RESEARCH ARTICLE

GJB2 Gene Mutations Causing Hearing Loss in Consanguineous Pakistani Families

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ARTICLE INFO

Received: Dec 03, 2014
Accepted: Dec 21, 2014
Online: Dec 27, 2014

Keywords
Compound heterozygosity Connexin26 Deafness GJB2 Hearing loss

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ABSTRACT

Because of the availability of highly consanguineous population and large family size, Pakistani population has been a rich source for genetic investigations of autosomal recessive disorders such as deafness. In this study, we recruited 20 consanguineous Pakistani families segregating autosomal recessive hearing loss. Sequencing the GJB2 gene revealed six mutations in 10 families which were previously reported to cause genetic deafness in various populations across the world. Seven of these families were homozygous while three were compound heterozygous for two different mutations. The high ratio of compound heterozygosity in these families indicates that GJB2 mutations are prevalent in our population. According to our findings p.Trp24* was found to be the most frequent GJB2 mutation in Pakistan. Therefore, screening our population for this mutation can play a crucial role in lowering down the incidence of hearing loss in Pakistan.

INTRODUCTION

Profound hearing loss in the childhood has far-reaching lifelong consequences for both children and their families, especially in terms of educational and employment prospects (Mohr et al., 2000; Schroeder et al., 2006). Approximately 1.2 -1.7 of 1000 live births in the world suffer from permanent bilateral congenital hearing loss (Newton, 1985). Due to late onset and delayed diagnosis, this frequency further rises by the age of six years. In developing countries, prevalence is greater because of a lack of immunization, greater exposure to ototoxic agents, and consanguinity. About half of the disabling cases of hearing loss worldwide are preventable (Kral and O'Donoghue, 2010). Approximately half of these cases are attributed to genetic causes of which 70% are isolated while the rest of the 30% have additional disability such as cognitive impairment (Van Naarden et al., 1999) or cardiac dysfunction (Baig et al., 2011). Like other genetic disorders, hearing impairment too has high incidence among consanguineous populations (Zakzouk, 2002).

Pakistan, where >60% marriages take place between cousins, has thus a high prevalence of deafness (Hussain and Bittles, 1998). Congenital deafness changes the functional properties of the auditory system and impairs the cortical development, affecting the mutual interaction of the cortical areas (Gilley et al., 2008; Kral et al., 2006). In the congenitally deaf people, even if the hearing is restored later in life, auditory functions and speech perception cannot be comprehensively established because some aberrant developmental steps in synaptic counts, plasticity and network properties have taken place without hearing. Stimulation of the auditory system during maximal receptiveness is therefore, pivotal to its normal development (Kral et al., 2006; Sharma et al., 2007). Hearing loss is a heterogeneous disorder and to date more than 1200 causative variants have been reported in over 70 genes implicated in nonsyndromic hearing loss [NSHL, (Shearer and Smith, 2012)]. The genetic heterogeneity is a clear indicator of the complexity of the hearing process. The growing knowledge about different causative genes for hearing loss has enabled...
us to understand the basic genetic architecture of deafness. Genes that transport ions across membranes to maintain appropriate solute concentration and pH are the most important which is evident from mutations in the various gap junction proteins and potassium ion-channel genes. Gap junctions are clustered channels between contacting cells through which direct intercellular communication via diffusion of ions and metabolites can occur (Tekin et al., 2001; Willecke et al., 2002). To date 21 gap junction genes have been identified of which 19 can be grouped as orthologous pairs (Sohl and Willecke, 2004). Other genes which are important are regulatory genes and genes that play a role in structural integrity. Of the gap junction genes, mutations in one gene, GJB2 (OMIM 121011), have been found to cause up to half of the autosomal recessive nonsyndromic hearing loss (ARNSHL) cases (Estivill et al., 1998; Green et al., 1999; Kelley et al., 1998b). GJB2 gene encodes connexin 26, a protein component of intercellular gap junctions. These gap junctions play important physiological roles in the cochlea. In this study, we present mutations in GJB2 gene responsible for ARNSHL in ten consanguineous Pakistani families (Figure 1).

