

Review Studies of *GJB2* Gene in Patients with Hearing Impairment in Pakistan

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Abstract

Deafness is inherited as one of the most frequent type of neurosensory disorder. The specific physiologic mechanisms of the different types of hearing loss are still unknown. Recent studies have listed numerous causative agents for hearing loss. Genetic factors contribute to a greater extent in hearing disability. *GJB2* gene is one of the most promising candidates. 35delG is the most common mutation which accounts for about 50% of all *GJB2* gene mutation. After collection of data and isolation of blood, extraction of DNA was performed for each sample. Direct sequencing of *GJB2* gene was performed. Primary sequencing data of the representative sample concluded that *GJB2* gene is not mutated in the studied family. There are other candidates for hearing disability; further investigations are needed for assessment of other members of the selected family or other genes that are responsible for hearing loss. Human *GJB2* protein was compared with that of mouse and rabbit. The data from multiple alignment shows that there is only alteration at nine different points of rabbit compared to human *GJB2* protein while this variation is sixteen times when we compared human *GJB2* protein sequence with that of mouse.

Keywords: Deafness; *GJB2* gene; Hereditary deafness; X-linked recessive

Introduction

Hearing loss (HL) or hearing impairment (HI) is the most common and frequent neurosensory disorder in human populations [1]. It is defined as partial or complete loss of hearing due to which the individuals are unable to develop normal speech, language and communication skills [2].

Prevalence of deafness

Hearing loss occurs at the frequency of 1 in 2000 to 1 in 650 births [1]. Nearly 10% of worldwide genetic deafness is estimated to be caused by consanguineous marriages [3].

Usher syndrome (USH), is a type of hearing loss. It is highly heterogeneous in nature that is associated with bilateral nonsyndromic autosomal recessive sensorineural deafness and *retinitis pigmentosa* (RP) or rod-cone dystrophy [4-6]. The worldwide prevalence of Usher syndrome (USH), causing HL and blindness is up to 1 in 23,000 [7,8]. It affects 1.86 in 1000 newborn babies in which half of it is due to genetic causes [1]. It is responsible for autosomal recessive HL at the frequency of 1-10% in children and about 3-5 in 10,000 of general population [7]. In European populations, Usher syndrome accounts for 3-4 in 100,000 [7,9]. There are different types of USH i.e., USH1, USH2, USH3. USH1 affected people (25-44%), USH2 cases (56-75%) and USH3 (2%) were found in Europe [10-13]. The prevalence of Usher syndrome in the United States is predicted to be 4.4 per 100,000 individuals [7] and in Scandinavia, it is 3.5 in 100,000 people [12,14,15]. Epidemiological study has shown that about 25% people are diagnosed with presbycusis (age related hearing impairment ARHL) at the age of 60 years, while this ratio has increased twice (50%) with increase in the age (80 years) [16,17]. It was estimated that out of 600 million worldwide deaf people about 400 million stay in the developing countries [18]. The prevalence frequency of noise induced HL (NIHL) was reported to be 7% and 21% in the Western population and developing countries, respectively [19]. The estimated prevalence of deafness in the UK is 14% [20] (Davis, 1987). It is thought that there are approximately 35,000 young people and deaf children in the UK. Nearly 850 affected children are born annually and 1-2 in 1000 neonates will be suffering from HL (www.

earlysupport.org.uk). 20% (nearly 52 million) of affected adults and 36% of 55 years old deaf individuals was estimated in Europe [21]. According to estimation, deaf patients were found to occur in a prevalence ratio of 14.3% and 16.9% in Denmark and Sweden, respectively [22,23]. From a multicenter survey conducted in the North America, the number of deaf individuals was estimated to be about 25 million in 300 million people [24]. The prevalence of prelingual HL in the USA is reported to be 4-11 in 10,000 children [25].

The inheritance pattern of deafness is autosomal recessive in Pakistani population [25]. According to estimation, bilateral profound HL is up to 1.6 in 1000 and 70% of deafness is seen in consanguineous Pakistani families [26]. Nearly 30% of genetic deafness is predicted to be caused by consanguineous marriages in Pakistan and in the Middle East [3]. It is also found that nearly 60% of Pakistani marriages are consanguineous as a result of cultural conditions and about 80% of them are between the first cousins [27]. 5% cases of severe to profound HL are caused by mutation of *MYO15A* gene in Pakistan [2]. Prevalence of X-linked heterogeneous nonsyndromic deafness is up to 1-5% [28,29]. DFNB locus genes were thought to be involved in recessive, prelingual HL. The prevalence of DFNB4 locus is up to 7.2% in this population [30]. A recessive deafness locus DFNB49 was first identified at 5q13 chromosome in two Pakistani populations [31]. The estimated prevalence frequency of autosomal recessive nonsyndromic deafness loci DFNB23, DFNB39, DFNB7/11, DFNB8/10, DFNB9/48, DFNB3/12, DFNB2, DFNB1 and DFNB4 was found to be 1.83%, 2.19%, 2.65%, 3.01%, 3.01%, 3.93%, 5.66%, 5.94% and 7.58% respectively [32].

