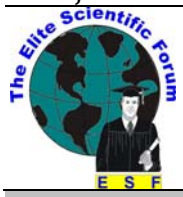
Pak. j. life soc. Sci. (2014), 12(3): 126-131 E-ISSN: 2221-7630;P-ISSN: 1727-4915



Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH ARTICLE

GJB2 Gene Mutations Causing Hearing Loss in Consanguineous Pakistani Families

Shehla Anjum[1], Aysha Azhar[2], Muhammad Tariq[2], Shahid Mahmood Baig[2,*], Hanno J Bolz[3], Mazhar Qayyum[1], Syed Muhammad Saqlan Naqvi[1] and Ghazala Kaukab Raja[1]
[1]Department of Biochemistry, Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan
[2]Human Molecular Genetics Laboratory, Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan
[3]Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany

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

Keywords
Compound heterozygosity
Connexin26
Deafness
GJB2
Hearing loss

***Corresponding Author:**
shahidbaig@nibge.org

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 -1.7of 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 sequences 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

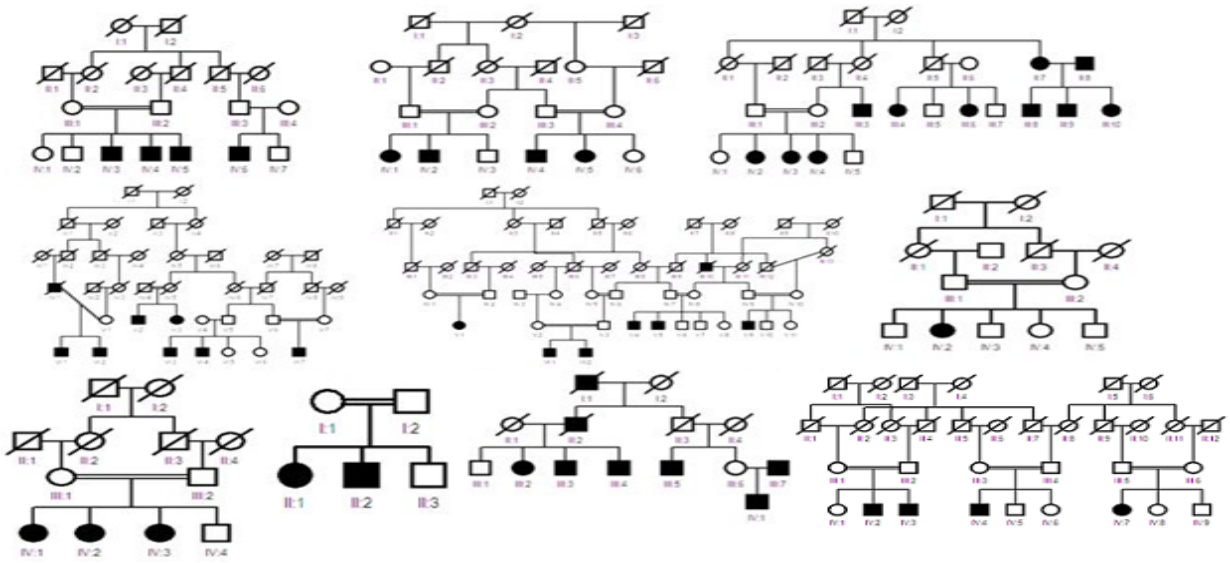


Fig. 1: Pedigrees of Pakistani families segregating autosomal recessive hearing loss linked to *GJB2* gene.

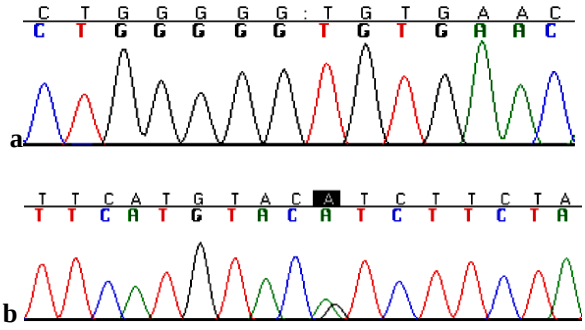


Fig. 2: Chromatograms showing mutations a: c.35delG and b: p.Val153Ile

(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 Ca^{2+} (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 Ca^{2+} 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; Lerer 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 p.Thr8 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.