MATERIALS AND METHODS

Families
We analyzed 20 large consanguineous families segregating autosomal recessive hereditary hearing loss. These families belong to different areas of Pakistan and were identified through a network of audiologists, centres for special education, regional schools and colleagues. The study was approved by the Institutional Ethical Committees of PMAS-Arid Agriculture University, Rawalpindi, Pakistan; National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan and Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany. Informed consent was obtained from all family members willing to participate in this research. All the available and consenting patients, their parents and phenotypically normal siblings were examined to record phenotypes and clinical data. Hearing loss in all the patients was profound and congenital. Parents of all the patients were cousins and none of the families had any previous history of deafness. Families were questioned to exclude syndromic and environmental cases. Clinical data about noise exposure, illness, accidents, antibiotics usage, abnormal pigmentation of hair and/or skin, night blindness and infections was also recorded. Patients were checked for goitre and excluded by palpation. Pedigrees were drawn and consanguinity confirmed by random interviewing family members especially the elders of the family. A venous blood sample of 3-6 mL was taken from all the consenting individuals in each family. DNA was extracted from lymphocytes by phenol chloroform method (Miller et al., 1988).

Sequencing
GJB2 gene is a strong candidate for autosomal recessive genetic deafness (Estivill et al., 1998; Green et al., 1999). Therefore, this gene was directly sequenced in these families. GJB2 has two exons but the open reading frame lies within the exon 2. The second exon was PCR amplified from genomic DNA using primer pairs corresponding to exon 2, the exone/intron splice junction and 20 bps on either side of the exon. The amplified products were ethanol purified and sequencing reaction was performed with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The sequencing products were run on an ABI 3700x1 analyzer (Applied Biosystems). Chromatograms were analyzed with the help of computer software package Sequencher® v.5.0 (Gene Codes, MI, USA). Sequence data from both affected and phenotypically normal individuals were compared to published reference sequence for corresponding genes to identify mutation(s).

RESULTS

The coding region of GJB2 gene was first sequenced in probands and later confirmed in all the patients, their parents and healthy siblings. Seven of these families revealed homozygous mutations whereas patients in three families were compound heterozygous for different mutations in GJB2. A total of six different mutations were found including three nonsense mutations c.370C>A (p.Gln124*), c.71G>A (p.Trp 24*), c.231G>A (p.Trp77*), two missense mutations c.23C>T (p.Thr8Met) and c.457G>A (p.Val153Ile) and one deletion c.35delG (p.Gly12Valfs*1). GJB2 gene was also sequenced in 200 phenotypically normal Pakistani subjects and none of these mutations was found on any of the 400 chromosomes, indicating that these mutations are rare in population.

DISCUSSION

GJB2 gene defects are the most frequently reported genetic cause of nonsyndromic hereditary hearing loss (Cohn and Kelley, 1999). GJB2 encodes CX26, a 26kDa (226 amino acid) gap junction protein which is a member of the connexin family of proteins. In the rodent cochlea it is expressed in supporting cells and basal cells of stria vascularis of the Corti organ.
GJB2 gene mutations causing hearing loss

Fig. 1: Pedigrees of Pakistani families segregating autosomal recessive hearing loss linked to GJB2 gene.

Fig. 2: Chromatograms showing mutations a: c.35delG and b: p.Val153Ile

(Kikuchi et al., 2000). The function of CX26 gap junctions is crucial for K⁺ recycling pathway in Corti organ. The ion content of cochlea is unique, there is high concentration of K⁺ (150 mmol/dm³), low concentration of Na⁺ (1 mmol/dm³) and low concentration of Ca²⁺ (0.02 mmol/dm³) outside in endolymph. The influx of K⁺ through mechanically gated ion channels of hair cells induce depolarization of hair cell and Ca²⁺ influx through basal membrane and this causes release of neurotransmitter. So the recycling of K⁺ is critical for the proper functioning of cochlea. The association of GJB2 mutations with nonsyndromic hearing loss could; therefore, be explained in view of the signaling mechanism which is unique to cochlea (Kikuchi et al., 1995). It has already been reported by several researchers that 30-50% of nonsyndromic hereditary hearing loss is caused by mutations in GJB2 gene in different populations (Gasparini et al., 2000; Hamelmann et al., 2001; Lerner et al., 2000; Tekin et al., 2003; Yan et al., 2003). Some of the variants reported in this gene are particularly prevalent in specific ethnic group. The mutation p.Trp24*, for instance, has been reported to be predominant on the Indian subcontinent (Ghosh et al., 2004). In the current study, too, this mutation was found to be the most frequent causative GJB2 gene variant in our study population. Of the 20 families that were screened in this study four were found to segregate this mutation (20%). Other mutations found in this study were c.35delG (12.5%), p.Gln124* (7.5%), p.Val153Ile (5%), p.Trp77* (5%) and pThr8 Met (5%).

