Mutational Spectrum of GJB2 gene with its Promoter region in Congenital non syndromic hearing impairment.

THESIS SUBMITTED

TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH

For Awarding the Degree as

DOCTOR OF PHILOSOPHY
IN
MEDICAL ANATOMY

Under Faculty of Medicine By

Dr. KUMARASWAMY REVANAKIMATH

Under the Supervision of

Dr. (Prof.) VENKATESHU K. V.

Department of Anatomy

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August 2023

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I, **Dr. Kumaraswamy Revanakimath**, do hereby declare that the thesis entitled **"Mutational Spectrum of GJB2 gene with its Promoter region in Congenital non syndromic hearing impairment"** characterizes original research work carried out by me for the award of Doctor of Philosophy in Medical Anatomy under supervision of **Dr. Venkateshu K V.**, Professor, Department of Anatomy and Co-Supervision of **Dr S M Azeem Mohiyuddin**, Professor, Department of ENT, Sri Devaraj Urs Medical College, A constituents of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka, India. The work has not been submitted for any other degree or diploma of this or any other Universities. The particulars given in this thesis are true to the best of my knowledge.

Date: 21/08/2023

Place:

Dr.Kumaraswamy Revanakimath

CERTIFICATE

This is certify that the thesis entitled **"Mutational spectrum of GJB2 gene with its promoter region in congenital non syndromic hearing impairment"** submitted for the requirement of award of Degree, Doctor of Philosophy by **Dr. Kumaraswamy Revanakimath (Reg. No. 15PhD0102)** is the record of original research work carried out by him under supervision of **Dr. Venkateshu K. V.**, Professor, Department of Anatomy and Co-Supervision of **Dr. S M Azeem Mohiyuddin**, Professor, Department of ENT, Sri Devaraj Urs Medical College, A constituents of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka, India.

This research work included in the thesis has not been submitted for the award of any degree, diploma, associateship, fellowship or other similar titles in this universities or any other Universities of Institution of Higher learning.

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No part of this thesis has been previously formed the basis for the award of any degree or fellowship.

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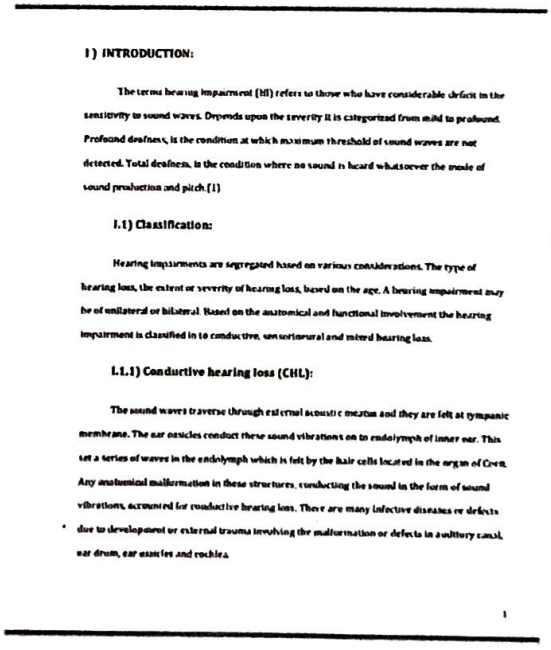

1) INTRODUCTION:

The terms hearing impairment (HI) refers to those who have considerable deficit in the sensitivity to sound waves. Depends upon the severity it is categorized from mild to profound. Profound deafness, is the condition at which maximum threshold of sound waves are not detected. Total deafness, is the condition where no sound is heard whatsoever the mode of sound production and pitch.[1]

1.1) Classification:

Hearing impairments are segregated based on various considerations. The type of hearing loss, the extent or severity of hearing loss, based on the age. A hearing impairment may be of unilateral or bilateral. Based on the anatomical and functional involvement the hearing impairment is classified in to conductive, sensorineural and mixed hearing loss.

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The sound waves traverse through external acoustic meatus and they are felt at tympanic membrane. The ear ossicles conduct these sound vibrations on to endolymph of inner ear. This set a series of waves in the endolymph which is felt by the hair cells located in the organ of Corti. Any anatomical malformation in these structures, conducting the sound in the form of sound vibrations, accounted for conductive hearing loss. There are many infective diseases or defects due to development or external trauma involving the malformation or defects in auditory canal, ear drum, ear ossicles and cochlea.

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<1% match (Vinay N/A. "Prevalence of Dead Regions in Subjects with Sensorineural Hearing Loss", Ear and Hearing, 04/2007)
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http://www.sriramachandra.edu.in

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"Hearing Loss: Mechanisms, Prevention and Cure", Springer Science and Business Media LLC, 2019

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I) INTRODUCTION: The terms hearing impairment (HI) refers to those who have considerable deficit in the sensitivity to sound waves. Depends upon the severity it is categorized from mild to profound. Profound deafness, is the condition at which maximum threshold of sound waves are not detected. Total deafness, is the condition where no sound is heard whatsoever the mode of sound production and pitch.(1) I.1) Classification: Hearing impairments are segregated based on various considerations. The type of hearing loss, the extent or severity of hearing loss, based on the age. A hearing impairment may be of unilateral or bilateral. Based on the anatomical and functional involvement the hearing impairment is classified in to conductive, sensorineural and mixed hearing loss. I.1.1) Conductive hearing loss (CHL): The sound waves traverse through external acoustic meatus and they are felt at tympanic membrane. The ear ossicles conduct these sound vibrations on to endolymph of inner ear. This set a series of waves in the endolymph which is felt by the hair cells located in the organ of Corti. Any anatomical malformation in these structures, conducting the sound in the form of sound vibrations, accounted for conductive hearing loss. There are many infective diseases or defects due to development or external trauma involving the malformation or defects in auditory canal, ear drum, ear ossicles and cochlea. I.1.2) Sensorineural hearing loss (SNHL): The inner ear, the cochlea, is the seat of organ of Corti, responsible for converting the sound waves to neuronal impels. Further these impels are carried by cochlear nerve to auditory centers through auditory pathway. Any pathology involving the nerve, auditory centers and auditory associated area leads sensorineural hearing loss. Most often the cochlear hair cells damage are observed in the cases of sensorineural hearing loss. As age advances the hearing hair cells tend to get damaged. It is evident that auditory issues are more often among elderly age group. I.1.3) Mixed hearing loss: It a condition where anatomical structures involved in conducting system and the neuronal malfunction due to various reasons. Chronic ear infection may deform the anatomy of tympanic membrane, or damage to the ossicles, or both. I.1.4) Prelingual deafness: Incident of deafness before an

ABBREVIATIONS

HI	**HEARING IMPAIRMENT**
CHL	**CONDUCTIVE HEARING LOSS**
HL	**HEARING LOSS**
IHC	**INNER HAIR CELLS**
OHC	**OUTER HAIR CELLS**
GJB	**GAP JUNCTION BETA**
CX	**CONNEXIN**
DNA	**DEOXYRIBOSE NUCLIC ACID**
NSHL	**NON SYNDROMIC HEARING LOSS**
DFNA	**NONSYNDROMIC DEAFNESS AUTOSOMAL RECESSIVE**
DFNB	**NONSYNDROMIC DEAFNESS AUTOSOMAL DOMINANT**
EDTA	**ETHELENEDIAMINETETRAACETIC ACID**
ASHA	**AMERICAN SPEECH-LANGUAGE-HEARING ASSSOCIATION**
PCR	**POLYMERASE CHAIN REACTION**
RFLP	**RESTRICTION FRAGMENT LENGTH POLYMORPHISM**
TAE	**TRIS ACETAE EDTA**
TE	**TRIS EDTA**
ELB	**ERYTHROCYTE LYSIS BUFFER**
dNTPs	**DEOXYRIBOSE NUCLEOTIDE TRIPHOSPHETS**
DNA	**DEOXYRIBOSE NUCLIC ACID**
SDS	**SODIUM DODECYL SULFATE**
SNPs	**SINGLE NUCLIOTIDE POLYMORPHISM**
PHC	**PRIMARY HEALTH CENTER**
iPSC	**INDUCED PLERIPOTENT STEM CELLS**

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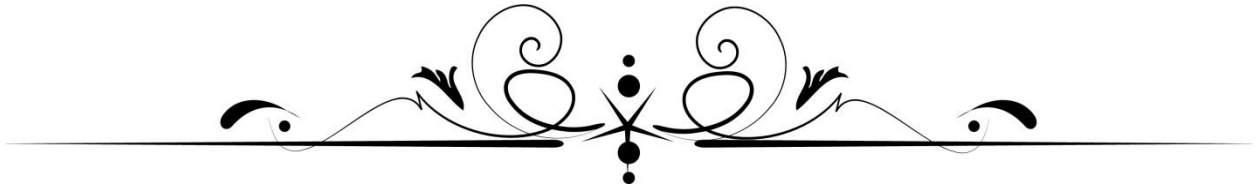

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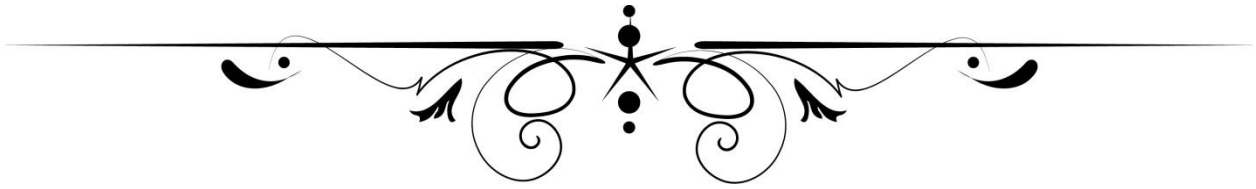

I.INTRODUCTION:

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I.1.3) Mixed hearing loss:

It a condition where anatomical structures involved in conducting system and the neuronal malfunction due to various reasons. Chronic ear infection may deform the anatomy of tympanic membrane, or damage to the ossicles, or both.

I.1.4) Prelingual deafness:

Incident of deafness before an individual acquire a skills of spoken language. Deafness may be acquired by birth (congenital) or may be affected early in the life before one can able to learn the spoken language. Prelingual hearing impairment is considered to be hereditary but data shows contrary to this as there are high incidents of severe and chronic infections at neonatal period and alleged use of ototoxic drugs. It is also added by the trauma. In cases of prelingual hearing impairment there are significant improvements are observed with early intervention of cochlear implants. This could lead individual to comprehend the skills of spoken language.(2)

I.1.5) Post-lingual deafness:

The hearing impairment caused after an individual acquired the skills of spoken language. The defect is gradual in onset and commonly affected as injury to the conducting or sensorineural pathway due to chronic diseases of middle ear and ototoxic drugs. It is also noticed in cerebrovascular accidents causing cortical damage of auditory centers. As the defect is gradual in onset the affected individual may not experience the hearing impairment themselves. In most circumstances the family members and close associate notice the behavioral chances with respect to hearing sensitivity. Hence the disability appeared after an individual already comprehend the skills of spoken language the hearing capacity and speech abilities are better with the help of various hearing aids, lip reading techniques and cochlear implants. Data suggest that incident of congenital deafness is lower than the post-lingual deafness.

I.1.6) Unilateral and Bilateral hearing loss:

These are the conditions wherein the hearing loss is noticed either of the ears or only in one ear. Typically such an individual experience difficulty in hearing while there is nosier background and fails to localize the direction of the sound.

I.2) ANATOMY OF HUMAN EAR:

I.2.1) External ear:

External ear comprises ear pinna and external auditory canal. The primary purpose of the external ear is to gather and transmit sound waves to tympanic membrane which is placed at the lateral end of the canal. Tympanic membrane is a semitransparent fibrous layer separates middle ear from the external ear. The sound waves are felt at this membrane and transformed in to sound vibrations which further transmitted to inner ear through ear ossicles. The handle of the malleus is attached to tympanic membrane. The integrity and tightness of the tympanic membrane is asserted by the tensor tympani muscle through handle of the malleus.

I.2.2) Middle ear:

Middle ear located in the petrous part of the temporal bone. The middle ear cavity is bound anteriorly by carotid wall, posteriorly by mastoid wall, laterally by tympanic wall, medially by labyrinthine wall, roof is formed by tegmental wall and floor is by jugular wall. The cavity contains ear ossicles, malleus, incus and stapes, paly important role in physical transmission of sound waves in the form of vibrations. The cavity also contains two muscles, Tensor tympani muscle which maintains the appropriate tension of the tympanic membrane helping it to receive the sound waves effectively. Stapedius is another muscle attached to stapes and helps in regulation of the intensity of vibrations.

I.2.3) Inner Ear:

It is found in the highly intricate membranous labyrinth that is part of the bony labyrinth in the petrous part of the temporal bone. The bony labyrinth is a potential canal

that is brimming with the perilymph, a clear fluid. The membranous labyrinth is a tube like structure filled with endolymph suspended in the perilymph. The inner ear comprises six sensory organs. The cochlea, concerned with hearing, comprising the organ of Corti, the auditory sense organ. The rest five are vestibular end organs, which maintains acceleration and equilibrium.(3,4)

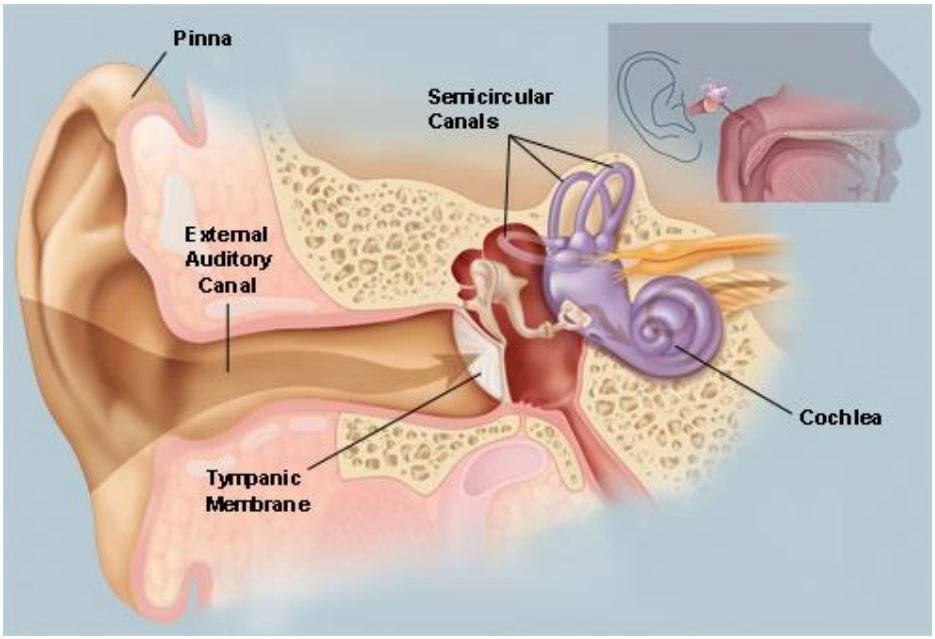


Figure.1: Schematic picture of ear. (Source: http://www.webmed.com/picture of ear)

I.2.3.1) Microscopic anatomy of cochlea:

The organ of Corti is the sensory receptor for audition. It is located on the basilar membrane of the cochlear duct filled with endolymph. The organ of Corti mainly has inner and outer hair cells. The inner hair cells are of single row and play a key role in audition and transmission of sound waves in to nerve impulses which are carried by cochlear nerve to the auditory centers. The outer hair cells are set with rows of three there are contractile cells that helps in amplification of the stimulus perceived by the inner hair cells. Apart from the sensory hair cells organ of Corti facilitated with other supporting cells.