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Received December 20, 2017; **Accepted** December 28, 2017; **Published** December 29, 2017

Citation: Farooqi N, Khan O, Ellaham S, Jalil SF (2017) Review Studies of *GJB2* Gene in Patients with Hearing Impairment in Pakistan. J Mol Genet Med 11: 316 doi:10.4172/1747-0862.1000316

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Structure and Function of Auditory System

The anatomy of the human ear, the organ of auditory system and balance, consists of the outer, middle and inner ear also called the labyrinth [33]. The external ear is composed of auricle or pinna, ear canal, and tympanic membrane or eardrum membrane [34]. Diagram of human ear illustrating the outer, middle and inner ear (modified from www.webmd.com).

The external ear serves to protect the inner delicate parts of the auditory system. It prevents the auditory system from harmful infections and from clogging by the entrance of foreign antigen or bacteria. It consists of squamous keratinized epithelial cells and protects the inner and middle ear [32]. After receiving the sound waves, external ear transfers them to the eardrum in the middle ear through the ear canal [34]. Three smallest and tiny bones of the middle ear (called the ossicles: malleus, anvil and stapes or hammer, anvil and stirrup, respectively) carry the sound waves through the oval window into a snail shaped spiral bony organ, 35 mm in length, the cochlea in the inner ear [34]. Cochlea detects the sound stimulus through auditory receptors called organ of Corti [34].

The pressure of the sound waves vibrates the tectorial membrane on the top of the hair cells [35]. These vibrations in the organ of Corti produce sound transduction which is detected by receptor cells called the hair cells [36]. The hair cells and the fluid move as a result of these vibrations (www.earlysupport.org.uk). These cells produce electrical impulses or signals that are conveyed by nerves to the brain where they are interpreted in the left side of the brain (inferior colliculus or IC) [36]. The IC (a hemispherical mass of nerves) forms the greatest part of the midbrain. It serves as a principal auditory center and helps in the integration of hearing reflexes (inner.com/nerve 88-new.ht ml) or it is a part of the midbrain that functions as an important auditory center in the body. It exists as a pair of lower lobes which helps in processing the auditory signals from both the ears. Its three subdivisions are called the external, lateral and central cortex. Its metabolic activity is higher as compared to other parts of the brain. It also contributes in processes like signal integration, pitch detection, frequency recognition and processing sensory signals from the superior colliculi (health.yahoo.net/human-body-maps/). The normal hearing threshold is 15 decibel (dB) [36]. The frequency range of 20-20,000 pulses per second is audible by a normal human ear [32].

Genetic based hearing disabilities

Genetic hearing loss is a diverse and heterogeneous trait. There are numerous biotic as well as abiotic factors contributing in HL. Some of abiotic factors include viral and bacterial infections in infants, teratogens, high bilirubin content in blood, autotoxic drugs, head injury, loud noise and using antibiotic medications [37,38].

There are about 100 different candidate genes of HL [39]. Genetic or hereditary deafness is up to 50% that can be either syndromic (30%) or non-syndromic (70%) [40,41]. In syndromic hearing loss (SHL), the individuals are affected with other clinical features beside deafness, while in NSHL, no additional signs can be seen in individuals [36]. Syndromic deafness can be dominant, recessive and X-linked [42]. Based on the position of damage and type of ear defect, syndromic HL can be conductive, sensorineural or mixed HL [36]. Conductive HL occurs when sound waves are unable to pass properly from the outer and middle ear to the cochlea. It is caused by congenital abnormalities like atrophy, dysplasia, malformation and clogging of the middle and outer ear, otitis media or glue ear, the three small bones of the middle ear are unable to move as a result of the middle ear fluid

or glue ear. Sensorineural HL occurs in the cochlea or the auditory nerve [43]. Nonsyndromic HL is sensorineural impairment [42]. Unlike conductive HI, sensorineural HL is permanent and cannot be treated (www.earlysupport.org.uk). Moreover, NSHL shows autosomal recessive (AR), autosomal dominant (AD), X-linked (XL), Y-linked (YL) and mitochondrial mode of inheritance, the most severe and important form being the autosomal recessive i.e., 75% of NSHL is shown as AR pattern in affected families [37,41]. While AD cases are of 10-20%, 1-5% cases are inherited as X-linked recessive and genetic deafness is inherited by 1% human genome [44]. All these forms show monogenic traits while digenic inheritance has also been reported [43]. Gap junction $\beta 2$ (*GJB2*) or *Connexin 26* (*Cx26*) is one of the prominent candidate of hearing loss. Therefore, it was screened for mutations with direct sequencing method.

