Hearing Impairment

Subjects: Others
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Hearing impairment (HI), also referred to as hearing loss, is the partial or total loss of hearing. *GJB2* is the most common gene associated with HI and it belongs to a family of genes that encodes connexin proteins. Over seven connexins (*GJB3, GJB4, GJC3, GJC1, GJB2, GJB6,* and *GJA1*) have been globally studied for their association with HI, however, the majority did not have a clearly established association with the condition. Although there were studies that reported variants in *GJB3*, *GJB4*, *GJC3*, and *GJC1* in hearing-impaired patients, there is a need for more studies to clearly describe their role in the development of HI. *GJB2, GJB6*, and *GJA1* on the other hand have been identified as HI genes that should be considered in routine clinical investigations. Recent data has shown that the coding region variants of *GJB6* (except the large genomic deletions) do not contribute to the development of HI.

Keywords: connexin ; gap junction protein ; gene variant ; GJB2

1. Introduction

Hearing impairment (HI) is the most common sensorineural disability worldwide, with a global prevalence of 1.3 per 1000 population [1][2]. It occurs in about 1 per 1000 live births in high-income countries, with a much higher incidence of up to 6 per 1000 in the lower-income countries [3]. According to the World Health Organization, 466 million people are living with HI and about 900 people will be affected by the year 2050 [4]. Depending on the degree of severity, HI can be classified as mild, moderate, severe, or profound when the pure tone average ranges from 26 to 40 dB, 41 to 60 dB, 61 to 80 dB or is over 81 dB, respectively (Deafness and Hearing Loss, n.d.). It is estimated that approximately 50% of congenital profound HI cases are of genetic origin [5]. If there are no other distinguishing clinical findings, HI is classified as non-syndromic [6]. About 80% of non-syndromic HI (NSHI) cases are inherited in an autosomal recessive mode, while an autosomal dominant pattern of inheritance is observed in 18% of cases [7]. In the remaining 2% of cases, the mode of inheritance is either X-linked or mitochondrial [7].

Non-syndromic HI is extremely heterogeneous, with approximately 170 loci and 121 genes identified so far [8]. Studies in European and Asian populations have identified mutations in connexin genes as the major contributors to NSHI [9][10]. Connexins (Cx) are a homogeneous family of proteins expressed in a large variety of tissues in the human body and known for their assembly into intercellular channels, called gap junctions [11]. Twenty-one different human connexin genes have been reported so far, each coding for a transmembrane protein with the same protein topology [11]. Connexins have four transmembrane domains (TM), TM1–TM4, connected by two extracellular loops (E), E1, and E2, which mediate docking [12]. The N- and C-termini, and a loop connecting TM2 and TM3 are on the cytoplasmic side of the plasma membrane [12].

Connexins are synthesized in the endoplasmic reticulum (ER) and oligomerize in the ER/Golgi or trans-Golgi network to form hexameric hemichannels or connexons [11]. Connexons are transported to the plasma membrane, where they can act as functional channels by themselves, or move to regions of cell contact and find a partner hemichannel from an adjacent cell to form a complete gap junction channel [12]. Gap junctions play an important role in cell-cell communication and homeostasis in various tissues, by mediating a direct exchange of ions and other small molecules up to 1 kDa (including a variety of second messengers, metabolites, but also small linear peptides) between the cytoplasms of adjacent cells [11].

To date, mutations in four connexin genes including *GJB2* (Cx26), *GJB3* (Cx31), *GJB4* (Cx30.3), and *GJB6* (Cx30) have been associated with sensorineural HI [13][14][15]. These four connexins were shown to be expressed in the inner ear, and some studies supported their role in potassium removal and recycling in the ear, as well as a possible role for nutrient passage in the cochlea [16]. GJB2-related sensorineural HI can occur alone or in association with hyperproliferative skin disorders, as in the case in Keratitis-ichthyosis-deafness syndrome and Bart-Pumphrey syndrome [17][18][19]. It is has been shown that digenic inheritance of recessive deafness by mutations in GJB2 and GJB6, or *GJB2* and *GJB3* can occur [11]. In other words, deafness can be caused by the addition of a mutation in one allele of GJB2 and one allele of GJB6 or

GJB3, indicating an interaction of these connexins in the cochlea [11]. GJB6 coding region variants have been proven not to cause HI using mouse models, however, the large deletions of the GJB6 gene especially GJB6-D13S1830 were implicated as causal factors of HI. The cis-acting element upstream of the *GJB2* and GJB6 gene is disrupted by the large genomic deletions abolishing the expression of the *GJB2* gene which is responsible for the development of HI [20].

GJB2 and GJB6 genes have been well studied in Europeans and Asians, with c.35delG identified as the most prevalent GJB2 mutations associated with NSHI [9]. However, the other NSHI-causing connexin genes (i.e., GJB3 and GJB4) have not been extensively studied [11][21].

2. Contribution of Connexins Genes in HI

Connexin channels regulate the transport of small signaling molecules between cells to aid the proper functioning of the tissue/organ systems in the body [22]. We found that more than 570 studies were conducted globally on connexin-related HI investigations with most studies performed in Asia, while relatively few have been done in Africa.

Most studies used targeted sequencing, but the decline in next-generation sequencing cost has accelerated the discovery of novel disease gene variants through available high-throughput targeted panels or whole-exome sequencing technologies investigating several gene targets in a single test [23][24][25]. Indeed, there was a clear migration from non-sequencing approaches such as denaturing high-performance liquid chromatography (DHPLC), multiplex ligation-dependent probe amplification (MLPA), PCR, restriction fragment length polymorphism (RFLP), and single-strand conformational polymorphism (SSCP) to sequencing techniques or a combination of sequencing and non-sequencing techniques.