The hair cells at their apical surface present microvilli filled with actin filament. The microvilli are of stereocilia category forms a unit of hair bundle constituted by 30 to 300 stereocilia. The hair bundles functions as mechanoreceptive structure. The stereocilia are organized in a typical staircase appearance. The tip of the smaller stereocilia is coupled with the next larger stereocilium by a special tip link. The links are noticed along the entire length of stereocilia. The stability of the hair cells are ensured by tight junctions placed at apical part of the stereocilia and walls of the associated supporting cells forming reticular lamina **(Figure.2).** The endolymph and perilymph possess difference in their ionic compositions. The architecture of the hair bundle and other surfaces of the hair cell are submerged in liquids of different ionic compositions.(5)

The endolymph is mainly composed of large amount of potassium estimated to 150 mM, and pitiable amount of calcium 0.02mM and negligible amount of sodium 1mM. Contrary to this the perilymph has very low level of potassium 3.5 mM, abundance of Sodium 140 mM, and calcium concentration 1 mM, compared to the endolymph.

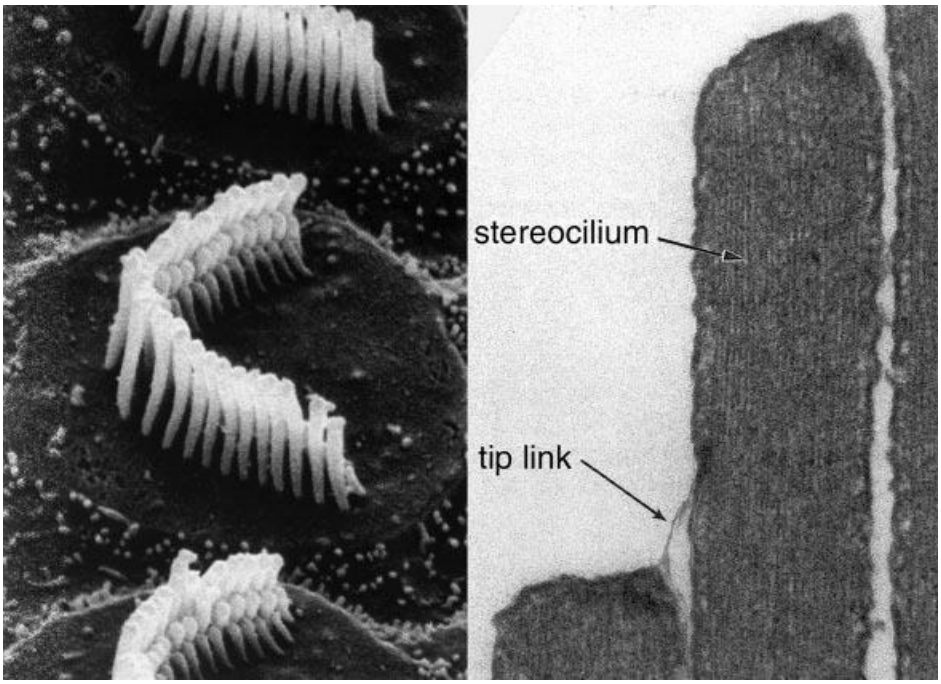


Figure.2: Stereocilia and their tip links.

A different types of epithelial cells line the membranous labyrinth of the cochlea. On the lateral wall of the cochlea resides stria vascularis comprising two type cells marginal cells and basal cells. These two cell types held together by tight junctions and establish a two cell barriers and lodges intrastrial space between them. The intrastrial space composed of blood capillaries and sporadic layer of intermediate cells. The basal cells are coupled with gap junctions to surrounding intermediate cells and also to fibrocytes of the contiguous connective tissue. This shows there is constant exchanges between those cells.(6)

However, strial marginal cells dose not establish any connections with other cells through gap junctions. The section of the potassium ions in to cochlear endolymph by stria vascularis elicit the endocochlear potential.(7)

I.2.3.2) The functional anatomy of cochlea:

The ear ossicles especially footplate of stapes articulates with the oval window of cochlea and conduct the sound vibrations by inward movement of the membrane covering the oval window this creates the compression of the perilymphatic fluid. The fluid is decompressed passively causing outward movement covering the oval window. This phenomenon set up the liquid waves in the endolymph. The liquid waves also produces movement of the basilar membrane, the peaks of the fluid waves depends on the sound frequency. The movement in the basilar membrane causes movement of the hair cells of organ of Corti.

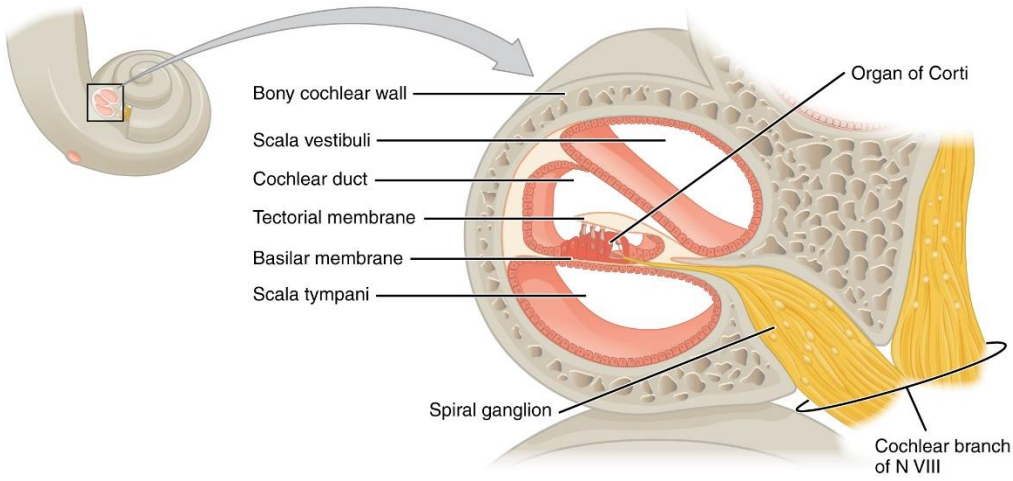


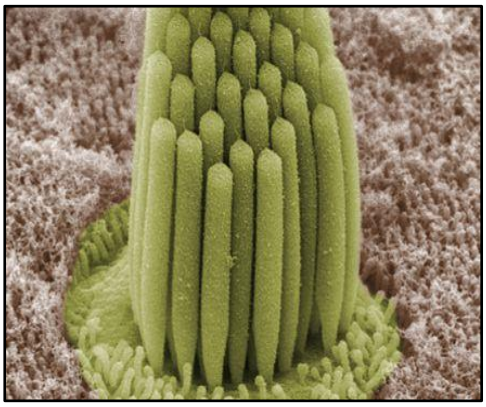
Figure.3: Organ of Corti and its associated structures. (Source: www.medicalanatomy.net/organ of Corti)

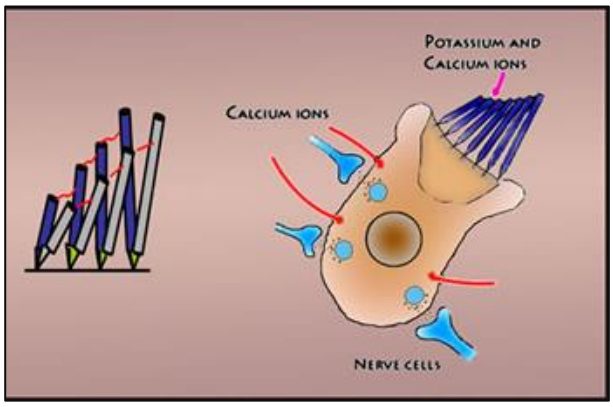
The apical surface comprising hair cell move concurrently with the vibrations produced by the movement of basilar membrane. Further tectorial membrane lying on the hair cells deviates the stereocilia bundle. The stereocilia are connected with tip links and movement in them creates a stretching effect and tension causing mechanotransduction cationic channels situated at the tip of the stereocilia to open.(8,9)

The polarization of the cells are driven by entry of potassium ions. The polarization leads to electric gradient between endolymph and hair cells. It estimated to be150 mV between them. The depolarization of the hair cells allows calcium ion to enter cells through basal membrane. This makes the synaptic vesicles to fuse with the plasma membrane. The synoptic vesicles releases the neurotransmitters. This manifests action potential which is carried by the auditory nerve to cerebral cortex and associated structures.

The functional studies confirms that the frequencies of sound are assessed and perceived at specific position of the cochlea.(10) The apical transducer current brings depolarization in the outer hair cells leading to contraction of their lateral

membrane.(11,12) The sequential and recurring changes in the dimensions of the outer hair cells are hypothesized to magnifies the auditory stimulation by increasing the basilar membrane vibrations.





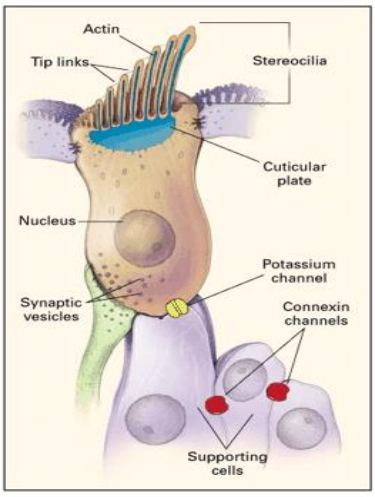


Figure.4: The outer hair cell with hair bundles and their connections. Movement of the perilymph produces movement of stereocilia, causing stretch of tip links thus play role in opening of potassium channels. (Williems 2000)

I.3) The causative factors of hearing loss:

Hearing loss or impairment is considered to be the most frequent sensory disorder. Nearly one child in 500–650 is born with hearing impairment.(13,14) Major proportion of the diseased are due to defect in the single gene. The inheritance exhibit typical pattern and shows 80% are autosomal recessive type, 20% are autosomal dominant type and 1% of mitochondrial and X-linked origin.(15)

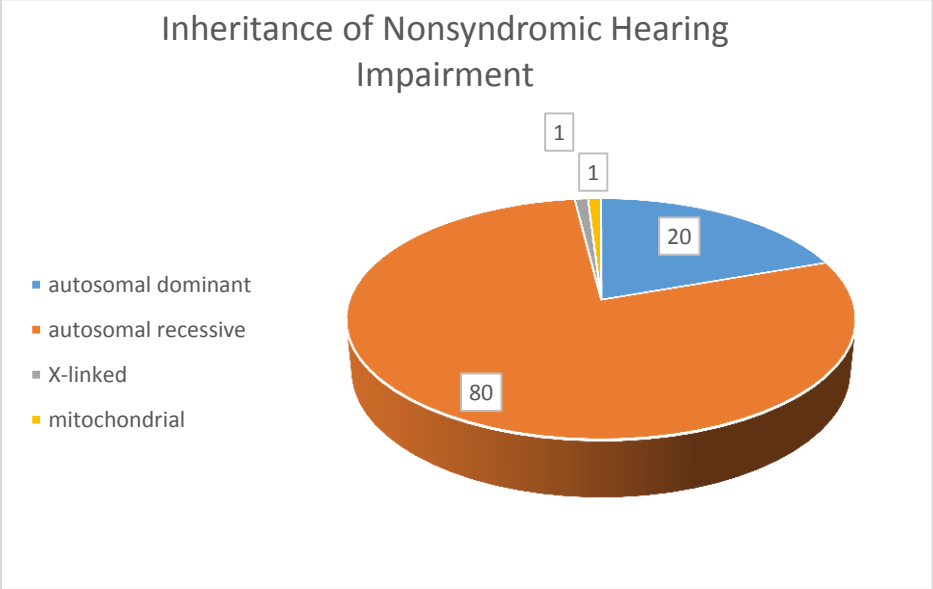


Figure.5: Proportions of genetic causes of Nonsyndromic hearing impairment.

Hearing impairment is associated in both syndromic and nonsyndromic cases. In the Syndromic form along with hearing impairment there will be association of some other systemic defects or deformities. Statistical wise syndromic form of hearing impairment is observed in approximately 20-30% and nonsyndromic form up to 70–80% of cases. Nonsyndromic are those there will not be any phenotypic abnormalities.(16)

Up till now, as many as 35 deafness associated genes are identified and more than 70 loci are marked for the nonsyndromic hearing impairment. Based on the impact of the mutation it is classified in to autosomal dominant and autosomal recessive types. As per the data the more than 75% of the nonsyndromic cases are autosomal recessive type with severely affected before an individual acquire the spoken language.(17)

The expression and regulation of the gene depends on many factors, like transcription factors (TFs), cellular trafficking proteins, components of Extra and intracellular components and many more. Irrespective of syndromic or nonsyndromic disruption of these factors leads to hearing impairment.(18)

I.3.1) Molecular genetics of nonsyndromic hearing impairment:

The nonsyndromic hearing impairment is a recessive disorder and the hereditary pattern came in to light only in the sixteenth century by Johannes Schenck.(5,19) Furthermore, identifying the putative genes started much later as hearing impairment exhibits great genetic heterogeneity, lack of clinical data and also lack of technical assistance for mapping the chromosomes.

Hearing impairment exhibit widespread genetic heterogeneity. However, DFNB1 locus on the long arm of chromosome 13 involved in more than 50% of the cases of non-syndromic autosomal recessive disorder. This locus comprises *GJB2* and *GJB6* genes commonly linked with hearing impairment.

The GJB2 gene is located on the long (q) arm of chromosome 13 between positions 11 and 12. More precisely, from base pair 20,761,601 to base pair 20,767,113 on chromosome 13.