Structure and function of *GJB2* gene

GJB2 (Gap junction $\beta 2$) or *Connexin 26* (*Cx26*), is a small gene of molecular weight 26 kDa, comprising 5.5 Kb or 5500 bp. It consists of two exons (about 2311 bp) separated by an intron. Exon 2 is protein encoding. The size of encoded mRNA is 4.2Kb while the protein has 226 amino acid residues [45].

GJB2 is expressed in various body organs including the limbus, basement membrane and spiral prominence of the mammalian cochlea and hepatocytes [46,47]. It encodes certain transmembrane gap junction proteins known as *Connexins* (having similar topology) [48]. The passage of small metabolites (<1000 Dalton) and inorganic ions between the adjacent cells is regulated by these proteins [49,50]. They are also important for the growth and differentiation of keratinocytes in the epidermis [51]. These proteins are present in the fibrocytes of the cochlear duct and the epithelial supporting cells, lining the sensory ear cells of the cochlea [52]. Six molecules of *connexin* oligomerise to form a hemi-channel connexon. Two connexons of the adjacent plasma membranes form a channel which makes the gap junctions through disulphide linkages.

To briefly explain, gap junction channels allow the transfer of different solute molecules across the plasma membrane of adjacent cells. These include ions, metabolites, peptides, nucleotides and secondary messengers. These channels play vital role in many biologically important processes like auditory transduction by recycling K^+ ions in the cochlea, electrical signaling in the nervous system, growth of epidermal keratinocytes, cardiac development, the immune system, fertility, homeostasis and maintenance [53]. There is a high concentration of K^+ ions, low Na^+ ions and Ca^{+2} ions concentration outside in endolymph. The gap junctions help in the potassium recycling from the hair cells into the endolymph at the upper surface of the sensory ear cells during the sound transduction. Influx of K^+ and Ca^{+2} ions causes the hair cells to depolarize and releases the neurotransmitter. This recycling helps in the normal function of cochlea and sensorineural hearing [42,45]. In case of genetic alteration in *GJB2*, abnormal synthesis of *connexin*, impaired potassium cycle, cell death and finally bilateral non-syndromic HL occurs [54,55]. The second *connexin* gene (*GJB6*, 35-kb downstream of the *GJB2* gene on 13q12 chromosome) is expressed in the same inner-ear structures and both the gap junction proteins are functionally related [56]. The cell-to-cell interactions are mediated by different *connexins* that organize themselves in a complex way within connexons. This complex arrangement is referred to as “*connexin code*” [57].

Contribution of *GJB2* gene mutation in deafness

GJB2 gene is located on DFNB1 locus, initially identified by linkage analysis using the markers D13S143, D13S175, D13S292 in a large

Tunisian family. It was mapped to chromosome 13q12-q13 in two consanguineous families [58]. After three years, it was shown that *Cx26* gene mutations are responsible for HL [59]. Approximately, 90 different *GJB2* variants have been identified (The *Connexin-Deafness* Homepage: <http://davinci.crg.es/deafness/>). It has been reported that autosomal recessive nonsyndromic hearing loss (ARNSHL) results from *GJB2* gene mutations in many cases [60,61]. It causes 50% HL in children in some populations [62]. *GJB2* gene mutation is responsible for 50% of profound HL cases, severe HL cases: 30%, moderate: 20% and 1-2% of mild HL [63]. In addition, a high frequency of this gene mutation has been observed in some of the ethnic groups [64]. Similarly, 50% of autosomal recessive non-syndromic deafness in the Caucasians is due to *GJB2* mutations [65]. At position 30-35 of coding region of *GJB2* gene, there is a small repeated sequence of six G residues. If there is deletion of one G residue in codon 10, then the mutation is referred to as 30delG or 35delG [46]. It was identified by the method proposed by Storm et al. [66]. 35delG, the most frequent mutation in the Caucasians, is responsible for 70% of *GJB2* mutations. The most prevalent nonsense mutation W24X truncating *Cx26* protein (24 amino acids), was first reported in a Pakistani family [65]. The *GJB2* mutations which cause syndromic HL include: delE42, [67], G12R, D50N and S17F [68], G59A [69], N54K [70], R75Q [71], R75W [72], D66H [73], G130V [74]. While autosomal dominant non-syndromic HL is caused by C202F [75], R143Q [76], W44C [77], R184Q [78], D179N [79] and G21R gene mutation. It lies in the first intracellular domain [80]. C202F mutation is located in the M4 transmembrane domain of the *GJB2* protein. This domain functions in the oligomerisation of *connexins*. Heterozygous W44C mutation is present in the extracellular loop E1 (allows the interactions between adjacent cells connexons) of the protein. Other known *GJB2* mutations are E47X, I20T, R184P, L90P, delE120 and V95M [81].

