.5%)</td>
<td>+/+ 0/200 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/-</td>
<td>0/200 (0%)</td>
<td>+/- 0/200 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-/-</td>
<td>199/200 (99.5%)</td>
<td>-/- 200/200 (100%)</td>
<td></td>
</tr>
<tr>
<td>G to A 380 bp</td>
<td>R127H</td>
<td>IC2</td>
<td>+/-</td>
<td>2/200 (1%)</td>
<td>+/+ 0/52 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/-</td>
<td>56/200 (28%)</td>
<td>+/- 19/52 (36.5%)</td>
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<tr>
<td></td>
<td></td>
<td>-/-</td>
<td>142/200 (71%)</td>
<td>-/- 33/52 (63.4%)</td>
<td></td>
</tr>
</tbody>
</table>

+/- : Homozygous mutants; +/- : Heterozygous; -/- : Homozygous Normal
IC : Intracellular domain; EC : Extracellular domain; TM : Transmembrane domain
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homozygotes (1.0%) and also as heterozygotes (28.0%). In controls only heterozygotes were found with a higher frequency (36.5%) as compared to that found in probands.

None of the proband and control samples revealed the presence of 35delG and 167delT.

DISCUSSION

Variants of several connexin genes, in particular of the Cx26-encoding GJB2 have been shown to underlie distinct forms of hearing impairment specially ARNSHI. Connexin26, the gap junction protein (GJB2) has an important role in recycling of potassium ions depolarizing hair cells. These hair cells allow potassium ions across the supporting cells through the gap junction of the stria vascularies and back to endolymph (Kikuchi et al. 2000).

Defect in Cx26 gene hence disrupt this potassium ion flow causing complete or partial loss of this protein leading to hearing loss (Martin et al. 1999; Yeager et al. 1998).

Among the variants identified, a causative role was initially established for 35delG which causes frameshift generating a premature stop codon (Zelante et al. 1997). It is predominantly seen among the subjects of ARNSHI in Caucasian and other Mediterranean populations from Italy (88.0%) and Spain (55.0%) (Estivill et al. 1998), 55% in Australia (Wilcox et al. 2000), and Lebanon (94.0%) (Mustapha et al. 2001). 35delG mutation was found in 100% of the DFNB1 linked cases. The 35delG mutation has a carrier frequency of 3% in the white population (Estivill et al. 1998; Lench et al. 1998). In fact, the 35delG mutation is thought to occur as a hotspot mutation, (Denoyelle et al. 1997; Kelley et al. 1998; Morell et al. 1998) although it has been recently suggested based on Single Nucleotide Polymorphisms (SNPs) that its frequency could be secondary to a founder effect (Van Laer et al. 2001).

From India so far occurrence of 35delG mutation is not reported indicating relatively low frequency of this mutant allele in this population (Maheswari et al. 2003; Ramshanker et al. 2003), as found in other populations viz., African Americans, Asian Americans, Egyptians, English and Jordanians (Van Laer et al. 2001). Absence of 35delG as found in the present study suggests that the incidence of this mutation may be rare adding to the population diversity for this gene.

Next to 35delG mutation, deletion of T at 167 position in the Cx26 gene is found to be the cause of NSHI which was predominantly reported in Ashkenazi Jews which is attributed to be due to a founder effect based on haplotype analysis (Lerer et al. 2000). The carrier rate of this mutation in Ashkenazi Jews in the control group was found to be 4.03% (Morell et al. 1998; Lerer et al. 2000; Sober et al. 2000) while 35delG was 0.73% (Morell et al. 1998). In the present study none of the samples revealed the presence of the mutation for 167delT suggesting absence or low incidence of this mutation.

In this study only one (0.5%) proband with W77X mutation involving a premature stop codon (Try to Opal: W77X) due to G to A substitution at codon 77 resulting in a nonsense mutation (W77X) was identified. Similar mutation was previously reported in 3 NSHI families from Pakistan, (Kelsell et al. 1997) in two families and one individual case from India (Scott et al. 1998; Ramshanker et al. 2003).