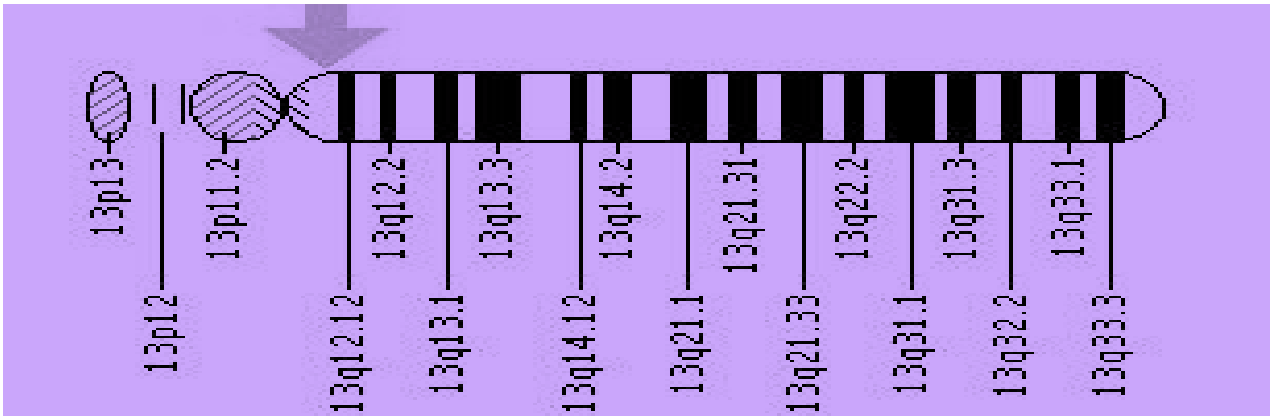


Figure.6: Location of the GJB2 and GJB6 genes. (Source: www.genetic home reference.com/genes/GJB)

The official name of *GJB2* gene is "gap junction protein, beta 2, 26kDa. The GJB2 gene transcribe and translate to form gap junction proteins called as connexin26. There are several varieties connexin proteins. Connexin26 protein involved in forming functional gap junctions that plays important role in transporting charged ions, nutrients, and signaling molecules.

Connexin26 is widely involved in formation of gap junctions in many of tissues of the body. However, these proteins are highly expressed in supporting cells of organ of Corti and also on skin. In cochlear epithelium connexin26 gap junctions specially transports K+ ions and some micro molecules between the cells that are in contact with each other.(20)

The process of transformation of sound vibrations in to nerve impulses hold the key phage of the hearing and also determine the sensitivity to the sound. This process is primarily dependent on ion homeostats and maintenance of K^+ ions in the Cochlea.(21) Any mutational changes in the GJB2 gene sequence leads to nonfunctional or malfunction gap junctions, causing impaired K^+ levels in the cells of cochlea. The increased and decreased level of potassium ions in the cells may bring adverse effect and may destroy the cells responsible for hearing.(20)

I.3.2) Microanatomy of Connexin26:

Under the electron microscope the Connexins shows 4 transmembrane domains having N- and C-terminals of variable length suspended in the intracellular fluid (Figure.7). In each connexin the size of the C-terminal region is different. Connexin26 comprises both a short N terminal and long C-terminal region. Usually the connexons are

homomeric and composed of 6 indistinguishable connexins linked with noncovalent bonds.(21)

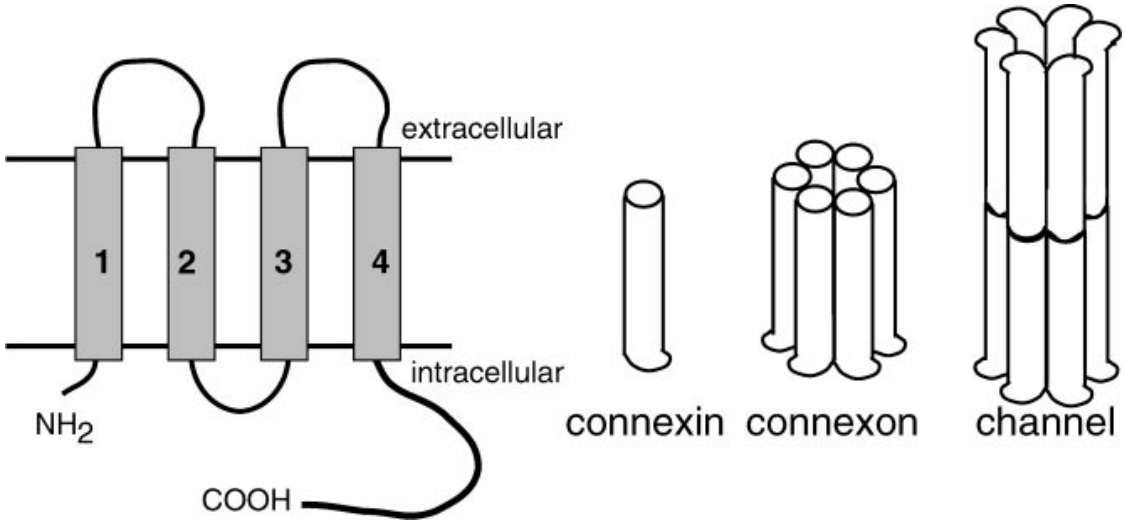
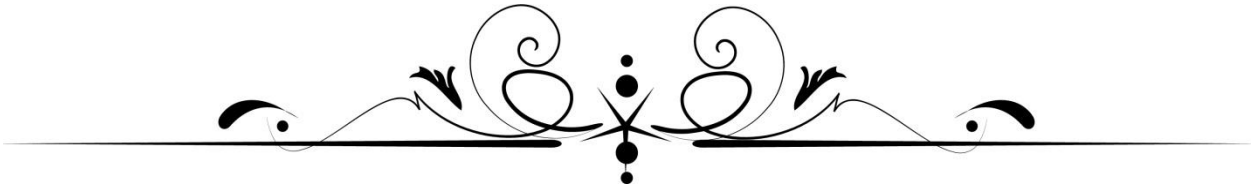
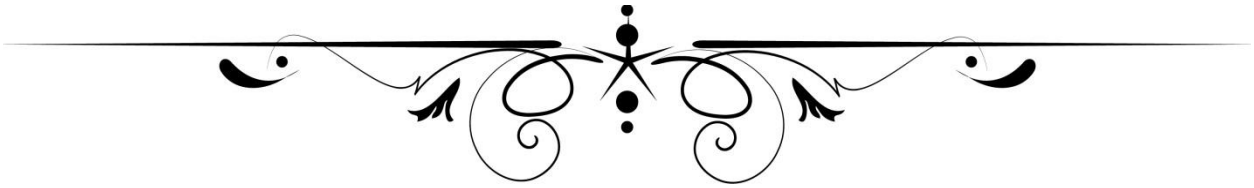


Figure.7: Schematic representation of connexins.

Positively charged ions or molecules are more permeable to Connexin26 than negatively charged ones. It lacks consensus sequences that kinases can phosphorylate.(22) The second transmembrane domain's proline-87 and the first extracellular loop have both been linked to the voltage control of gating. After Cx26 was immunostained, gap junctions in the rat cochlea were examined using light microscopy and electron microscopy.(23) The foundation of two distinct cellular networks is Cx26. One links the neighboring epithelial cells to the supporting cells of the Corti organ. The other is made up of mesenchymal cells, basal, intermediate, and endothelial cells from the stria vascularis, as well as fibrocytes. Although their function is uncertain, these networks have been hypothesized as the structural underpinnings of a transcellular K+ circulation.(3,23) The experimental study to assess the pathogenic effect of Cx26 was carried out by knocking out GJB2 gene in mouse failed to elucidate as the mutant mice could not survive.(24)



REVIEW OF LITERATURE



II) REVIEW OF LITERATURE

Prosper Meniere, the French physician, in 1846 was the first person to study the inheritance pattern of hearing loss. As mentioned in his research "Upon marriage between relatives considered as the cause of congenital deaf-mutism," it is evident that "deaf-mutism" is the autosomal recessive disorder.

Later In 1994, a study on profound prelingual deafness cases of Tunisian families succeeded to identify the locus DFNB1, for an autosomal recessive form of deafness. They also reported DFNB1 located on chromosome 13q12.(25)

Once the DFNB1 locus was identified several studies were carried out to evaluate the mutagenic effect of GJB2 gene and study results shows high prevalence of GJB2 in autosomal recessive deafness. As the defect is recessive disorder a study conducted on consanguineous families of Pakistan and identify two different nonsense mutations and concludes the association of GJB2 gen in hearing impairment.(26)

Subsequently separate analytical studies conducted at Spain and Italy and found 79% of the cases are linked to the DFNB1 locus.(27) Mutational screening of GJB2 gene endorse the high prevalence of putative Connexin26 among deaf cases. It has been estimated that DFNB1 accounts for 30% to 60% in separate deafness in Europe and United States(28,29)and ~20% in Japan.(30)

Approximately more than 50 different of GJB2 gene variants / mutations were reported. Conversely, there are very few mutations are highly prevalent and they will be discussed separately.

A. 35delG :

A missense mutation, 35delG. There is a stretch of six guanine (G) nucleotides at position 30-35. Premature stop codon 13 is produced when a single guanine (G) nucleotide is deleted from a DNA sequence.

Table-1 shows that populations in Europe, the Middle East, North Africa, North and South America, Australia, and European-Mediterranean populations all have a high prevalence of the mutation 35delG.(28) This mutation appears to be uncommon in Asian groups(31) and has not been seen in deaf Chinese children(32) Baluchi populations.(33) Even across siblings, the degree of hearing loss in 35delG homozygous people varies greatly. Either flat or sloping with a preferential loss at high frequencies characterize audiometric curves.(29,32)

B. 167delT :

The 167delT is a missense mutation. Due to the deletion of single nucleotide Leucine is read as Arginine in a coding region (p.Leu56Arg), this creates premature stop codon. Eventually leads to abrupt stoppage of protein formation causing production of non-functional protein complexes.

The 167delT mutation (Table-2) is frequently detected among deaf Ashkenazi Jews and is associated with a specific haplotype, which indicates a single origin of the mutation and considered to be pathogenic,(34) found to be very rare in Asian populations.(31,35,36)

C. 235delC :

The c.235delC is the frameshift mutation due to deletion of single nucleotide at the coding region of GJB2 resulting truncation of connexin proteins. Due to the mutation,

the codon for Leucine is read as stop codon p.Leu79Cysfs. The functional studies suggests abnormal connexins failed to maintain the ion homeostasis required for the eliciting action potential at the hair cells. Thus the mutation is considered to be pathogenic.

The 235delC mutation (Table-3) seems to account for a large proportion of the CX26 mutations either homozygous or heterozygous in the Japanese population.(32,37–39) A study shows increases the risk of NSHL for 235delC mutation of GJB2 gene in the East Asian and South-east Asian populations, but non-significantly associated with the NSHL in Oceania and European populations, suggesting a significant ethnic specificity of this NSHL-associated mutation.(40)

D. p.W24X

The c.71G>A is a nonsense mutation caused by substitution of Adenine(A) in place of Guanine (G) at 71st position of coding part of GJB2 gene. Because of the mutation the codon for Tryptophan is read as stop codon thus causing premature stoppage of production connexin proteins. Functional analysis of the mutation proves to be pathologic as the truncated proteins were failed to execute the normal expected function.(41)

The p.W24X (Table-4), is found to be more prevalent in Indian subcontinents and among Caucasian populations.(34–36,42) However it has been rarely seen in Mongolians, Syria and China.(16,32,43,44)

E. p.W77X

Along with W24X a similar mutation p.W77X (Table-5) has been reported by Indian researchers in both homozygous and heterozygous conditions.(31,45) The p.W77X

is a nonsense mutation caused by substitution of Adenine (A) in place of Guanine(G) at position 231. The codon for amino acid Tryptophan is replaced with stop codon. c.231G>A (p.Trp77X) anticipated to results in the abnormal proteins, which leads to manifestation of hearing deficit. There are not enough substantial evidences of the impact of this mutation on the functional connexins. However recent studies claimed the mutation is pathogenic, based on the functional assessment submitted to ClinVar database.

Table – 1: Population based studies showing 35delG mutation

Sl. No	Studies	Population	Total no. of subjects	Presentations	
				Hetero zygous	Homo Zygous
1	Saber Masmoudi et al., 2000.(46)	Tunisia	70	4	10
2	G Minarik et al., 2003.(47)	European Gypsies	54	07	00
3	M RamShankar et al., 2003.(36)	India	205	00	00
4	Rikkert L. et al., 2005.(34)	Turkey Ashkenazi Jews Africans	1531	335	889
5	Posukh O, et al. 2005.(48)	Russia	74	03	07
6	P V Ramchander. et al., 2005.(31)	India	200	00	00
7	Anoosh Naghavi. et al., 2008.(35)	Baluchi	100	00	00
8	Pu Dai. et al., 2009.(32)	Chinese	2063	04	02
9	G. Padma. et al., 2009.(42)	India	303	00	00
10	Mustafa Tekin et al. 2010.(43)	Magnolia	534	00	00
11	Walid Al Achkar et al. 2011.(44)	Syrian	50	6	15
12	Nina Danilenko et al. 2012.(49)	Belarus	391	51	178
13	K Cryns et al. 2013.(16)	Belgian Italian Spanish American	277	67	166

Table – 2. Population based studies showing 167delT mutation

Sl. No	Studies	Population	Total no subject	Presentations	
				Hetero Zygous	Homo Zygous
1	M RamShankar. et al. 2003.(36)	India	205	0	0
2	Rikkert L. et al. 2005.(34)	Turkey Ashkenazi Jews	1531	45	17
3	P V Ramchander, et al. 2005.(31)	India	200	00	00
4	Anoosh Naghavi, et al. 2008.(33)	Baluchi	100	00	00
5	Pu Dai. et al. 2009.(32)	Chinese	2063	00	00
6	G. Padma, et al. 2009.(42)	India	303	1	00
7	Mustafa Tekin et al. 2010.(43)	Magnolia	534	00	00
8	Walid Al Achkar et al. 2011.(44)	Syrian	50	5	00
9	K Cryns et al. 2013.(16)	Belgian Italian Spanish American	277	11	00

Table – 3. Population based studies showing 235delC mutation

Sl. No	Studies	Population	Total no subject	Presentations	
				Hetero Zygous	Homo Zygous
1	Park HJ, et al. 2000.(37)	Korea	147	5	5
2	Kudo T, et al. 2000.(30)	Japan	63	1	3
3	Abe S, et al. 2000.(38)	Japan	35	4	1
4	Ohtsuka A, et al. 2003.(39)	Japan	1227	92	16
5	Posukh O, et al. 2005.(48)	Russia	74	4	1
6	Schimmenti LA, et al. 2008.(50)	Minnesota, USA	95	0	1
7	Dai P, et al. 2007.(32)	China	3004	255	233
9	Chen D, et al. 2009.(51)	Beijing, China.	100	10	16
10	Padma G, et al. 2009.(42)	Hyderabad, India	456	0	1
11	Batissoco AC, et al. 2009.(40)	Brazil	300	1	0
12	Pu Dai et al. 2009.(32)	Japan	2063	66 113 (compound)	164