Clinical features of deafness

Diagnosis of deaf individuals is related to the characteristic signs and symptoms of deafness. Up to 151 different forms of inherited deafness have been discovered [82]. Hereditary deafness is associated with more than 500 syndromes [83]. On the basis of clinical symptoms like onset of retinitis pigmentosa, severity of deafness and vestibular response, the autosomal recessive Usher syndrome has been classified into type 1, type 2 and type 3, the most severe being the type 1 [84]. Narrow blood vessels, pale optic disc and balance problems are also observed in individuals affected with USH [85]. Beside these, symptoms like structural anomalies of nasal cilia [86,87], olfactory loss is also found in the USH patients [88]. The characteristic features of X-linked Alport syndrome are ocular problems, hematuria, glomerulonephritis, renal failure and sensorineural deafness. These symptoms are more common in men than women. Uremia can also lead to death in men [89]. Based on the clinical symptoms like cleft palate, osteoarthritis, sensorineural HL, vertebral abnormalities, unusual facial features and eye problems, autosomal dominant Stickler syndrome (STL) is divided into four types (type 1, 2, 3 and 4) [90]. The AD Neurofibromatosis type 2 (NF 2) is characterized by gliomas, meningiomas, unilateral sensorineural deafness, headache, imbalance, skin tumor, tumors of the hearing and balance nerve and tinnitus. Autosomal recessive Pendred disease is characterized by goiter (abnormal metabolism of iodine) [91]. The characteristic signs of autosomal dominant Branchio-oto-renal (BOR) syndrome include kidney problems, neck cysts, branchial and otologic anomalies (Mondini's dysplasia, stapes fixation), long narrow face and cup shaped pinnae [92]. Similarly, hereditary and acquired mitochondrial mutations are related to many signs and symptoms such as diabetes mellitus, myopathy, neuropathy, sensorineural HL,

presbycusis or ARHL and aging [93,94]. A few symptoms like night blindness, hair depigmentation, skin pigment changes, nausea, vomiting, severe dizziness, headache, pain, thyroid dysfunction, tinnitus, ear and respiratory tract infections are also seen in deafness [95]. The anomalies caused by Norrie disease (a syndrome associated with X-linked HL) include bilateral blindness, bilateral sensorineural HL, mental retardation and microphthalmia [96,97]. Poor growth, microcephaly, seizures, delay in sexual maturity, cardiovascular problems, pulmonary arterial hypertension and psychomotor retardation like complex symptoms are also seen in some patients [98-105]. Patients affected by AR Biotinidase deficiency syndrome shows the signs like deafness, hair loss, seizures, emesis, skin rashes and acidosis.

Audiological and vestibular function assessments

Before hearing tests, blood samples are subjected to DNA testing for diagnosis of *GJB2* alterations. With the advancement in technology, there are various approaches that can be used for testing of hearing threshold level of patients. These techniques are electrophysiological tests (Auditory brain-stem responses (ABR), searching for mutations in *connexin 26* gene, behavioral audiometry, evoked response audiometry, electrocochleography (EcochG), otoacoustic emission testing, tympanometry, pure-tone and speech audiometry, Pure tone air conduction audiometry, Early Speech Perception (ESP) test, Speech discrimination test, Visual Reinforcement Audiometry (VRA), Play audiometry, bone conduction audiometry.

Conclusion

As per the above studies, it can be concluded that while Romberg and Tandem Gait test, Electronystagmography (ENG) test are used for clinical assessment of vestibular function. ENG test graphically records eye movements to analyze the sources of dizziness, balance dysfunction and has further three parts; oculomotor evaluation, positional testing and caloric measurement.

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