REFERENCES

Baig SM, A Koschak, A Lieb, M Gebhart, C Dafinger, G Nurnberg, A Ali, I Ahmad, MJ Sinnegger-Brauns, N Brandt, J Engel, ME Mangoni, M Farooq, HU Khan, P Nurnberg, J Striessnig and HJ Bolz, 2011. Loss of Ca(v)1.3 (CACNA1D) function in a human channelopathy with bradycardia and congenital deafness. Nature Neuroscience, 14: 77-84.

Bajaj Y, T Sirimanna, DM Albert, P Qadir, L Jenkins and M Bitner-Glindzicz, 2008. Spectrum of *GJB2* mutations causing deafness in the British Bangladeshi population. Clinical Otolaryngology, 33: 313-318.

Bayazit YA, BB Cable, O Cataloluk, C Kara, P Chamberlin, RJ Smith, M Kanlikama, E Ozer, EA Cakmak, S Mumbuc and A Arslan, 2003. *GJB2* gene mutations causing familial hereditary deafness in Turkey. International Journal of Pediatric Otorhinolaryngology, 67: 1331-1335.

Biyikli TA, 2012. Prevalence of the IVS1(+1) G→A and 35delG mutations in the *GJB2* gene of Turkish patients with nonsyndromic hearing loss. Turkish Journal of Biology, 36: 6.

Bukhari I, G Mujtaba, S Naz, 2013. Contribution of *GJB2* mutations to hearing loss in the Hazara Division of Pakistan. Biochemical Genetics, 51: 524-529.

Cohn ES and PM Kelley, 1999. Clinical phenotype and mutations in connexin 26 (DFNB1/*GJB2*), the most common cause of childhood hearing loss. American Journal of Medical Genetics, 89: 130-136.

Denoyelle F, D Weil, MA Maw, SA Wilcox, NJ Lench, DR Allen-Powell, AH Osborn, HH Dahl, A Middleton, MJ Houseman, C Dode, S Marlin, A Boulila-ElGaied, M Grati, H Ayadi, S BenArab, P Bitoun, G Lina-Granade, J Godet, M Mustapha, J Loiselet, E El-Zir, A Aubois, A Joannard, J Levilliers, EN Garabedian, RF Mueller, RJ Gardner, C Petit, et al., 1997. Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene. Human Molecular Genetics, 6: 2173-2177.

Estivill X, P Fortina, S Surrey, R Rabionet, S Melchionda, L D'Agruma, E Mansfield, E Rappaport, N Govea, M Mila, L Zelante, P Gasparini, 1998. Connexin-26 mutations in sporadic and inherited sensorineural deafness. Lancet, 351: 394-398.

Gasparini P, R Rabionet, G Barbujani, S Melchionda, M Petersen, K Brondum-Nielsen, A Metspalu, E Oitmaa, M Pisano, P Fortina, L Zelante and X Estivill, 2000. High carrier frequency of the 35delG deafness mutation in European populations. Genetic Analysis Consortium of *GJB2* 35delG. European Journal of Human Genetics, 8: 19-23.

Ghosh M, R Vijaya, M Kabra, 2004. Genetics of deafness in India. Indian Journal of Pediatrics, 71: 531-533.

Gilley PM, A Sharma and MF Dorman, 2008. Cortical reorganization in children with cochlear implants. Brain Research, 1239: 56-65.

Green GE, DA Scott, JM McDonald, GG Woodworth, VC Sheffield and RJ Smith, 1999. Carrier rates in the midwestern United States for *GJB2* mutations causing inherited deafness. The Journal of the American Medical Association, 281: 2211-2216.

Hamelmann C, GK Amedofu, K Albrecht, B Muntau, A Gelhaus, GW Brobby and RD Horstmann, 2001. Pattern of connexin 26 (*GJB2*) mutations causing sensorineural hearing impairment in Ghana. Human Mutation, 18: 84-85.

Hussain R and AH Bittles, 1998. The prevalence and demographic characteristics of consanguineous marriages in Pakistan. Journal of Biosocial Science, 30: 261-275.

Kelley PM, DJ Harris, BC Comer, JW Askew, T Fowler, SD Smith and WJ Kimberling, 1998a. Novel mutations in the connexin 26 gene (*GJB2*) that cause autosomal recessive (DFNB1) hearing loss. American Journal of Human Genetics, 62: 792-799.