Deletion of C at 235bp which is reported to be a common mutation in Japanese population (Kudo et al. 2000) was detected in only one case (0.5%) of NSHI in this study. None of the 200 controls detected positive for the mutation. The mutation causes frameshift at codon 79, and truncation of the protein disrupting the recycling of endolymphatic potassium ions. This mutation has been detected mostly in the individuals of Japanese origin, at a frequency of 7.8% in cohorts with NSHI and at a frequency of 0.0% to 1.0% in control cohorts (Fuse et al. 1999; Kudo et al. 2000; Abe et al. 2000; Park et al. 2000). GJB2 hearing impairment genes shows a specific spectrum of mutations in Japan including a frequent founder mutation 235delC inherited from a common ancestor (Ohtsuka et al. 2003). Similar founder effect of this mutation in Chinese was also postulated (Liu et al. 2002).

R127H mutation in which an arginine is replaced by histidine due to a G to A transition at codon 127 (Arg127His) maps to the second cytoplasmic domain of GJB2. This mutation was previously described in hearing impaired patients as the sole mutation found in Cx26, affecting a residue that is not highly conserved among β-connexins (Estivill et al. 1998; Rabionet et al. 2000) implying nonpathogenic nature of this mutation. Compound heterozygous genotypes R127H/M34T or R127H/W24X were reported by Roux et al. (2004) in two families where normal sibs of the probands also had similar genotypes. This observation and reports of parents who were normal with
R127H/R127H homozygosity indicate that the mutation could be a poly-morphism not causative of NSHI. However the functional studies of this variant were inconsis-tent (Thonnissen et al. 2003; Wang et al. 2003). The frequency of carrier rate for this mutation in France was found to be 1/75 (1.3%), not signifi-cantly different from that of the deaf population. These findings support combined genotypes with variants such as M34T, V37I, and or R127H could have a phenotypic expression modulated by environmental factors or modifier genes (Roux et al. 2004). In Indian hearing population, R127H allele was found at a high frequency 17.5% in previous study (Ramshanker et al. 2003), 36.5% in the present study, strongly suggesting that this is not a causative mutation (Roux et al. 2004).

W24X is found to be a common allele among the mutations screened, causing ARNSHI (13.33%) in North India (Maheswari et al. 2003) and 18.1% in South India (Ramshanker et al. 2003). Incorporation of a stop codon in this mutation at codon 24 in Cx26 gene results in the formation of a protein that is just one-tenth the length of the wild type protein (Kelsell et al. 1997). Haplotype analysis of markers flanking the GJB2 gene showed W24X as the most common mutation found in the Indian population, probably occurring as a founder effect (Ramshanker et al. 2003). In the present study, of the 200 probands screened there were 13 (6.5%) who were homozygous and 1 (0.5%) heterozygous suggesting variation in the incidence of the mutation between North and South Indian populations.

Present study is the first of its kind from Andhra Pradesh, India. So far there seems to be no reports on the prevalence of W24X mutation among deaf from other populations. The high frequency of W24X mutation restricted to Indian subcontinent and absence or low prevalence of 35delG, 167delT and 235delC could be a population specific feature contributing further to the ethnic diversity observed globally.

The results of the present study brings out the need to focus in future on the screening of newborns for various mutations in Cx26 for early intervention and language development taking advantage of residual hearing. It’s also imperative to screen the population for W24X mutation to identify the carriers and counsel them appropriately to avoid the occurrence of the NSHI in their progeny. Counseling the affected against assortative mating is also equally important in bringing down the incidence of NSHI in this population.

ACKNOWLEDGEMENTS

We acknowledge the Department of Atomic Energy for providing financial support for this project and Ramchander is thankful to Council for Scientific and Industrial Research for the award of a Senior Research Fellowship. We are thankful to all the subjects who participated voluntarily in the present study.

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