JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

MUTATIONAL ANALYSIS OF GJB2 GENE CAUSING CONGENITAL NONSYNDROMIC HEARING IMPAIRMENT IN INDIA: A REVIEW

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ABSTRACT

Hearing impairment is a major disorder ranging from slight, moderate to profound. The genetic cause of the non syndromic hearing impairment is exceptionally heterogeneous. Despite of this, a change in the connexin genes is a major contributor. Several mutations in the GJB2 gene and deletion in GJB6 are found to be related with hearing impairment. The analyses found that the average frequency of GJB2 mutation is varying among different regions.

Materials and Methods: In this manuscript, we have reviewed 12 previous publications. In which around 2546 probands were included to analyse the prevalence and type of mutation in the GJB2 gene.

Results: Our study found several mutations and the common mutations are c.71G>A, c.230G>A, c.235DelC, c.167delTA, and 35delG. The most common mutation in GJB2 is c.71G>A (p.W24X) followed by 35delG. The etiology of hearing impairment is of multi-factorial and K+-toxicity is the most accepted hypothesis to explain the cause of the hearing impairment.

Conclusion: The mutations in the GJB2 gene are responsible for the contribution of up to around 36%. Regarding GJB2 mutation c.71G>A (p.W24X), a tryptophan stop codon is the most common mutation.

Keywords: GJB2, connexin genes, hearing impairment, Indian population and sensory disorder

INTRODUCTION

Hearing impairment refers to the hearing loss range from slight to profound. It is the most common problem among sensory disorders, and leading a cause of disability worldwide (WHO). Hearing loss affects 5% of the world population and 60% of cases are due to preventable causes (WHO). Hearing Impairment occurs more in developing than developed countries and several genes are causing the hearing impairment. They can cause 70% non-syndromic isolated hearing impairment and 30% syndromic that can be associated with other medical abnormalities [1]. A study done in 1995 suggests that 60% of the cases with hearing impairment are well established with genetic etiology [2] , and among them 85% of these cases exhibiting autosomal recessive, dominant, X-linked or mitochondrial inheritance showing significant genetic heterogeneity. Hearing impairment is common among the sensory congenital disorders with the prevalence of 1–3 in 1000 live births in the general population, and 50–60% of these cases have genetic etiologies [3]. There is a group of proteins called connexins, play an important role in intracellular communication of ear [4]. These proteins are then coded by genes called gap junction genes and among them GJB2 and GJB6 are commonly associated with hearing impairment. The GJB2 and GJB6 genes codes for gap junction protein beta 2 gap junction protein beta 6. The GJB2 and GJB6 are the predominant isoform and co-expressed in an epithelial supporting cell of cochlea and function as a gap junction. It was found mutations in these genes are more frequent with hearing impairments [5]. GJB2 gene has a simple genomic structure with 680 base pairs [6] that has a role of homeostasis in cochlear fluids that is endolymph and perilymph by recycling of potassium ions in the inner ear [7]. The mutation in connexion 26 is primarily known for the causation of prelingual hearing impairment and it was the first reported by Kelsell *et al* in 1997 causing autosomal recessive non-syndromic deafness [8]. Mutations in the GJB2 and GJB6 genes are mostly found to be autosomal-recessive nonsyndromic hearing loss in many populations. The mutation includes frame shift or deletion resulting in a short non-functional truncated form of the protein [9]. Mutations in these genes account for up to 50% of all cases of the prelingual deafness in many tested populations [10] and it also affects 1 in 1000 of congenital hearing loss. These genes are responsible for 60% case of hearing impairments with genetic origin and 70% of non-syndromic hearing loss [11] .

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

METHODS:

Literatures search:

Literature is searched from PubMed, Web of Science, Google Scholar, and Science Direct databases in English for articles available before Dec 2019. The following keywords and headings were used at the same time in each set: (“hearing impairment’’ or ‘‘deafness’’ or ‘‘hearing loss’’ or ‘‘Connexin 26 or Cx26”) and (“Mutational analysis”). The bounds were chosen to yield a broad comprehensive review of literature.

Inclusion criteria and data extraction:

The suitable published papers included in our study that met the inclusion criteria as follow: (1) published in English; (2) performed on Indian communities with hearing impairment subjects (profound, moderate to the severe case); (3) designed to detect mutations of connexin genes; (4) detected pathogenic variants.