Kelley PM, DJ Harris, BC Comer, JW Askew, T Fowler, SD Smith and WJ Kimberling, 1998b. Novel mutations in the connexin 26 gene (*GJB2*) that cause autosomal recessive (DFNB1) hearing loss. American Journal of Human Genetics, 62: 792-799.

Kenna MA, BL Wu, DA Cotanche, BR Korf and HL Rehm, 2001. Connexin 26 studies in patients with sensorineural hearing loss. Archives of Otolaryngol Head & Neck Surgery, 127: 1037-1042.

Kikuchi T, JC Adams, Y Miyabe, E So and T Kobayashi, 2000. Potassium ion recycling pathway via gap junction systems in the mammalian cochlea and its interruption in hereditary nonsyndromic deafness. Medical Electron Microscopy, 33: 51-56.

Kikuchi T, RS Kimura, DL Paul and JC Adams, 1995. Gap junctions in the rat cochlea: immuno-histochemical and ultrastructural analysis. Anatomy and Embryology, 191: 101-118.

Kral A and GM O'Donoghue, 2010. Profound deafness in childhood. New England Journal of Medicine, 363: 1438-1450.

Kral A, J Tillein, S Heid, R Klinke and R Hartmann, 2006. Cochlear implants: cortical plasticity in congenital deprivation. Progress in Brain Research, 157: 283-313.

Lerer I, M Sagi, E Malamud, H Levi, A Raas-Rothschild and D Abeliovich, 2000. Contribution of connexin 26 mutations to nonsyndromic deafness in Ashkenazi patients and the variable phenotypic effect of the mutation 167delT. American Journal of Medical Genetics, 95: 53-56.

Maheshwari M, R Vijaya, M Ghosh, S Shastri, M Kabra and PS Menon, 2003. Screening of families with autosomal recessive non-syndromic hearing impairment (ARNSHI) for mutations in *GJB2* gene: Indian scenario. American Journal of Medical Genetics, 120A: 180-184.

Marlin S, EN Garabedian, G Roger, L Moatti, N Matha, P Lewin, C Petit and F Denoyelle, 2001. Connexin 26 gene mutations in congenitally deaf children: pitfalls for genetic counseling. Archives of Otolaryngology, Head & Neck Surgery, 127: 927-933.

Mese G, E Londin, R Mui, PR Brink and TW White, 2004. Altered gating properties of functional CX26 mutants associated with recessive non-syndromic hearing loss. Human Genetics, 115: 191-199.

Miller SA, DD Dykes and HF Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research, 16: 1215.

Minarik G, V Ferak, E Ferakova, A Ficek, H Polakova and L Kadasi, 2003. High frequency of *GJB2* mutation W24X among Slovak Romany (Gypsy) patients with non-syndromic hearing

loss (NSHL). General Physiology and Biophysics, 22: 549-556.

Mohr PE, JJ Feldman, JL Dunbar, A McConkey-Robbins, JK Niparko, RK Rittenhouse and MW Skinner, 2000. The societal costs of severe to profound hearing loss in the United States. International Journal of Technology Assessment in Health Care, 16: 1120-1135.

Morell RJ, HJ Kim, LJ Hood, L Goforth, K Friderici, R Fisher, G Van Camp, CI Berlin, C Oddoux, H Ostrer, B Keats and TB Friedman, 1998. Mutations in the connexin 26 gene (*GJB2*) among Ashkenazi Jews with nonsyndromic recessive deafness. The New England Journal of Medicine, 339: 1500-1505.

Mustapha M, N Salem, V Delague, E Chouery, M Ghassibeh, M Rai, J Loiselet, C Petit and A Megarbane, 2001. Autosomal recessive non-syndromic hearing loss in the Lebanese population: prevalence of the 30delG mutation and report of two novel mutations in the connexin 26 (*GJB2*) gene. Journal of Medical Genetics, 38: E36.

Newton VE, 1985. Aetiology of bilateral sensori-neural hearing loss in young children. The Journal of Laryngology and Otology. Supplement, 10: 1-57.