Table – 4. Population based studies showing p.W24X mutation

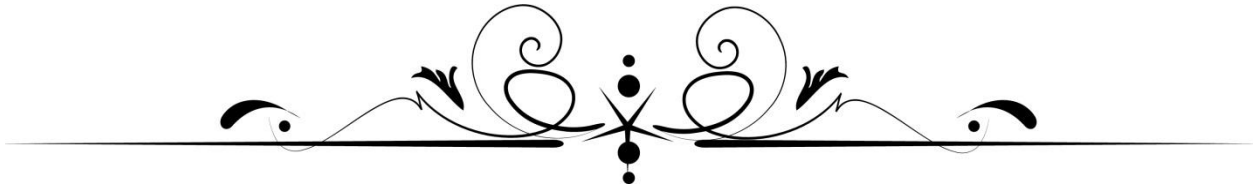
Sl. No	Studies	Population	Total no subject	Presentations	
				Hetero Zygous	Homo Zygous
1	M RamShankar, et al. 2003.(36)	India	205	6	36
2	Rikkert L, et al. 2005.(34)	Turkey Ashkenazi Jews	1531	108	6
3	P V Ramchander, et al. 2005.(31)	India	200	1	13
4	Anoosh Naghavi, Et al. 2008.(35)	Baluchi	100	00	4
5	Pu Dai. Et al. 2009.(32)	Chinese	2063	00	00
6	G. Padma, et al. 2009.(42)	India	303	4	24
7	Mustafa Tekin et al. 2010.(43)	Magnolia	534	00	00
8	Koumudi Godbole, et al. 2010.(45)	India	288	00	∼ 60
9	Walid Al Achkar et al. 2011.(44)	Syrian	50	00	00
10	K Cryns et al. 2003.(16)	Belgian Italian Spanish American	277	00	00
11	Maheshwari et al. 2003.(52)	India	45	02	04

Table – 5. Population based studies showing p.W77X mutation

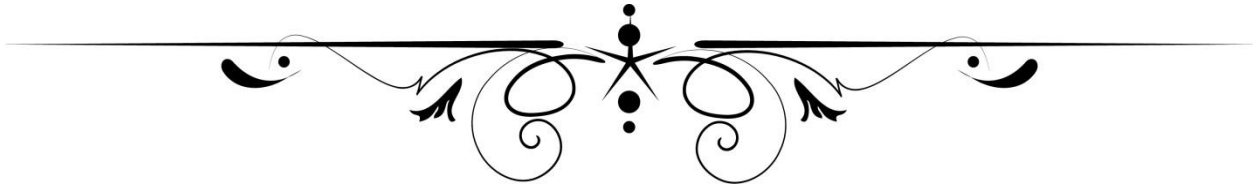
Sl No	Studies	Population	Total no subject	Presentations	
				Hetero Zygous	Homo Zygous
1	M RamShankar, et al. 2003.(36)	India	205	00	01
2	Rikkert L, et al.2005.(34)	Turkey Ashkenazi-Jews	1531	06	00
3	P V Ramchander, et al. 2005.(31)	India	200	01	00
4	G. Padma, et al. 2009.(42)	India	303	00	01
5	Koumudi Godbole, et al. 2010.(45)	India	288	17	00

Table – 6. Population based studies showing c.IVS1+1 G>A mutation

Sl No	Studies	Population	Total no subject	Presentations	
				Hetero Zygous	Homo Zygous
1	M RamShankar, et al. 2003.(36)	India	205	1	00
2	K Cryns et al. 2003.(16)	Belgian Italian Spanish American	277	8	00
3	Rikkert L, et al.2005.(34)	Turkey Ashkenazi Jews	1531	16	00
4	Anoosh Naghavi, Et al. 2008.(35)	Baluchi	100	00	00
5	Pu Dai. Et al. 2009.(32)	Chinese	2063	00	00
6	G. Padma, et al. 2009.(42)	India	303	1	1
7	Mustafa Tekin et al. 2010.(43)	Turkey	307	3	3
8	Koumudi Godbole, et al. 2010.(45)	India	288	00	00



NEED FOR THE STUDY



III) NEED FOR THE STUDY:

The World Health Organization is always been proactive in supporting planning and executing community based health programs. The Ear and Hearing care is one such program incorporated with PHCs (Deafness and hearing loss, Fact sheet, N°300, Updated February 2013) so that data can be collected to -

- Identify the chief causes and genetic pattern of hearing loss.
- Identify the magnitude of the population affected and being affected.
- It is also to assess the physical, social and emotional impact on the affected individuals.
- Develop Strategies and laydown the guidelines to tackle and address the chief causes of hearing loss that can be preventable effectively.
- To develop the programs that can be implemented in the community / population identification and management of the hearing loss.

There is a obvious ethnic variations in the incidence of mutations in the GJB2 gene. There are very few studies on GJB2 gene mutations in Indian population(31,35,42). Further the studies differ in their opinion within the populations about the prevalence and putative mutations in GJB2 gene.

With a frequency of 1 in 200 to 1 in 1000 bp throughout the human genome, polymorphisms are not uncommon.(30) The promoter polymorphisms have the ability to influence gene expression, which could have phenotypic or even pathogenic implications.(31) Not all variations in the promoter sequence impact transcriptional

regulation. The regular processes of gene activation may be interfered with by disrupting the organized recruitment of TFs at the promoter, leading to a decrease or rise in the level of mRNA and consequently protein, depending on the position and nature of the mutation in the promoter region of a gene.(53) The ability of cis-acting DNA sequence motifs to bind to the trans-acting protein factors that typically interact with them can be altered or eliminated by promoter alterations.(54)

However, it is unclear how much of the total number of disease-causing mutations promoter mutations contribute to. For example, most missense mutations result in qualitative flaws. Mutant alleles can occasionally even act as dominant alleles because the afflicted protein may compete with other normal proteins. By using functional research, more and more promoter polymorphisms have been identified. Some of them may have pathological significance. Thalassemia, Bernard-Soulier syndrome, pyruvate kinase deficiency, familial hypercholesterolemia, and hemophilia are a few diseases that can be brought on by promoter mutations.(54)

In 2007, a study was conducted in Portuguese population and claims for identifying the first pathogenic associated with hearing loss-related mutation -3438C→T impairing the basal promoter activity of the GJB2 gene.(55) In concern with the issue raised, in 2008 another study from Poland wavering the possible explanation for the excess monoallelic mutations causing deafness, but they also postulate the chances of difference between Portuguese and Poland populations.(56) With the knowledge of ethnic variation in the prevalence of mutational spectrum of GJB2 gene and as there are no studies or reports focusing over noncoding and promoter region from the Indian population we would like to explore the unexplored novel mutations in promoter regions.

IV. SUMMARY OF THE RESEARCH PROPOSAL:

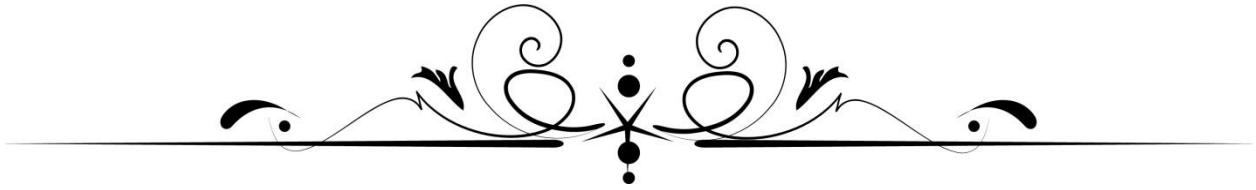
In order to rule out the GJB2 gene mutations as the founder gene for hearing impairment, it is proposed to explore the other mutations in coding and promoter region by subjecting the DNA for sequencing. It also envisages to carry out the project in larger population so that the data might provide to arrive at prevalence rate of mutations in GJB2 gene and possible mutations at promoter region that could explain the deafness with heterozygous genotype.

IV.1) Research hypothesis:

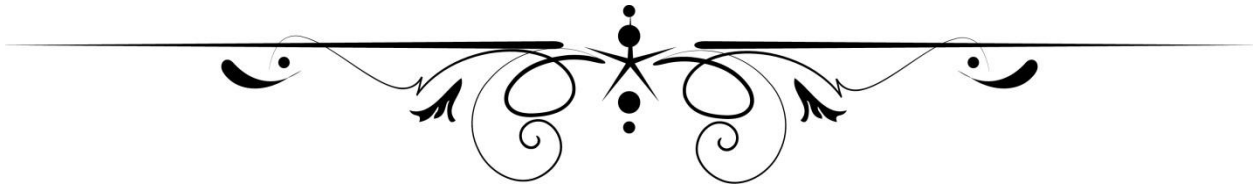
Association of promoter region variations of GJB2 gene in congenital non-syndromic hearing impairment.

IV.2) Research question:

Are, promoter region mutations accountable as causative in heterozygous presentation among probands of congenital nonsyndromic hearing impairment?

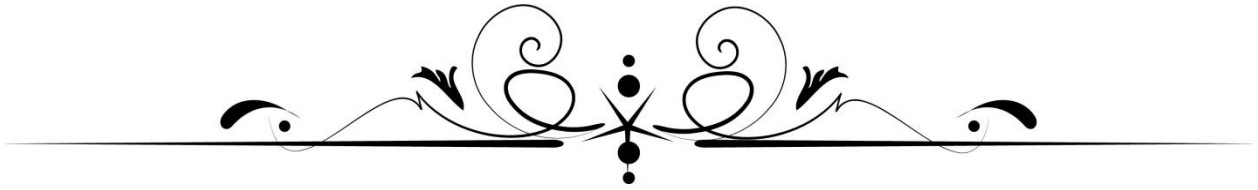


AIMS & OBJECTIVES

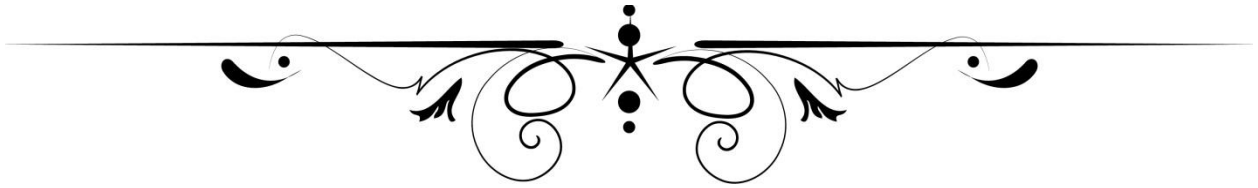


V. AIM and OBJECTIVE OF THE STUDY:

1. To find out the DNA base pair changes at promoter region of *GJB2* gene.
2. To find out the prevalence of most common mutations in GJB2 gene among south Indian population.
3. To establish the association of promoter region variations as causative factor in heterozygous presentation among probands of hearing impairment.



MATERIALS AND METHODS



VI. MATERIALS AND METHODS:

VI.1) Design of the study:

Cross sectional Study in which the congenital nonsyndromic hearing impairment children genotype will be compared with the controls samples as the study intended to carry out with population specific assessment and also with the available reference genomic sequence.

VI.2) Sample size:

Sample size for the study is estimated on prevalence rate of congenital hearing impaired population all together since prevalence rate for the promoter gene mutations of GJB2 gene is not available.

Sample size is estimated to be 98 and is calculated with absolute error of 6% with a prevalence rate of 10% in general population with 95% of confidence level.

$$n = \frac{Z^2_{\alpha} PQ}{(d)^2} = 98$$

D= Absolute error = 6%

Z_{α} = Standard normal deviate at 95% = 1.96

P= 10%

Q= 90%

Total sample size is 98 and they are divided in to two groups that are 50 cases and 48 controls samples.

Source of sample: Residential schools for differently abled children.

VII. 3) Inclusion and Exclusion criteria:

a. **Inclusion criteria:**

i. Patient population**:** Mild, Moderate and profound hearing impairment probands of age between 2-20 years.

ii. Control population: Normal healthy controls without any hearing impairment.

b. **Exclusion criteria:**

i. Patients with a history of acquired aetiology,

ii. Genetic syndromes associated with hearing impairment,

iii. Oto-acoustic trauma

iv. Hearing impairment caused by alleged prenatal and neonatal history.

VI.3.1) Enrollment of participants:

Letters addressing deaf organizations, and centers were prepared, explaining the genetic etiology of deafness, the study being performed in our laboratory and its advantages. The participant or family members, were briefed the intended work. (Appendix-2) After understanding completely they were invited to sign consent forms (Appendix-3). Prior appointment was taken from the residential school for the deaf to conduct basic evaluation of the participants. When approached first time, a questionnaire (Appendix-4) was filled up which contained details about HL (hearing loss), presence of physical signs and symptoms suggesting syndromic conditions, information about other deaf members in the family and ethnic origin. The Sri Devaraj Urs Medical College, the constituent college of the Sri Devaraj Urs Academy of Higher Education, institutional ethical committee gave its approval to the project (Appendix-1).

IV.4) Clinical assessment:

Meticulous history of the hearing loss was obtained from the parents or guardian of the subject. (Appendix-4) Specifically age of the at the time of identification of defect, the magnitude of the deficit, unilateral and bilateral presentation, alleged prenatal history, any rehabilitation assistance, medication, structural deformities ear, and other defects that might point to a syndrome, such as defects of eyes, kidney, heart, thyroid or any other results of clinical tests performed in the past. Detailed information about deceased family members also was obtained. After clinical history ensured that the HL was not a result of non-genetic causes (e.g., trauma, infection, drugs) cases were enrolled for the study after informed written consent.

IV.5) Audiological assessment:

All the subjects suitable to be enrolled for the study were subjected to external ear examination otoscope was used to look for ear wax, intactness of tympanic membrane, and external ear infections. Those subjects who were found to have other middle ear abnormalities were excluded from the study.

Immittance audiometry included tympanometry and acoustic reflex measurements which were carried out to analyze the middle ear functioning. Tympanogram type was noted and the acoustic reflexes were obtained at 500 kHz, 1 kHz, 2 kHz and 4 kHz. Subjects with 'A' type tympanogram and absent reflexes were considered for the study.

Pure-tone audiometric thresholds were measured using Grason Stadler Incorporation, Model 68 (GSI-68) diagnostic audiometer to find out the degree and type of hearing loss. The air conduction thresholds for frequencies ranging from 0.25 kHz to

8 kHz were measured. Using a Radio Ear B71 bone vibrator, bone conduction thresholds were measured for frequencies ranging from 0.25 to 4 kHz. Carhart & Jerger's (1959) modified Hughson-Westlake method was used to measure the audiometric thresholds. Audiogram obtained for one of the subjects is shown in the Figure 8.

Severity of HL was classified according to ASHA as follows:

<16 dB, normal hearing;

16 dB to 25 dB, slight HL;

26 dB to 40 dB, mild HL;

41 dB to 55 dB, moderate HL;

56 dB to 70 dB, moderately severe HL;

71 dB to 90 dB, severe HL;

>90 dB, profound HL (Clarke 1981).