Mutation p.Trp24* is a nonsense mutation which results in a truncated CX26 protein with 24 amino acids instead of 226. Individual homozygous for this mutation lacks any functional CX26 protein which negatively affects K⁺ recycling to endolymph. As a result the physiological response to sound stimuli is either absent or very weak (Minarik et al., 2003).

Mutation c.35delG (Figure 2a) causes a frameshift resulting in a premature stop codon [p.Gly12Valfs*1 (Zelante et al., 1997)]. This mutation is highly prevalent in Caucasians and other Mediterranean populations but it has a very low frequency in the Indian subcontinent (Bukhari et al., 2013; Maheshwari et al., 2003; Mustapha et al., 2001; RamShankar et al., 2003). Different opinions on this mutation being a result of a hotspot or a founder effect exist (Denoyelle et al., 1997; Kelley et al., 1998a; Morell et al., 1998; Van Laer et al., 2001).

Mutation p.Gln124* is a nonsense mutation which results in a premature stop codon and hence a truncated protein. This mutation has previously been reported in patients from Pakistan, India and Bangladesh suggesting that this mutation is common in the Indian subcontinent (Rickard et al., 2001a).
Mutation p.Val153Ile (Figure 2b) was found in association with mutations p.Thr8Met and 35delG on the other alleles in two different families. This mutation has been reported both as pathogenic (Bayazit et al., 2003; Marlin et al., 2001) and as a benign polymorphism (Rickard et al., 2001a; Santos et al., 2005; Wu et al., 2002b). Relatively high frequency of this mutation in heterozygous state in control samples supports the reports that this variation is a polymorphism (Biyikli, 2012). However, functional characterization of the V153I variants of CX26 in paired Xenopus oocyte expression system has shown that the formation of functional channels was not possible in these mutants (Mese et al., 2004). The amino acid Valin at position 153 is conserved among connexins from human (CX26, CX30, CX32); mice (Cx30) and (chicken Cx31); further supporting the possibility that mutation at this position is pathogenic (Wu et al., 2002a). Nonsense mutation p.Trp77* was found in patients of one family in heterozygous state with p.Gln124* on the other allele. This mutation has previously been reported in hearing impaired patients from the Indian subcontinent (Rickard et al., 2001b). Its carrier rate too is significantly high in the South Asian region (Bajaj et al., 2008). Similarly, mutation p.Thr8Met, was also found in compound heterozygous state with mutation p.Val153Ile on the other allele. Compound heterozygosity for these two mutations has already been reported in patients with hearing loss from USA. The uncertainty revolving around the pathogenicity of p.Val153Ile and hearing loss in patients with compound heterozygosity for the two mutations suggests that both of the variants result in mild recessively inherited deafness (Kenna et al., 2001). Our study supports previous reports of GJB2 gene being a common causative gene for hereditary hearing loss. The most common mutation in our study population is p.Trp24* which is in accordance with previous reports stating that this mutation is predominant in population from Indian subcontinent (Ghosh et al., 2004). Five other mutations identified in this study have also been reported in patients with hearing loss from across the world. In three of the twenty families investigated in this study, patients have been found compound heterozygous for two different causative mutations. This indicates that the frequency of GJB2 gene mutations is higher in Pakistani patients with hearing loss. Genetic counselling is needed to be offered to all patients for whom genetic testing is being considered because the test results are often not easy to comprehend for a population with low literacy rate. Population screening for highly prevalent mutations along with carrier screening and genetic counselling could play a significant role in bringing down the incidence of genetic deafness in Pakistani population.

Acknowledgements
The technical staff of all the three institutions i.e. Department of Biochemistry, Pir Mehr Ali Shah Arid Agriculture, University Rawalpindi, Pakistan; Human Molecular Genetics Laboratory, Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad Pakistan and Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany are greatly acknowledged for their excellent technical support for identification of families, collection of blood samples, isolation of DNA, molecular analysis and Sanger sequencing. The authors are very much thankful to the deaf families for their cooperation and consent to participate in this study and publish the research. The funding for this research was generously contributed by all the three institutions from their ongoing research projects e.g. HEC Dyslexia, SRC Collaborative Research Project, Cognitive Comorbidity and CMMC Microcephaly Project.

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