Connexin 26 gene *(GJB2,* OMIM:121011) located on chromosome 13q12.11 is known to be expressed in different tissues including the cochlear of humans [26], mouse, and rat [27]. GJB2 gene variants were the most common genetic factors associated with NSHI among several populations [28][29], however, they are rare in African, and African American populations [30]. Similarly, it was clear from our review that GJB2 is the most investigated gene and had the highest number of pathogenic variants identified among hearing-impaired patients. The most common pathogenic variants *(GJB2:* p.Gly12ValfsTer2, p.M34T, p.L79Cfs, p.V37I, p.H100RfsTer14, p.W24X, p.L56Rfs, and p.R143W) appeared to be localized to specific populations, due to a founder effect [10][31].

In a previous review by Chan and Chang in 2014, 216 original GJB2 research articles reporting not less than 10 probands were retrieved and analyzed [10]. In our current review, 571 original research publications on connexins associated with HI were considered of which 566 articles reported on *GJB2* associated HI. The previous report was from 63 countries [10], while in this study, we retrieved *GJB2* publications from 106 countries. The differences in the number of publications and countries involved can be explained by the time difference between the previous report and the present study. Also, we did not exclude case reports, contrary to the previous report. In contrast to the report from Chan and Chang, Australia, and not Africa, had the lowest contribution of GJB2 variants. Moreover, Asia was identified as the highest contributor while the previous report had Europe as the highest contributor of *GJB2* PLP to HI [10]; this can be attributed to the increasing interest and number of genetic researches in all parts of the world. Despite the above differences, the commonly reported PLP *GJB2* variants were similar in both studies. Furthermore, our study and the studies from Chan and Chang and Tsukada reported a similar ethnic-specific spectrum of the common PLP variants in *GJB2* [10][31].

The most common *GJB2* variant is p.Gly12ValfsTer2 (c.35delG) which is frequently reported among populations in Europe, the Middle East, Australia, North, and South America [10]. We observed widespread of this variant across the globe but it was almost absent in sub-Saharan Africa although there were studies from Ghana [28][32], Cameroon [33][34], and South Africa [33] that investigated this variant in African populations. Morocco is an exception, where five independent studies identified biallelic c.35delG mutation in hearing-impaired patients [35][36][37][38][39]. The spread of the variant from Europe and North Africa to North and South America seems to follow migration patterns [10].

Second to *GJB2:* c.35delG is *GJB2*- p.M34T (c.101T > C) which was found to be most prevalent in the United Kingdom (UK). The carrier rate of *GJB2:* p.M34T was calculated at 2.69% in the UK, which was almost twice the carrier rate of *GJB2-c.35delG* (1.36%). In the United States of America, the carrier rate for the GJB2: p.M34T variant was found to be 2.3% [40]. The high carrier rates of variants suggested the possibility of heterozygous advantage. However, the audiometric characterization of *GJB2*-p.M34T carriers was not different from homozygous hearing individuals. Hence there is no effect on the hearing ability of the carriers [41].

In the present review, we identified three variants *(GJB2:* p.L79Cfs/c.235delC, p.V37I/c.109G > A, and p.H100RfsTer14/c.299_300delAT) with very high allele frequencies from Asia compared to other continents. These variants were absent in sub-Saharan African countries but were found in a few cases in some North African countries. The

Chinese population was found to have a high prevalence of GJB2: c.235delC [42] with frequencies of about 14.7% homozygous among a hearing-impaired sub-population, and 16.1% heterozygous in the hearing population [43]. The carrier frequency of GJB2: c.235delC is similar to that of the entire Asian population [42][43], and a high prevalence of that variant was reported in Japan [44][45], Korea [46], and Taiwan [47].

The *GJB2:* p.V37I variant was described as a polymorphism by some researchers while others consider it a potential disease-causing missense mutation [48]. The high carrier frequency of the variant among hearing controls informs the polymorphism argument, however individual homozygous of the variant had HI [49]. Compound heterozygosity of the *GJB2:* p.V37I variant and other known GJB2 pathogenic variants produced mild to severe HI. It was proposed that the milder phenotype was due to the *GJB2*-p.V37I allele [50]. We have identified several independent studies that reported the variant in hearing-impaired individuals, implying that the variant is likely disease-causing. In addition, GJB2: p.V37I was predicted as pathogenic by CinVar, Varsome [51], and InterVar [52]. The majority of *GJB2:* p.V37I mutated alleles were identified among Asians and mostly Chinese [48][49]. The third most common *GJB2* variant associated with HI in Asia was c.299_300delAT with an estimated allele frequency of 3.89% [45][53]. Although this variant is very prevalent in China, it appears that this variant is not common in other populations [54].

The truncating GJB2 mutation p.W24X is the predominant mutation among the Indian and European Gypsy populations [55][56][57]. The *GJB2:* p.W24X was the most commonly observed mutation and accounted for about 95% of all GJB2 mutations found in the Indian population with a carrier frequency of 2.4% [57]. The mutation was proposed to be a founder effect and confirmed through haplotype analysis of the flanking markers of the *GJB2* gene [55][57].

GJB2: c.167delT was reported to be common in the Eurasian populations and postulated to have a single origin of allele due to the observed conserved haplotypes around the mutation [58]. Although the mutation was prevalent in the territories of the Middle East [58][59][60], we found a high number of alleles with PLP in the United States of America. The fourth most common *GJB2* mutation in the American population is *GJB2:* c.167delT and was found to account for about 3.6% of cases. The variant was more prevalent in the White-American population compared to other populations [61].

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