The pieces of literature were selected by excluding the studies that are done on age related and environmental factors associated with the hearing impairment.

The data that are extracted from the chosen papers are follows; the number of cases, the detection method, type of hearing impairment, subject ethnicity, and the frequency of gene mutations followed by author name and publication year.

Result:

A total of 12 studies comprising 2546 probands, range from profound, moderate to the severe case were incorporated and among them, 886 males, 681 females, and five research paper did not mention their gender, family history, and parental consanguinity in their manuscript. During the extraction of data, it was found that 633 probands had consanguinity and 389 non-consanguinity, 455 familial probands, and 444 non-familial probands mentioned in the articles. The pure tone audiometry (PTA) revealed that the probands are from type A, B, and C curve. This study analyses the type and prevalence of GJB2 gene mutation and found to be contributing up to 30-40% mutation in India. However, a study was done by Adhikary *et al*., 2015 found the mutation to be 46.54%, 30.24 heterozygous, and 16.3 homozygous mutations [12].

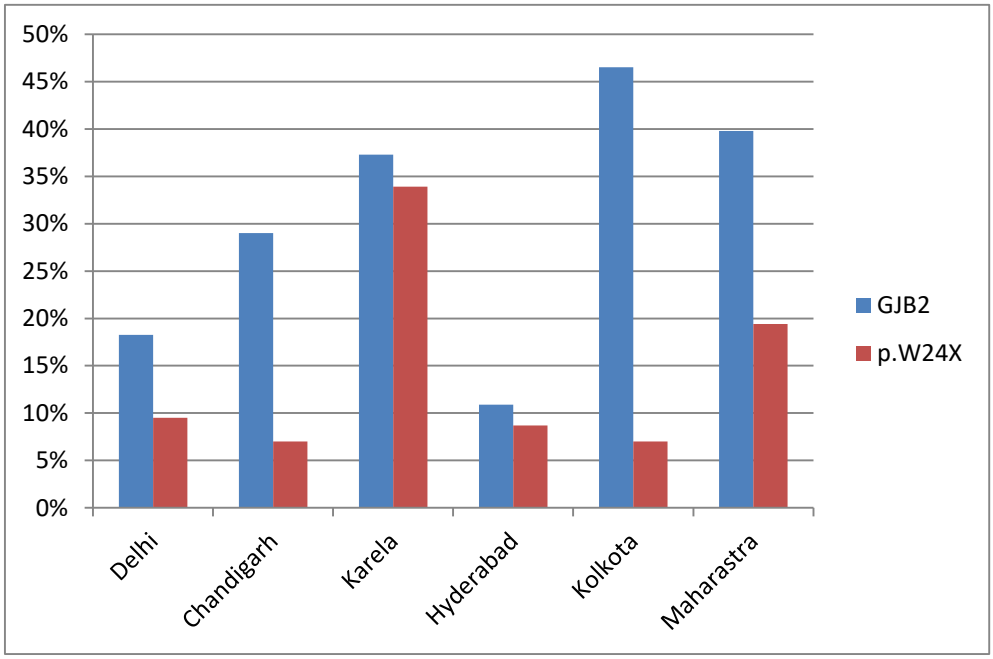

Figure1: Prevalence of GJB2 and W24X mutation

In this study, we found that a number of mutations in the GJB2 gene contributing to non-syndromic hearing loss patients as listed in table 1. The common mutation that are found to be associated with the hearing impairment are c.230G>A and c.231G>A (p.W77X), c.235DelC, c.167delTA and 35delG. The most common polymorphism is R127H and V153I. The results analysed the frequency of biallelic and heterozygote mutation in GJB2 which varies in different locations of India (table 2) and p.W24X is the most common mutation (figure 1). The p.W24X is a c.71G>A mutation that changes amino acid p.W24X, a tryptophan stop codon to be the most commonly found mutation during the analyses and accounting for up to 87% of mutant alleles and contributing from 7.5 to 33.9%

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

of genetic hearing impairment (table 3) followed 35delG mutation. Unlike GJB2, GJB6 does not show any data of mutation even the most common deletion of D13S1830, which is the most common in some countries. The homogeneity and heterogeneity of mutation vary in different populations due to different socioeconomic condition and the heterogeneity is high in North India whereas in the case of South India due to specific traditional intragroup marriage they may give rise to the homogeneity in the population. In addition to p.W24X, five other common mutations were recognised in the GJB2 gene viz., c.230G>A and c.231G>A (p.W77X), c.235DelC, c.167delTA, 35delG, and R127H. The present studies three novel mutations which include c.616A>G which cause mutation in c.616A>C (p.Asn 206His), c.95G>T which causes R32L and c.55 A>G which cause p. S19G.