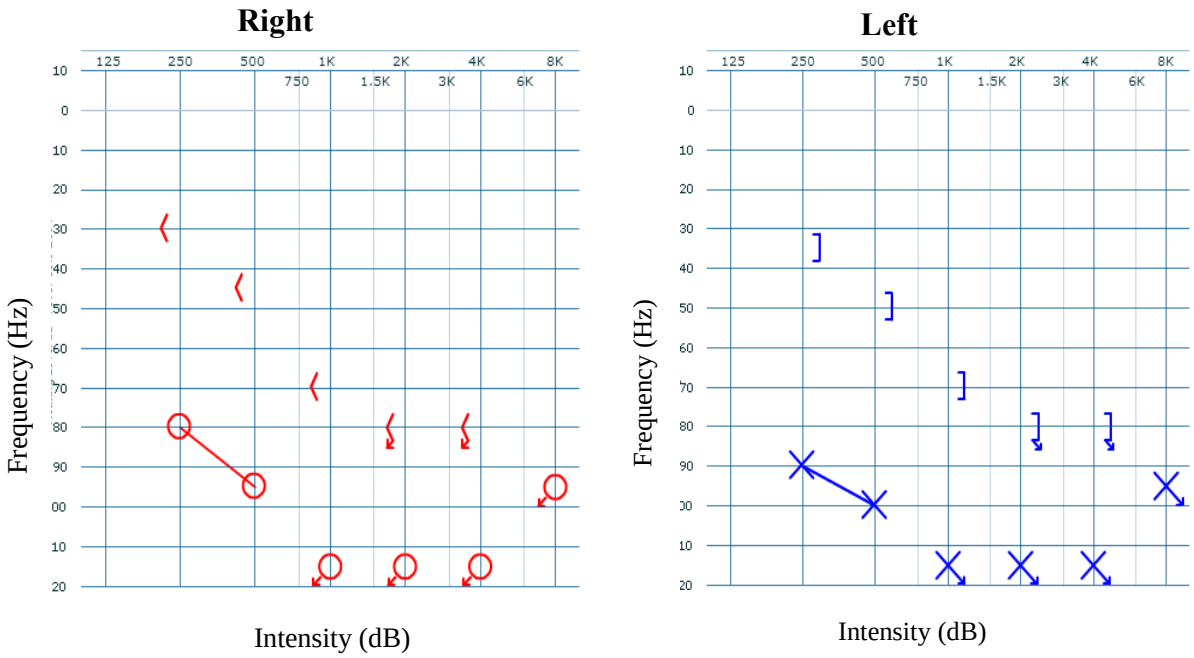


Figure 8: Audiometric thresholds of a subject for the right and left ear

After conducting the audiometric tests, those cases which were affected with profound hearing loss i.e. above 90 dB, were selected as per the inclusion criteria and all relevant information of each patient was recorded.

VI.6) Blood collection:

Consent form clearly emphasizes the need of 3ml of Blood sample on enrolling in to the study. Expert laboratory technician employed to draw blood sample in aseptic measures. 3ml blood was drawn and mixed with EDTA (0.1ml of 0.5M EDTA) to prevent clotting.

VI.7) Molecular analysis

IV.7.1) Genomic DNA extraction:

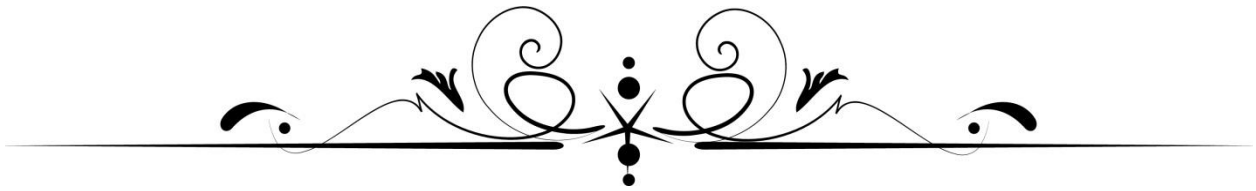
Erythrocyte lysis buffer was added to Blood sample, mixed and thoroughly and kept for 1 hour for the proper lysis of blood cells. This solution was centrifuged and supernatant was discarded. Again ELB was added and the same step repeated till the white buffy coat of white blood cells settles down. Following this SDS and Proteinase K were added and kept at 37^0C overnight. Next day NaCl and isopropyl alcohol were added and precipitated DNA was collected in tube containing isopropyl alcohol. The DNA was washed 3 times and supernatant was discarded and the DNA precipitation was allowed to air dry. The DNA was made to dissolve in TE buffer at 65^0C for 30min and placed in a rotator for overnight. The isolated DNA is preserved at -20^0C.(57)

Table-7: Materials used for Isolation of DNA by Salting-Out Technique

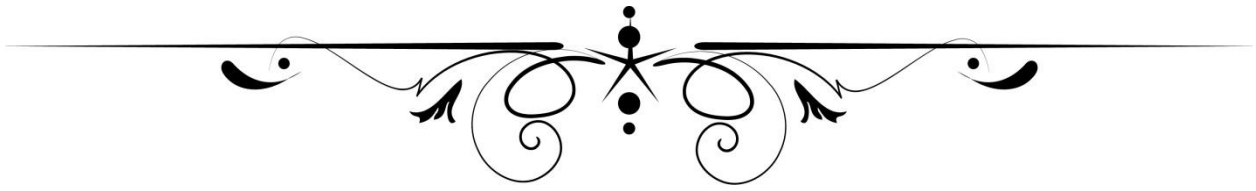
Material	Final Concentration	Use
RBC Lysis Buffer	0.15M - NH_4Cl 1Mm - NH_4HCO_3 0.1mM - EDTA (pH=7.4)	To lyse the white blood cells
SDS	20 %	To breakdown the cell membrane
Proteinase K	Proteinase K 5mg/ml	To hydrolyze the proteins
NaCl	5 mM	To precipitate DNA
Isopropyl alcohol	80%	To remove the salts
TE buffer		To dissolve the precipitated DNA

VI.7.2) Confirmation and quantification of the genomic DNA:

50µl of 10mM TE buffer taken as blank reading, DNA was quantified by taking 2µl of DNA in 48µl of 10mM TE buffer (pH8.0) by measuring UV –absorption of nucleic acid at 260nm using Perkin Elmer, Lambda 35 UV-VIS Spectrophotometer. The absorbance value was multiplied with 50 to get the concentration in µg/ml and purity of the DNA was calculated by taking A260/A280 ratio. Quality of the DNA was confirmed by running the 0.8 % agarose gel electrophoresis.



SECTION – A: PILOT STUDY



VII) SECTION – A: Pilot study

Mutational analysis of GJB2 gene by RFLP method.

A preliminary work was carried out with 22 subjects and 16 control samples to assess the prevalence and association of regularly found mutations p.W24X and c.235delC of GJB2 gene in congenital nonsyndromic hearing impairment patients from southern part of Karnataka.

VII.1) Polymerase chain reaction (PCR):

The genomic DNA, already extracted from the participants (cases and controls), were subjected to Polymerase Chain Reaction to GJB2 gene for mutational analysis. Specific primers (Table-8.) were used under the standardized PCR reaction mixture (Table-9) and conditions (Table-10) to amplify the regions of the GJB2 gene where p.W24X and c.235delC mutations are located.

Table-8: Primers for amplification of specific part of the GJB2 gene**.**

Mutation	Primers
p.W24X	1F: TCTTTTCCAGAGCAAACCGC 1R: GACACGAAGATCAGCTGCAG
c.235delC	1F: TGTGTGCATTCGTCTTTTCCAG 1R: GGTTGCCTCATCCCTCTCAT

Table-9: The composition of reaction mixture for PCR.

Components	Sample	Control
10mM Primer F	1 µl	1 µl
10mM Primer R	1 µl	1 µl
10x Buffer	5 µl	5 µl
2.5mM dNTP	5 µl	5 µl
Double distilled water	37 µl	37.6 µl
DNA (100ng)	0.6 µl	--------
Taq DNA polymerase	0.4 µl	0.4 µl
Total	50 µl	50 µl

Table-10: The standardized PCR condition.

1	96^0 C	5 min
2	96^0 C	15 sec
3	56.5^0 C	15 sec
4	72^0 C	1 min
5	96^0 C	5 min
6	Go to step 2 for 30 cycles	
7	72^0 C	10 min
8	Hold at 4^0 C	

VII.1.1) Purification of the PCR products:

PCR product were purified by mixing with sodium acetate and ethanol and kept for 30 min at -20^0 C then centrifuged at 12000 rpm, and the supernatant was discarded. This was once again washed with ethanol and solution centrifuged and supernatant was discarded and precipitation was allowed to air dry. After complete drying TE buffer was added to suspend the DNA.

VII.2) Agarose gels Electrophoresis:

The PCR products were subjected to agarose gel electrophoresis. 1% agarose gels prepared with 1X TAE buffer and 0.05% ethidium bromide. 3 µl of PCR product were placed on the gel and allowed to run in 1X TAE buffer at 80V for 30-45 minutes. DNA fragments were witnessed with the aid of ultraviolet light. Photographs were attained using the Molecular Imager Gel Doc^{TM} Image Lab Software.

VII.3) Restricted Digestion:

Purified PCR products were subjected to restriction digestion as shown in the table-11. Alu1 enzyme was employed for p.W24X mutation. For c.235delC mutations Apa1 enzyme was utilized. The reaction mixture kept for 4 hours of incubation at 37^{0}C and the reactions were terminated after 4 hours by inactivating the enzymes at 80^{0}C for 20 min. RFLP (Restriction Fragment Length polymorphism) analysis was carried out by Agarose gel electrophoresis of the digested products.

Table-11: Restriction digestion reaction mixture

Composition	**Restriction digestion - Cases**	**Restriction digestion - Controls**
PCR product (200ng)	8.6 µl	8.6 µl
10X Cut Smart Buffer	1µl	1µl
Water	-	0.4 µl
Enzyme	0.4 µl	-

VIII. 4) Results:

VII.4.1) Genomic DNA:

All the blood samples of the subjects and control group were subjected to DNA extraction as per the protocol detailed under materials and methods (section IV.7.1). The purity of the DNA were checked and all the samples (subjects and controls) exhibited purity in terms of absorption ratio 260nm to 280nm, invariably had the acceptable ratio between1.6 to 1.8. Agarose gel electrophoreses was carried out to see the quality of genomic DNA (figure.9).

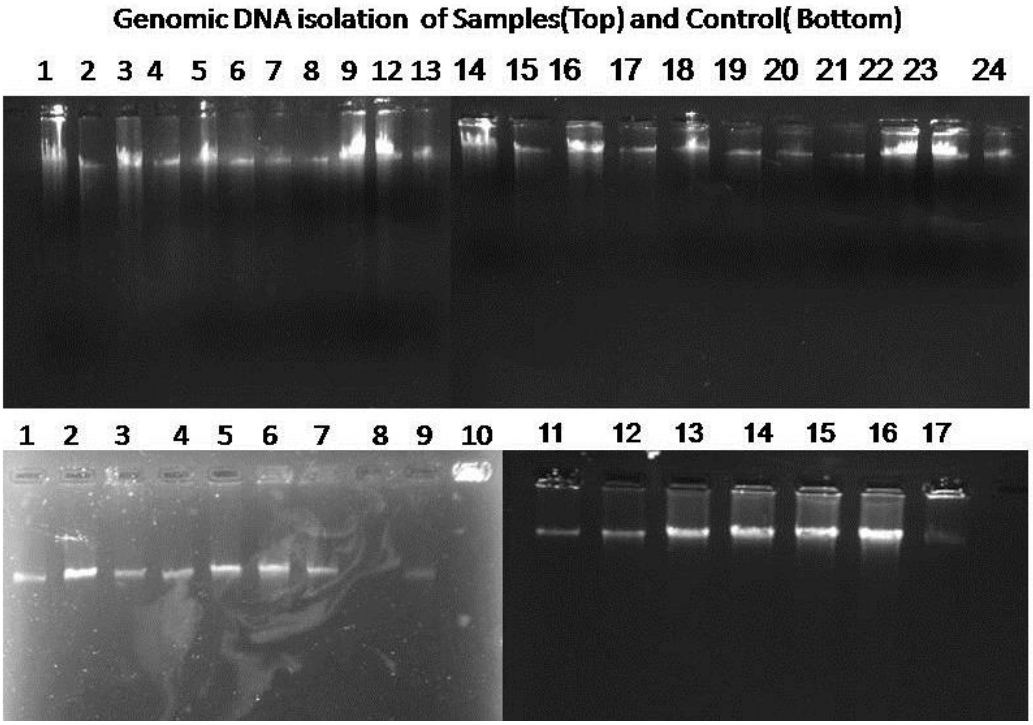


Figure.9: Genomic DNA of subjects (top) and control group (bottom).

VII.4.2) PCR results:

The PCR was carried out to amplify the GJB2 gene for the p.W24X mutation for all the samples as explained in methods. The expected PCR products were observed at 280bp (figure.10).

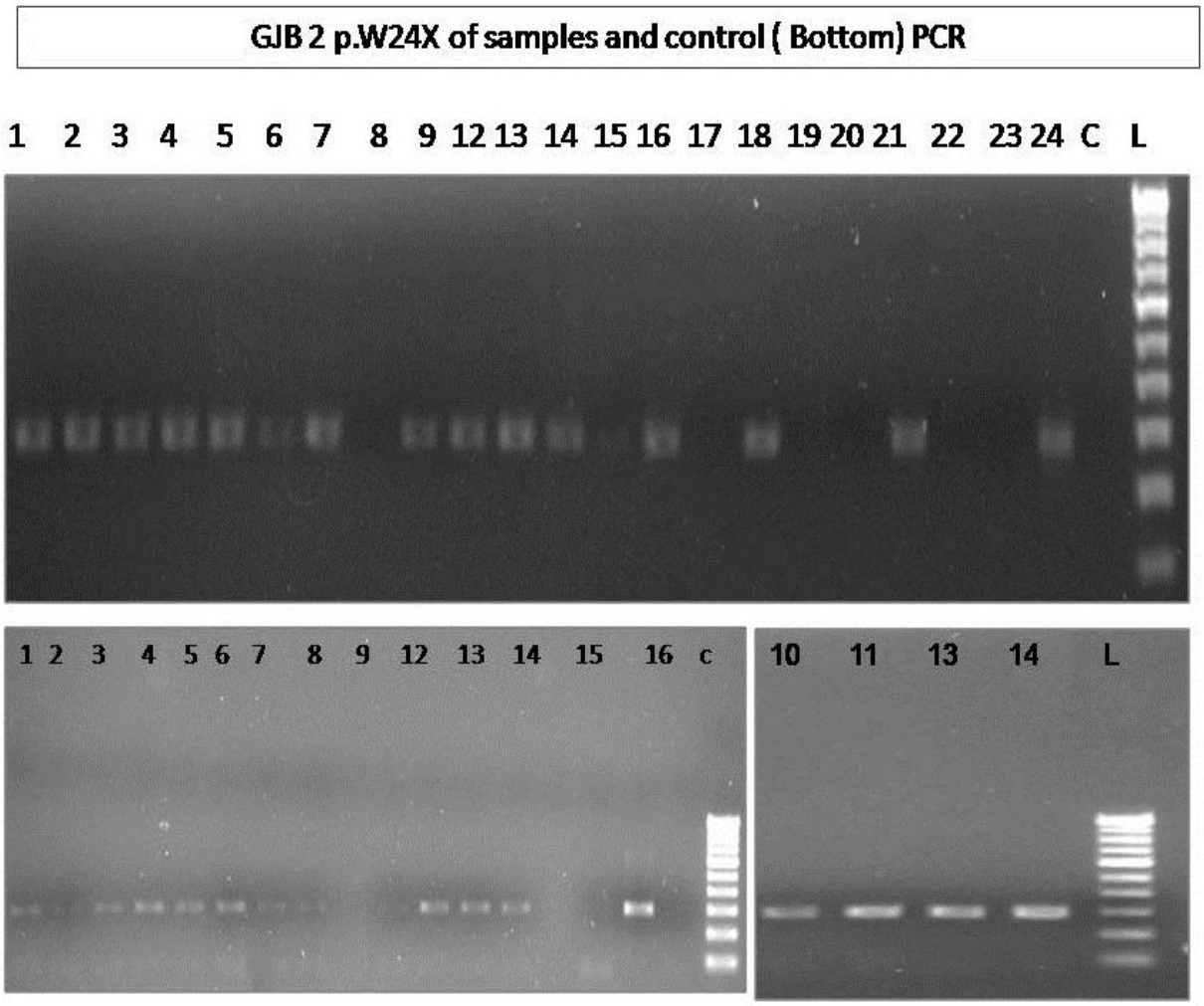


Figure.10: PCR products for p.W24X primers. The numbers indicates the samples and control groups respectively. L indicates 100bp ladder.