RamShankar M, S Girirajan, O Dagan, HM Ravi Shankar, R Jalvi, R Rangasayee, KB Avraham and A Anand, 2003. Contribution of connexin26 (*GJB2*) mutations and founder effect to non-syndromic hearing loss in India. Journal of Medical Genetics, 40: e68.

Rickard S, DP Kelsell, T Sirimana, K Rajput, B MacArdle and M Bitner-Glindzicz, 2001a. Recurrent mutations in the deafness gene *GJB2* (connexin 26) in British Asian families. Journal of Medical Genetics, 38: 530-533.

Rickard S, DP Kelsell, T Sirimana, K Rajput, B MacArdle and M Bitner-Glindzicz, 2001b. Recurrent mutations in the deafness gene *GJB2* (connexin 26) in British Asian families. Journal of Medical Genetics, 38: 530-533.

Santos RL, M Wajid, TL Pham, J Hussan, G Ali, W Ahmad and SM Leal, 2005. Low prevalence of Connexin 26 (*GJB2*) variants in Pakistani families with autosomal recessive non-syndromic hearing impairment. Clinical Genetics, 67: 61-68.

Schroeder L, S Petrou, C Kennedy, D McCann, C Law, PM Watkin, S Worsfold and HM Yuen, 2006. The economic costs of congenital bilateral permanent childhood hearing impairment. Pediatrics, 117: 1101-1112.

Sharma A, PM Gilley, MF Dorman and R Baldwin, 2007. Deprivation-induced cortical reorganization in children with cochlear implants. International Journal of Audiology, 46: 494-499.

Shearer AE and RJ Smith, 2012. Genetics: advances in genetic testing for deafness. Current Opinion in Pediatrics, 24: 679-686.

Sohl G and K Willecke, 2004. Gap junctions and the connexin protein family. Cardiovascular Research, 62: 228-232.

Tekin M, KS Arnos and A Pandya, 2001. Advances in hereditary deafness. Lancet, 358: 1082-1090.

Tekin M, T Duman, G Bogoclu, A Incesulu, E Comak, I Ilhan and N Akar, 2003. Spectrum of *GJB2* mutations in Turkey comprises both Caucasian and Oriental variants: roles of parental consanguinity and assortative mating. Human Mutation, 21: 552-553.

Van Laer L, P Coucke, RF Mueller, G Caethoven, K Flothmann, SD Prasad, GP Chamberlin, M Houseman, GR Taylor, CM Van de Heyning, E Fransen, J Rowland, RA Cucci, RJ Smith and G Van Camp, 2001. A common founder for the 35delG *GJB2* gene mutation in connexin 26 hearing impairment. Journal of Medical Genetics, 38: 515-518.

Van Naarden K, P Decoufle and K Caldwell, 1999. Prevalence and characteristics of children with serious hearing impairment in metropolitan Atlanta, 1991-1993. Pediatrics, 103: 570-575.

Willecke K, J Eiberger, J Degen, D Eckardt, A Romualdi, M Guldenagel, U Deutsch and G Sohl, 2002. Structural and functional diversity of connexin genes in the mouse and human genome. Biological Chemistry, 383: 725-737.

Wu BL, N Lindeman, V Lip, A Adams, RS Amato, G Cox, M Irons, M Kenna, B Korf, J Raisen and O Platt, 2002. Effectiveness of sequencing connexin 26 (*GJB2*) in cases of familial or sporadic childhood deafness referred for molecular diagnostic testing. Genetics in Medicine, 4: 279-288.

Yan D, HJ Park, XM Ouyang, A Pandya, K Doi, R Erdenetungalag, LL Du, N Matsushiro, WE Nance, AJ Griffith and XZ Liu, 2003. Evidence of a founder effect for the 235delC mutation of *GJB2* (connexin 26) in east Asians. Human Genetics, 114: 44-50.

Zakzouk S, 2002. Consanguinity and hearing impairment in developing countries: a custom to be discouraged. The Journal of Laryngology and Otology. Supplement, 116: 811-816.

Zelante L, P Gasparini, X Estivill, S Melchionda, L D'Agruma, N Govea, M Mila, MD Monica, J Lutfi, M Shohat, E Mansfield, K Delgrosso, E Rappaport, S Surrey and P Fortina, 1997. Connexin26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. Human Molecular Genetics, 6: 1605-1609.