Table 1: Distribution of type of mutation in the GJB2 gene in India

Mutation (Amino acid change)	North India	South India	East India	South-East	South-West
c.235delC	✓	×	×	✓	×
c.1A>G (p.M1V)	✓	×	✓	×	✓
c.71G>A (p.W24X)	✓	✓	✓	✓	✓
c.230G>A (p.W77X)	✓	×	✓	✓	✓
c.250G>C (p.V84L)	×	×	✓	×	×
c.439G>A (p.E147K)	×	×	✓	×	×
c.380G>A (p.R127H)	✓	×	✓	✓	×
c.250G>C (p.V84L)	×	×	✓	×	×
c.23C>T (p.T8M)	×	×	✓	×	×
c.55A>G (p.S19G)	×	×	✓	×	×
V1531/N	✓	×	×	×	×
p.M163V	×	×	×	✓	×
c.95G>T (R32L)	×	✓	×	×	×
c.ivs1(+1)G>A	×	×	×	×	✓
V27I	×	×	×	×	✓
35insG	×	×	×	✓	✓
c.167delT	✓	×	×	✓	×
c.148G>A (p.Asp50Asn)	✓	×	×	×	×
c.223C>T (p.Arg75trp)	✓	×	×	×	×
c.238C>T (p.Gln80Ter)	✓	×	×	×	×
c.283G>A (p.val95met)	✓	×	×	×	×
c.313_326del14 (p.lys105glyfster5)	✓	×	×	×	×
c.340G>A (p.Glu114lys)	✓	×	×	×	×
c.341A>G (p.Glu114gly)	✓	×	×	×	×
c.370C>T (p.Gln124ter)	✓	×	×	×	×
c.407dupA (p.tyr136ter)	✓	×	×	×	×
c.439G>A (p.glu147lys)	✓	×	×	×	×
c.616A>C (p.Asn206His)	✓	×	×	×	×
c.35delG (p.Gly12ValfsTer2)	✓	×	×	✓	×
Q124X	✓	×	×	×	×

Table 2: Distribution of GJB2 genes in India

Area		Study	Proband	GJB2 Bi Allelic (%)	GJB2 Heterozygote (%)
North India	**Delhi**	Singh *et al*.,2018 [13]	316	5.37	12.9
	Chandigarh	Khandelwal *et al*., 2009 [25]	100	0	29
	New Delhi	Bhalla *et al*., 2009 [26]	200	3.5	25.5
South India	**Karela**	Joseph and Rasool 2009 [21]	86	36	00
East	**Kolkata**	Adhikary *et al*., 2015 [12]	215	16.3	30.24
South East	**Hyderabad**	Padma *et al*.,2009 [14]	303	7.9	1.32
	Andra Pradesh	Ramchander *et al*.,2005 [19]	200	8.5	28.5
South West	**Maharastra**	Godbol *et al*., 2010 [27]	288	18.75	21.5
	Mumbai	Ramshankar *et al*., 2003 [18]	215	17.2	3.2

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

Table 3: Occurrence of a most common mutation in India

Study	Population	Proband	W24X%	W24X (Tryptophan stop codon)		
				Homo	Hetero	Compd. hetero
Singh *et al*., 2018 [13]	**New Delhi**	316	9.49%	2.53%	6.96%	00
Adhikary *et al*.,2015 [12]	**Kolkata**	215	7%	5.58%	1.4%	<0.004
Nayyar *et al*., 2011 [28]	**Pune**	27	25.9%			
Godbol *et al*., 2010 [27]	**Maharashtra**	288	19.4%			
Joseph and Rasool 2009 [21]	**Kerala**	86	33.9%	32.5%	0	<0.011
Bhalla *et al*., 2009 [26]	**Chandigarh**	200	4.5%	0.47%	3.3%	0.47
Padma *et al*.,2009 [14]	**Hyderabad**	303	8.9%	7.6%	1.3%	<0.003
Khandelwal *et al*.,2009 [25]	**Chandigarh**	100	7 %	0	7%	0.5
Ramchander *et al*.,2005 [19]	**Andra Pradesh**	200	7%	6.5%	0.5%	<0.005
Maheshwari *et al*., 2003 [20]	**Karnataka**	45	51%	33.3%	4.4%	13.3
Ramshankar *et al*., 2003 [18]	**Bangalore**	215	18.13%	16.7%	2.3%	0.46