Similarly the PCR was carried out for the c.235delC mutation and expected PCR products were observed at 780bp (figure.11).

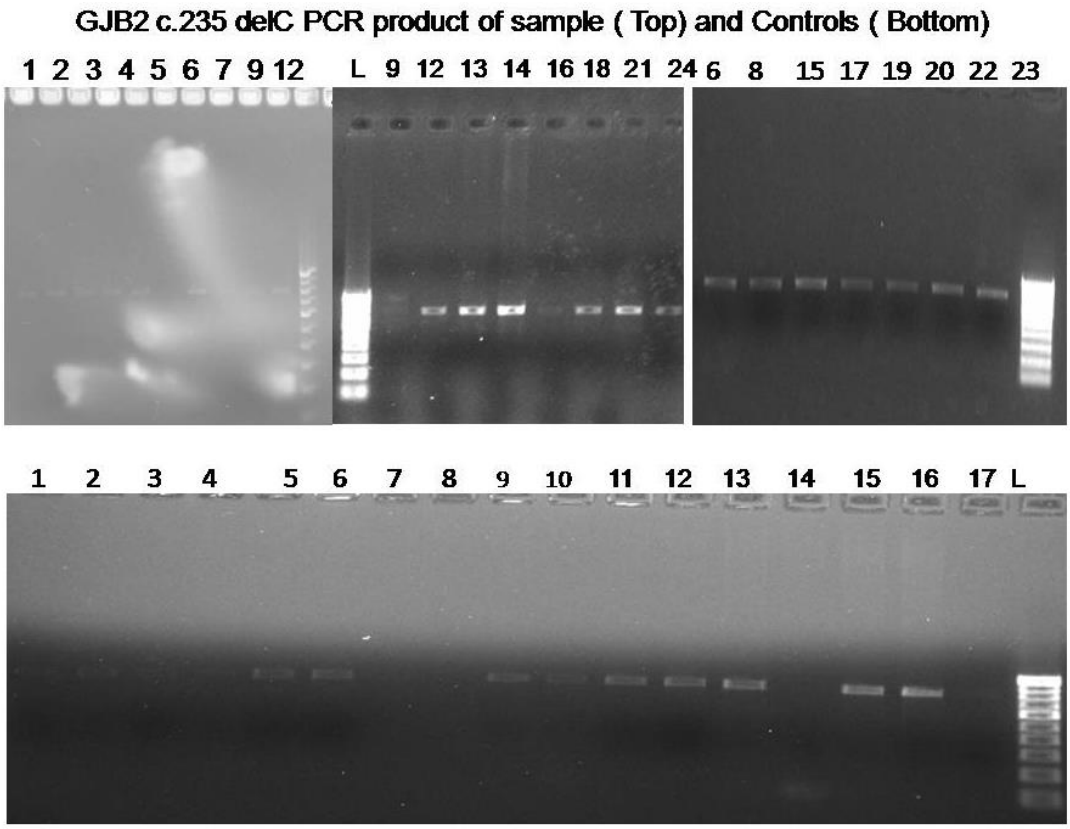
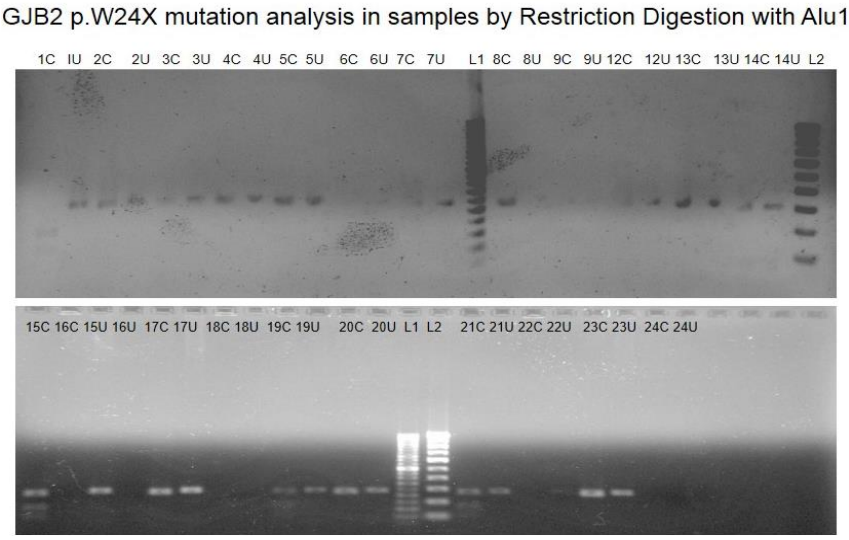


Figure.11: PCR products for c.235delC primers. The numbers indicates the samples and control groups respectively. L indicates 100bp ladder.

VII.4.3) RFLP results:

RFLP was performed for p.W24X mutation by using Alu1 restricted enzyme as detailed in methods (section IV.7.3). All of the samples' RFLP analyses reveal a band at 280 bp and controls that were typically anticipated. However, four samples no 1, 15, 19 and 21 among subjects two additional bands were noted. One at of 100 bp and another at 180 bp emphasizing the heterozygous mutation (figure.12).



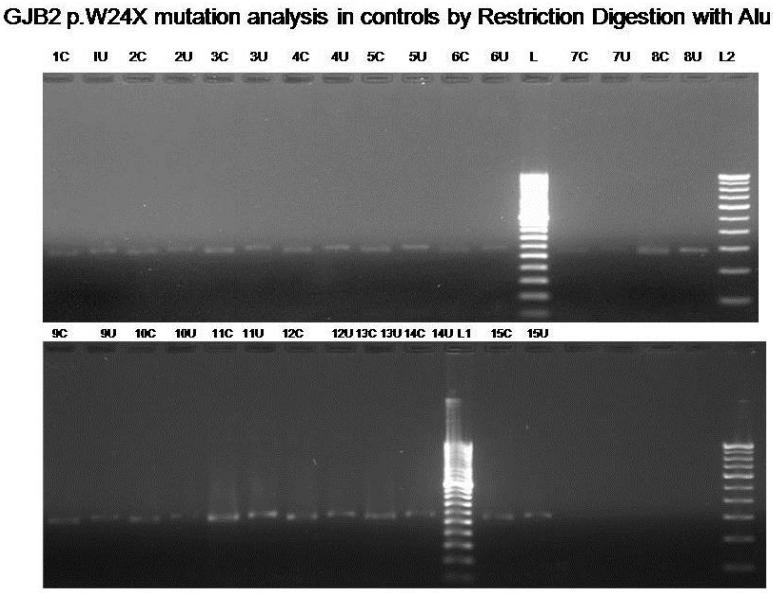


Figure.12: Restriction fragmentation by Alu1 for p.W24X mutation. Subjects (top two), controls (bottom two). The numbers indicates the samples and control respectively. L1 and L2 indicates ladder.

The PCR products were subjected to restriction digestion as described before (section IV.7.3) for identifying the presence of c.235delC mutation. The restriction enzyme identifies the digestion site and cleaves the DNA fragment in to two fragments of sizes of 300bps and 500bps. Due to mutation in the digestion site the restriction enzyme fails to identify the site. Leading a additional band in the gel picture suggesting undigested PCR products. Ten Subject samples that are number 8,13,14,15,16,17,20,22 and 23 exhibiting the additional band at 780bp along with a band at 300bp and 500bp. Having all the three bands in a same sample indicates the heterozygous condition for the mutation.(figure 13). The control samples show expected bands after digestion at 300 bp and 500bp as expected (figure.14).

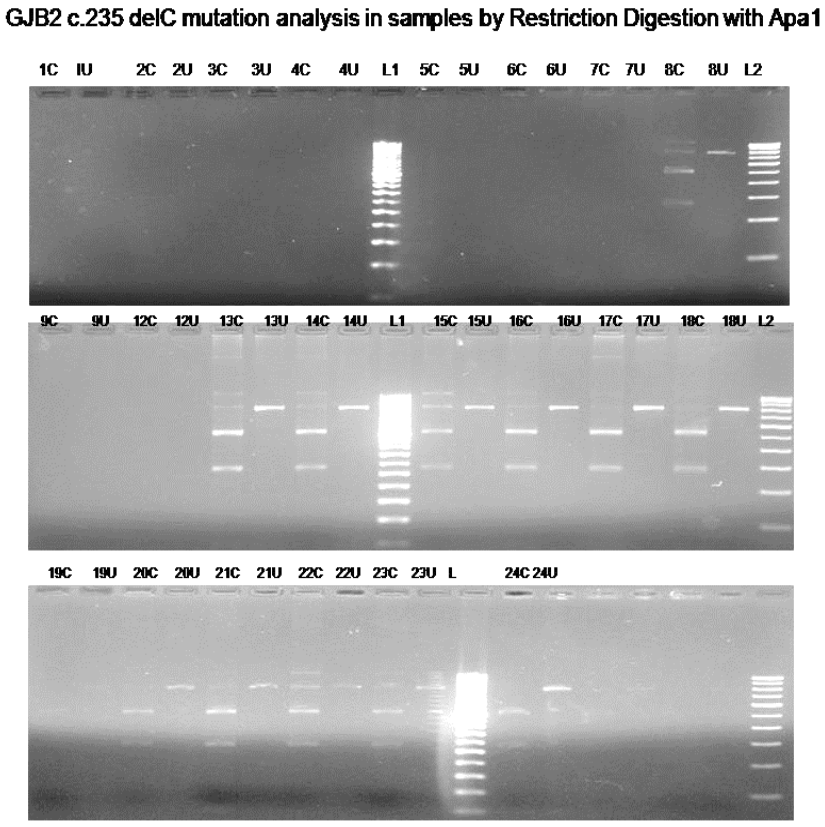


Figure.13: PCR products of c.235delC digested by Apa1 restriction enzyme. (Subject no- 8, 13, 14,15,16,17,20,21,22 and 23 showing mutations). The numbers with suffix C and U indicates RFLP and control conditions of samples. L1 and L2 indicates 50bp and 100bp ladder respectively.

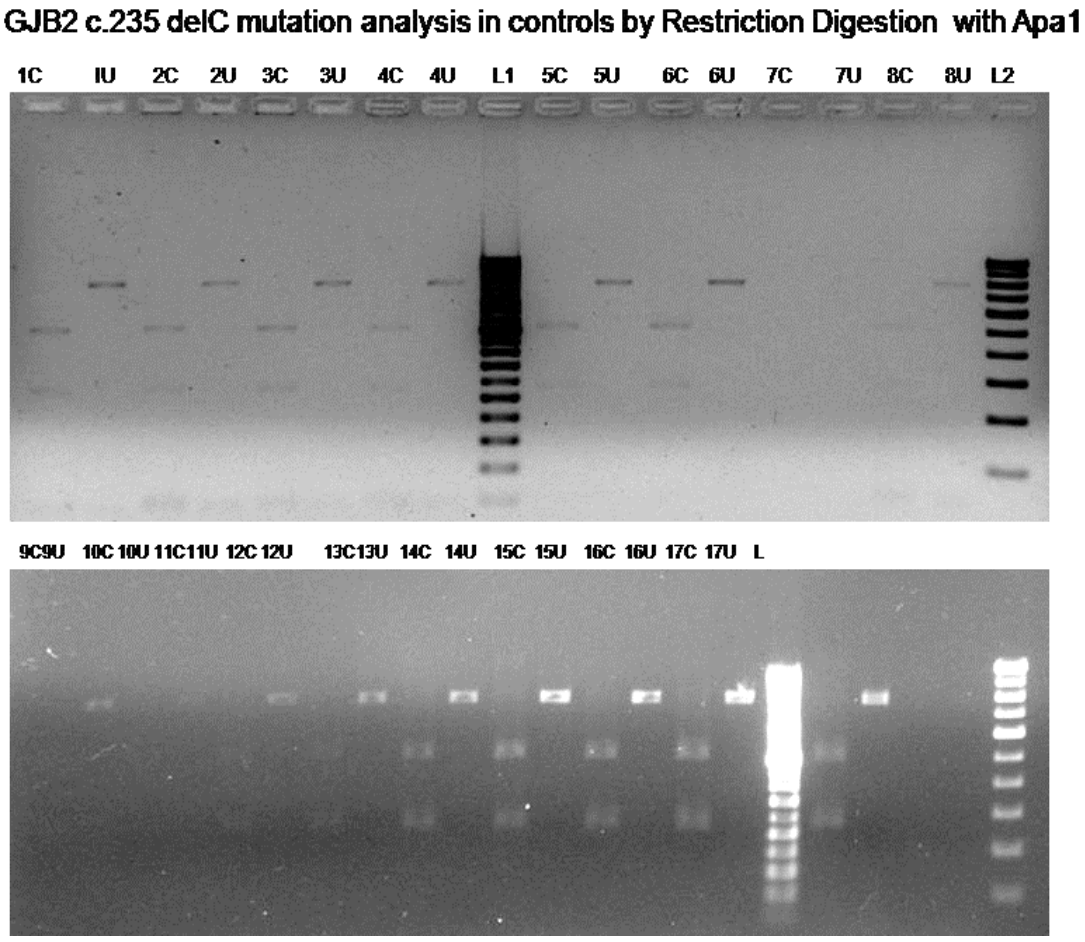


Figure.14: PCR products of c.235delC digested by Apa1 restriction enzyme.(CONTROLS) The suffix C and U indicates RFLP and control conditions respectively. The numbers with suffix C and U indicates RFLP and control conditions of samples. L1 and L2 indicates 50bp and 100bp ladder respectively.

VII. 5) Discussion:

To determine if a missense mutation is harmful, numerous criteria must be taken into account. Included among them are the location of the mutation, whether it occurs in a coding or noncoding area, which results in the synthesis of misbehaving or aberrant proteins. The functional effectiveness of a complex protein structure can be affected by a modification or aberrant amino acid at a critical place. It is exceedingly challenging to predict or assume the pathogenicity of particular missense mutations until they are proved by functional investigations since gap junction proteins are highly complicated in their protein structure and functions.

By focusing on the connexin26 mutations (p.W24x and c.235delC) and their prevalence in the Indian population, this study hopes to increase awareness of these conditions. Few research have been conducted to date in some parts of India, and none from the state of Karnataka, that attempt to identify the causal genes and their mutations and polymorphisms. There are more than 80 distinct GJB2 mutations known. However, among Caucasians, Ashkenazi Jews, Asians, and 167delT, 235delC, V37I account for 50% of mutant alleles, respectively.(58)

Several studies from the Indian subcontinent, including those from India, Pakistan, Bangladesh, and Sri Lanka, have reported the alterations in the coding region p.W24x that were the subject of this study.(26,59,60)

The GJB2 gene exhibits the p.W24X mutation, which is the 19.5% most prevalent mutation among those detected in the Indian population and likely a founder effect for HI. The prevalence of the same was estimated to 13.33% in North India,(58) and 18.1% in

south India.(36) In this present study prevalence of p.W24X mutation is 18.18%, and all the mutations were heterozygous.

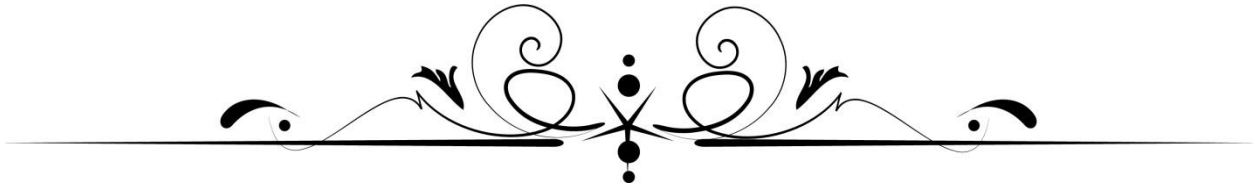
C.235delC was another frequently occurring mutation that was evaluated in GJB2. It is a frame shift mutation that was discovered using an Apa1-restricted enzyme. This enzyme recognizes the restriction site that is typically present in the gene's wild type and splits it into two fragments of 300 and 500 bases.

In the current investigation, 10 out of 22 (45.5%) participants discovered the presence of a band at 780 bp in addition to the 300 bp and 500 bp bands (Figure. 13). This suggests that a mutation has occurred, disappearing the restriction site, and preventing the restriction enzyme from recognizing it and cleaving the gene.

VII.6) Conclusion:

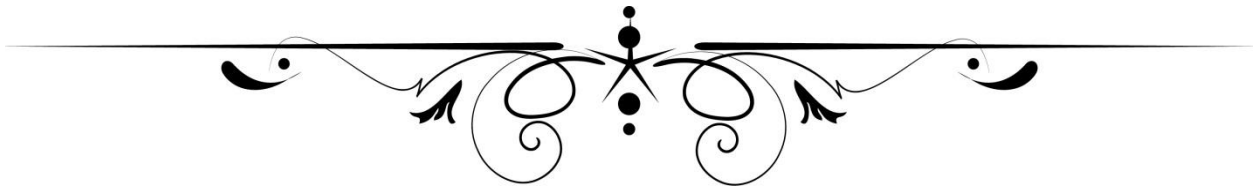
22 individuals with non-syndromic congenital hearing loss were chosen from the school for hearing impairment and underwent mutational analysis for the p.W24X and c.235delC type of GJB2 mutation using the RFLP method in our study. Out of 22 deaf cases, it was determined that 4 probands (18.18%) and 10 probands (45.45%) were heterozygous for the p.W24X and c.235delC types of mutations, respectively. No mutations were present in the control group, and both types of mutation carriers were present.

It is also important to emphasize that because the mutations found in this pilot study are heterozygous, they do not appear to be harmful. This suggests the possibility of additional mutations at the gene's unstudied region or as a result of mutations at other genes linked to hearing loss. It's possible that this substance or a number of mutations are responsible for the non-functioning heteromeric or heterotypic channels.



SECTION – B

MUTATIONAL SPECTRUM OF GJB2 GENE WITH ITS PROMOTER REGION



VIII) SECTION – B:

Mutational spectrum of GJB2 gene with its promoter region:

The previous studies including our preliminary work found lacking the explanation for the cause of deafness in those subjects having heterogeneity. It becomes necessary to find the explanation for the latter by the point of assuming variations in the non-exonic part and promoter region of GJB2 gene to include or exclude the involvement of GJB2 gene mutations in the congenital nonsyndromic hearing impairment.

There are many diseases, conditions and defects due to involvement of mutant promoter sites. This phenomenon has shown some possibilities of having mutations or SNPs that could probably answer for the heterozygous mutations in the coding region among the deaf population. Promoter region of the any gene is a key area which plays vital role on the regulation and expression of gene.(61) It is still complicated to understand even on several researches.(62) A study designed to comprehend the expression of connexins through the roles of coding region of connexins, factors responsible for transcription, and epigenetic regulation. They observed that they all are essential for the transcription but exact mechanism, interaction and concurrence between these regulatory mechanisms are yet to be cleared. (63)

The variation in the Connexin26 proteins were observed in a study conducted on the cell line of breast cancer. It was noticed that methylation status of the promoter regions has got some impact on expression of the gene.(64)

There were several instances where promoter region variants having either positive or negative influences on the expression of gene. Hence the present study assumed to put more light on possible mutation or polymorphism at gene regulating site

that is promoter region and estimate the prevalence of most commonly found mutations at coding region of GJB2 gene among south Indian population which may have a putative effect on the regulation of the gene.

The subjects and control population were educated about intent and need of the research in detail and were asked to consent voluntarily. 50 subjects and 48 controls were voluntarily enrolled for the study. The complete clinical and familial data of the participants were collected from parents and family members (Annexure-4). The objectives, design and protocol of the study was evaluated and approved by the institutional ethical Committee of Sri Devaraj Urs Medical College, Kolar, the constituent college of Sri Devaraj Urs Academy of Higher Education and Research. Karnataka.

VIII.1) DNA Isolation:

DNA was isolated by adopting Salting-Out Technique. Quantification of the genomic DNA carried out with Perkin Elmer Lambda 35 UV-VIS Spectrophotometer and Quality of the DNA obtained were assessed by 0.8 % agarose gel electrophoresis. (Described in section IV.7.1)

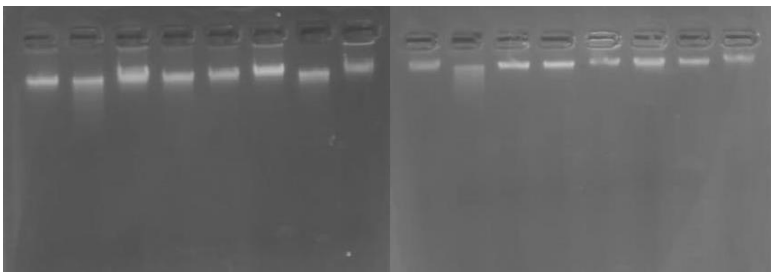


Figure.15: Gel doc image of genomic DNA

VII.2) PCR:

The PCR mix, temperature, and working conditions were standardized. The coding and promoter region of the GJB2 gene was amplified by using specific forward and reverse primers (Table-12 and 13)

Table-12: PCR primers to amplify basal promoter region of GJB2

No.	Oligo Name	Sequence (5`à 3`)	Tm (°C)	GC-Content
1	FORWARD	TGCTGTAGCAGGACAACCAG	59	55%
2	REVERSE	ACGTGGCATGTAGCAGTGAA	59	50%

Table-13: PCR primers to amplify exon region of GJB2 gene.

No.	Oligo Name	Sequence (5`à 3`)	Tm (°C)	GC-Content
1	FORWARD	GCATTCGTCTTTTCCAGAGC	59	55%
2	REVERSE	GGGAAATGCTAGCGACTGAG	59	50%

130 ng of Extracted DNA is used for amplification along with 10pM of each primer.

Composition of TAQ Master MIX:

PCR Amplification Mixture	Volume
DNA	3 ul
Primer	2 ul
dNTPs (2.5mM each)	4 ul
10X Taq DNA polymerase Assay Buffer	10 ul
Taq DNA Polymerase Enzyme (3U/ ml)	1 ul
Water	30 ul
Total reaction volume	50 ul

Cycling Conditions		
Initial Denaturation	3 minutes at 94°C	
Denaturation	1 minutes at 94°C	35 Cycles
Annealing	1 minutes 55°C	
Extension	2 minutes at 72°C	
Final Extension	7 minutes at 72°C	

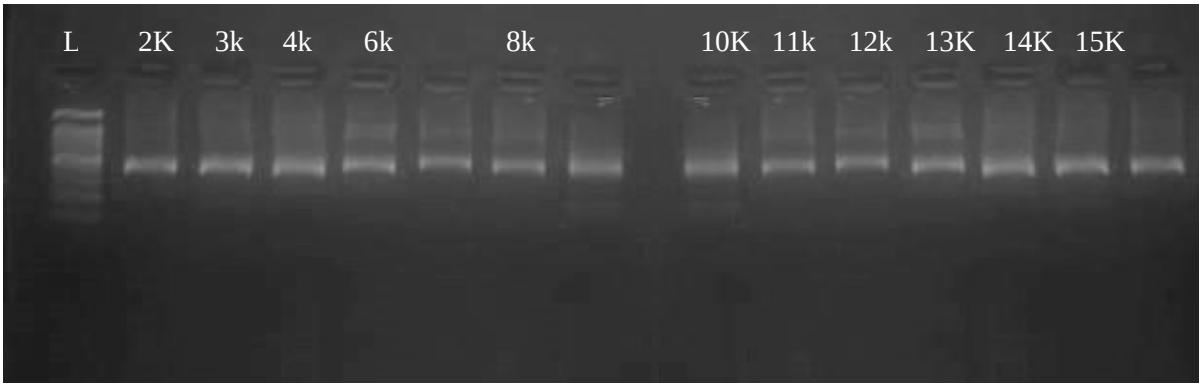


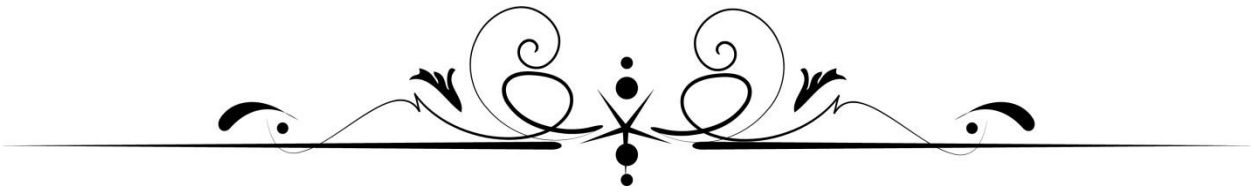
Figure.16: Gel doc image of PCR products.

VIII.3) Purification of PCR Products:

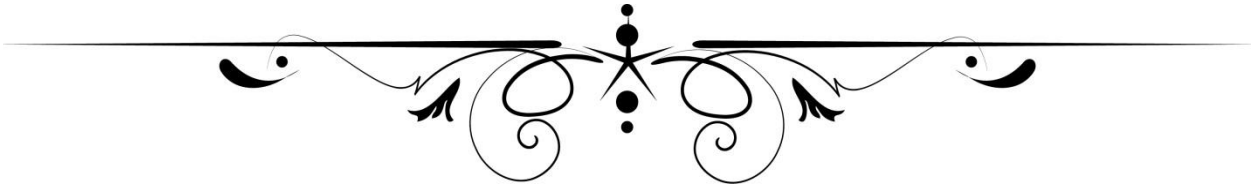
The PCR products were subjected to agarose gel electrophoresis. After completion the bands were sliced individually. 500ul of gel extraction buffer was added and incubated at 55°c-60°c in water bath. Later thoroughly mixed and transferred onto spin column and centrifuged for 2 minute, at 10000rpm. Repeated the step for two times and the supernatant was discarded each time. Again spun for 5 min at 10000 rpm (Drying step). Transferred the spin column into a fresh 1.5ml vial. Eluted according to the concentration (12-15ul). Centrifuged for 5 minute at 10000rpm. Re-eluted and spun for 6 minute at 10000rpm.

VIII.4) DNA sequencing:

Sequence specific primer pairs were designed to amplify the Promoter and Exon regions of *GJB2* gene with the help of Primer Quest tool, IDT DNA software.



RESULTS



The Sequencing mix Composition and PCR Conditions are as follows:

Big Dye Terminator Ready Reaction Mix	4µl
Template (100ng/ul)	1µl
Primer (10pmol/λ)	2µl
Milli Q Water	3µl
Sequencing Reaction mixture	10µl

PCR Conditions: (25 cycles)

Initial Denaturation	96°C for 5 min
Denaturation	96°C for 30 sec
Hybridization	55 °C for 30 sec
Elongation	60 °C for 1.30 min

DNA sequencing was performed with Big Dye Terminator v3.1 Cycle Sequencing Kit using ABI-3500 Genetic Analyzer. Chromatograms (Figure.18) were obtained for all the samples. Among which 4 subjects and 5 control's sequencing were shown bad sequencing quality due to idiopathic reasons.

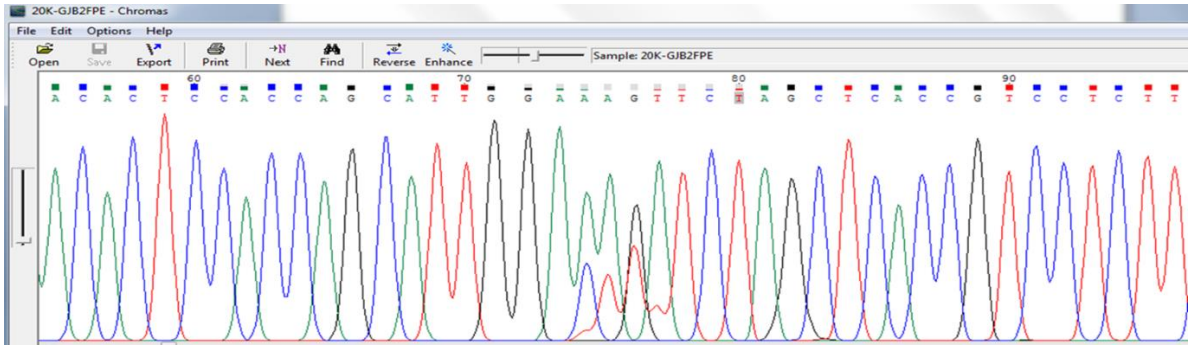


Figure.18: Sample Chromatogram picture

VIII.5) Results:

VIII.5.1) Demographic data analysis of subjects:

A) Male and female Gender ratio of the randomly selected subject participants involved in the research work is 64:36.

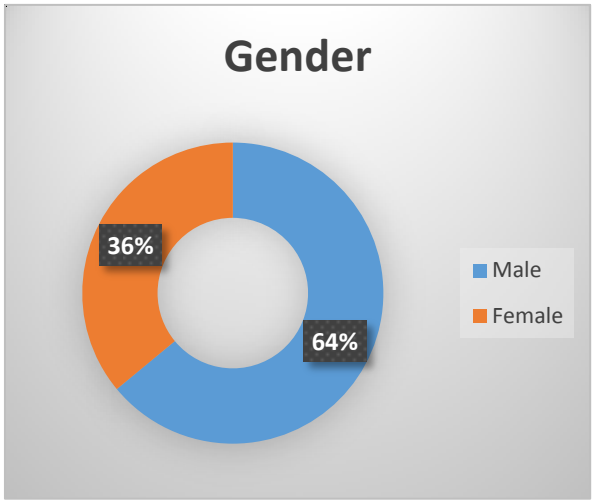


Figure.19: Gender ratio chart of the subjects.