DISCUSSION:

The study analyses the prevalence and kind of mutation in the GJB2 gene using a literature review and comprises of 2546 probands from five cities of India. Since the last decade, large scales of genetic screenings of hearing impairment have been done on the Indian population [13-15]. In this review, we examined a range of GJB2 and GJB6 mutations in comprising from all regions of India. India is a diverse country inhabiting linguistically and ethnically different people with Dravidian in South Indians [16] to Caucasians in North India [17]. Earlier the mutation in Indian patients comprised mainly of consanguineous in south India. Certain factors determine the prevalence of any disease such as the practice of a consanguineous marriage system as it is common in a certain community mostly in the south Indian community [18-20]. In another cohort study, Padma *et al* 2009 studied 303 non-syndromic hearing impairment patients and found 33(10.9%) patients found a mutation in GJB2, and among them, six were carriers for the mutant allele [14]. A work that done by Joseph *et al*., 2009 on 86 affected probands from 59 families of Kerela, India stated that the range for hearing impairments by GJB2 gene account for 36% and p.W24X is the most common mutation accounts for 32.5% in affected probands [21]. The grave and specific position of India is the existence of different ethnic groups that suggest heterogeneity throughout India. The etiology of hearing impairment is multi-factorial. Despite the differences in etiology, there is a common factor that is a pathological change along with aging that leads to hearing loss. Such change was seen in post-lingual progressive hearing impairments. Such hearing loss is due to damage in the mechano-sensory hair cells of the inner ear. There are several hypotheses to explain the hearing impairment. The most acceptable hypotheses state that the GJB2 gene produces a connexin protein known as Cx26. The deficiency of Cx26 protein may disturb inner ear gap junctions and will effect on recycling and sinking of expelled potassium (K+) ions after exciting the hair cell that causes accumulation of K+ ion in the extracellular space that ultimately leads to induces hair cell degeneration and hearing loss and the condition is called K+-toxicity.

Cx26 deficiency → Disrupt Gap junction → impaired K+ recycling → K+ accumulation → hair cell degeneration → hearing loss.

These connexin proteins are isoforms, expressed, and localized simultaneously both in the cochlea [22] where they create heteromeric gap junctions [23] and aids in the homeostasis of cochlea [24]. The hypothesis also states the role of MicroRNAs that have been showing a critical function in the development of inner ear and thus, changing in the gene may play a role in the development of hearing impairment.

The non-coding RNAs that are miRNAs pass through the gap junction of cochlear supporting cells and regulate the expression of genes that play a role in cochlear development [22]. It was found that Cx26 deficiency found to be cause cochlear developmental disorders. Zhu *et al* also studied association with Cx26 deletion that brought disorders in the cochlear developmental and found that the mutation of Cx26 only could disrupt intercellular transfer miRNA in the cochlea, although inner ear gap junctions still retained permeability after mutation in Cx26[22].

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

CONCLUSION:

The existence of various ethnic groups of people in India, there is the heterogeneity of mutations but due to specific traditional intragroup marriage may give rise to the homogeneity in some population of India. The mutations in the GJB2 gene are responsible for the contribution of up to around 36%. Regarding GJB2 mutation c.71G>A (p.W24X), a tryptophan stop codon is the most common mutation. The GJB2 shows a number of mutations unlike GJB2, GJB6 does not show any data of mutation.

ACKNOWLEDGMENT

The authors of this article would like to be thankful to all those authors of the scholarly article from which the literature has been reviewed. Mohd Murtaza acknowledges a fellowship from CSIR New Delhi, India.

CONFLICT OF INTEREST: No conflict of interest.

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