B) Analysis of the affected individual's history reveals 76% of their parents got married to close blood relatives. This shows the frequency of practice of the consanguineous marriages at Indian population.

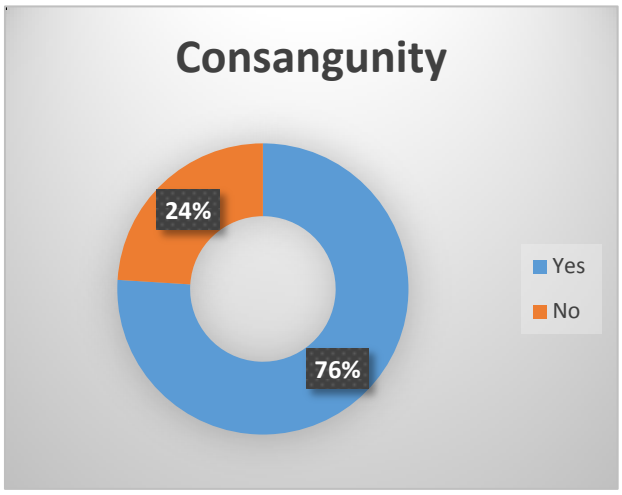


Figure.20: Proportions of consanguinity of the subjects' parents.

C) Congenital hearing impairment is an autosomal recessive disorder it is very much important and essential to assess the hearing condition of the affected participants. On analysis data shows 76% of the parents of affected are normal, 24% (14% father and 10% mother) of the parents were with hearing impairment and there were no participants having both of their parents are affected.

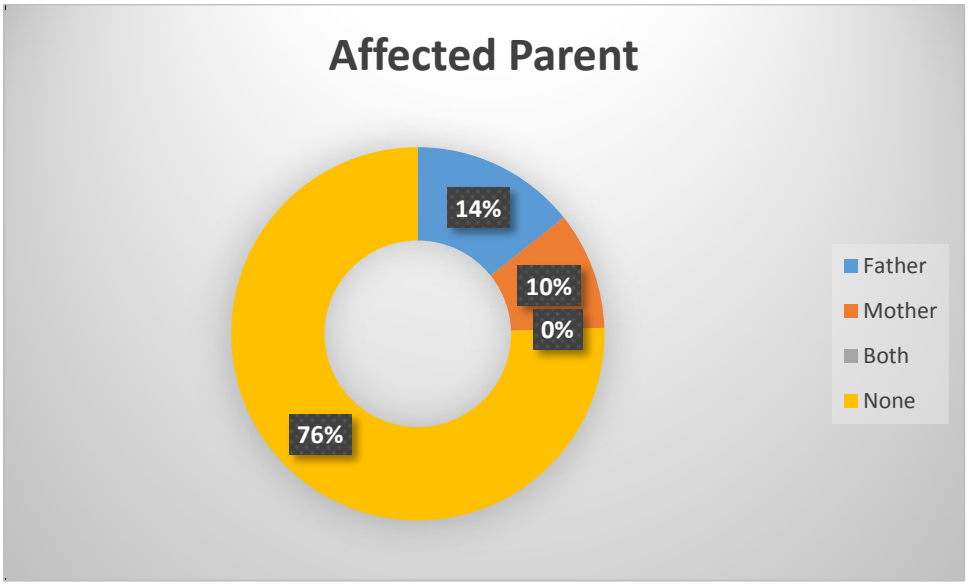


Figure.21: Proportions of affected parents of the subjects.

D) The pedigree charts (Figure.22) were prepared based on the information provided by the parents or guardian. This provide the vital information of pattern of inheritance of the hearing impairment. It was observed that 58% of the affected individual have at least one affected family member either from father's family or mother's family in few cases alleged history of hearing impairment at both parent's families.

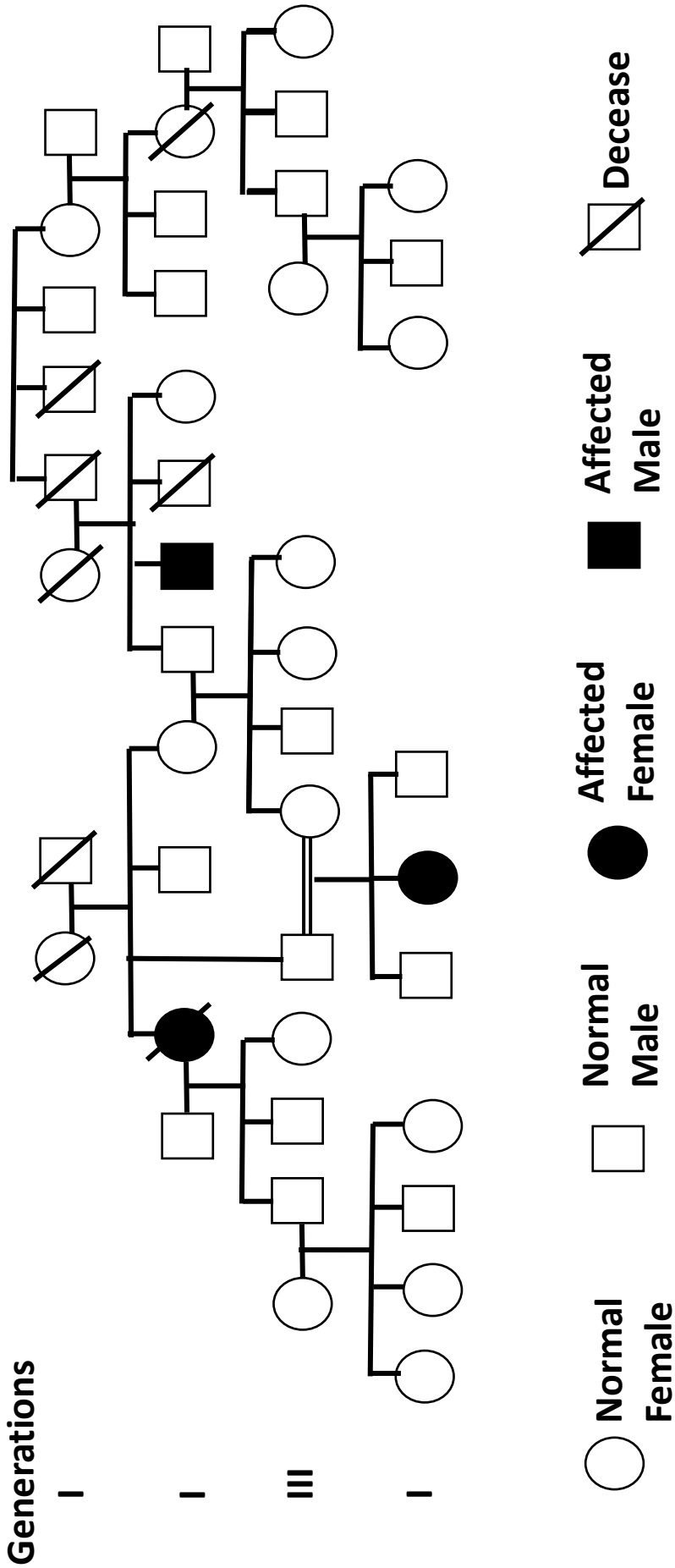


Figure.22: Pedigree chart sample

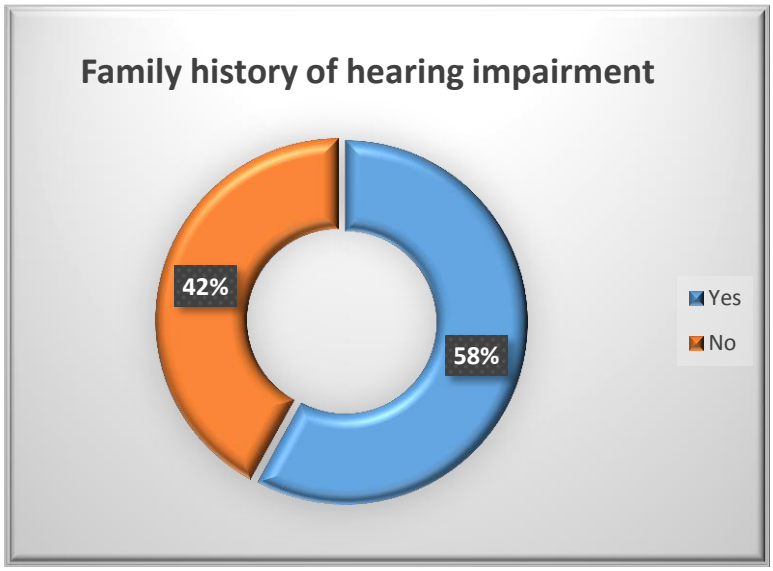


Figure.23: Proportions of alleged family history of the subjects.

VIII.5.2) SNPs / Mutations at Exon region:

Sequences of GJB2 gene coding region (42 cases and 43 controls) were obtained and by using the Clustal Omega software multiple gene sequences were aligned with reference gene sequence obtained from Genbank and analyzed for the base pair changes.

```
30K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGGGGACC  417
33K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  415
34K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  414
26K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCACATCGAAGGCTCCCTGTGGTGGACC  415
20K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  415
36K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  413
27K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  420
44K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  403
24K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  417
18K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  416
29K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  419
25K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  416
23K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  415
17K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  418
16K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  415
16H-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  413
15K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  417
14K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  416
19K-GJB2FPE.ab1          AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  415
9k-GJB2FPE.ab1           AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  416
NM_004004.6:179-859      AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  405
XM_011535049.2:207-887   AAGGACATCGAGGAGATCAAAACCCAGAAGGTCCGCATCGAAGGCTCCCTGTGGTGGACC  405
                         ********************************** ****************** *****
```

Figure.24: Image of Multiple alignment using CLUSTALW online tool

Individual Chromatograms of all the samples were screened for the nucleotide changes. Sequence analysis shown altogether five mutations p.W24X, p.R127H, p.W134G, p.V153I and p.613delT in the coding region of GJB2 gene, accounting for 38% mutation rate. Identified mutations were presented with both homozygous (21%) and heterozygous (17%) genotypes. Among the overall mutations identified proportion of the p.W24X mutation is 15.5%, p.R127H 7.1%, p.W134G, p.V153I and p.613delT are 2.4% each(Table:15). The individual mutations were subjected to dbSNP database to evaluate for any previously registered report, mutation type, and prediction of the effect.

Table-14: Mutations at exon region of GJB2 gene.

Sample ID	Mutations	Description	Nucleotide Change	Mutation prediction
20k, 24k, 25k, 27k, 30k, 34k, 40k, 45k.	p.W24X	Trp at 24 into Stop	c.71G>A	Pathogenic
11k, 18k, 26k, 43k.	p.R127H	Arg127 to His127	c.380G>A	Uncertain / Likely benign
30k	p.W134G	Trp134 to Gly134	c.400T>G	Novel mutation yet to verify the effect
29k, 40k	p.V153I	Val153 to Ile153	c.457G>R	Benign
46k	p.C213WfsX1	Cys213 to Trp213	c.639delT	Novel mutation yet to verify the effect

Table.15: Genotype and allelic frequencies of the mutations.

Mutations	Homozygous	Heterozygous	Allelic frequency
p.W24X	05	03	15.5%
p.R127H	02	02	7.1%
p.W134G	01	00	2.4%
p.V153I	00	02	2.4%
p.C213WfsX1	01	00	2.4%
Total mutations	09	07	29.7%

SNPs:

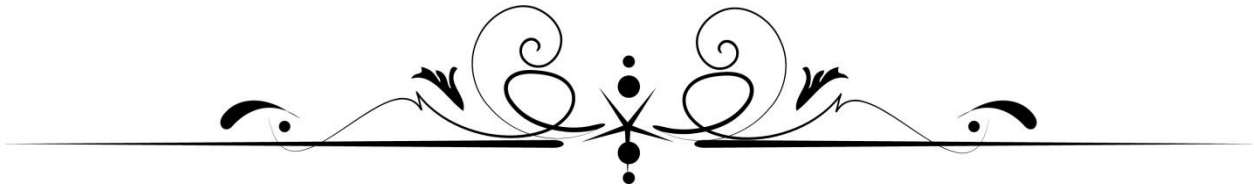
Apart from the above mentioned mutations there were no other nucleotide variants were not noticed at exon region of GJB2 gene in the cases and control samples. However, in the control sample number 23H there were two SNPs identified NM_004004.6:G95C and NM_004004.6:A160C.

VIII.5.3) SNPs / Mutations at Basal Promoter region:

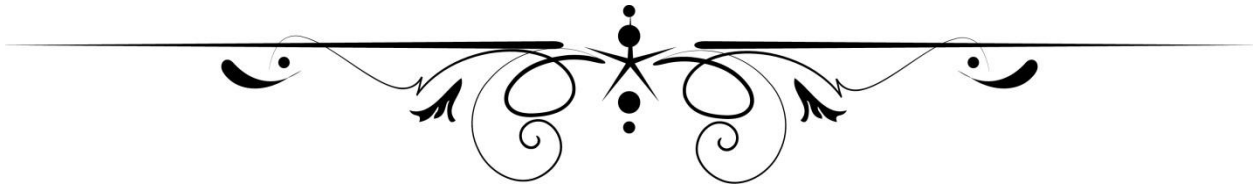
Multiple alignment of DNA sequences for the basal promoter region were analyzed for presence of any mutations / SNPs. There were five nucleotide variations were identified in the basal promoter region among cases and control samples (Table-16).

Table-16: Nucleotide variants at basal promotor region.

Sl. No.	Variants
1	NG_008358.1:7059 G>C
2	NG_008358.1:7170 A>G
3	NG_008358.1:7175 G>A
4	NG_008358.1:7220 G>R
5	NG_008358.1:7249 G>K



DISCUSSION



VIII. 6) Discussion:

The severity and prognosis of missense mutations rely on various factors, like position of the mutation causing abnormal proteins or malfunctioning proteins etc. However, the intricacy of the structure and function of gap junctions, makes it extremely difficult to forecast pathogenicity of some missense mutations.

Considering the role of promoter gene among any gene with respect to the regulation and its expression, this study is an effort to emphasize the knowledge on the connexin26 mutations and their prevalence in the Indian population and also to ascertain any DNA changes at promoter region of the gene. Till date, very limited information available with respect to Indian population as there are few studies and reports in some regions of India and none of them were included the population of Karnataka state. More than 200 different GJB2 mutations have been identified worldwide with specific prevalence rate in different populations.

With available current data among Indian population the studies shown several mutations and polymorphisms among which p.W24X, c.235delC, R127H, p.W77X, c.167delT etc. are being the major contenders. (31,36,42,45,52)

VIII.6.1) Identifying SNPs and Mutations:

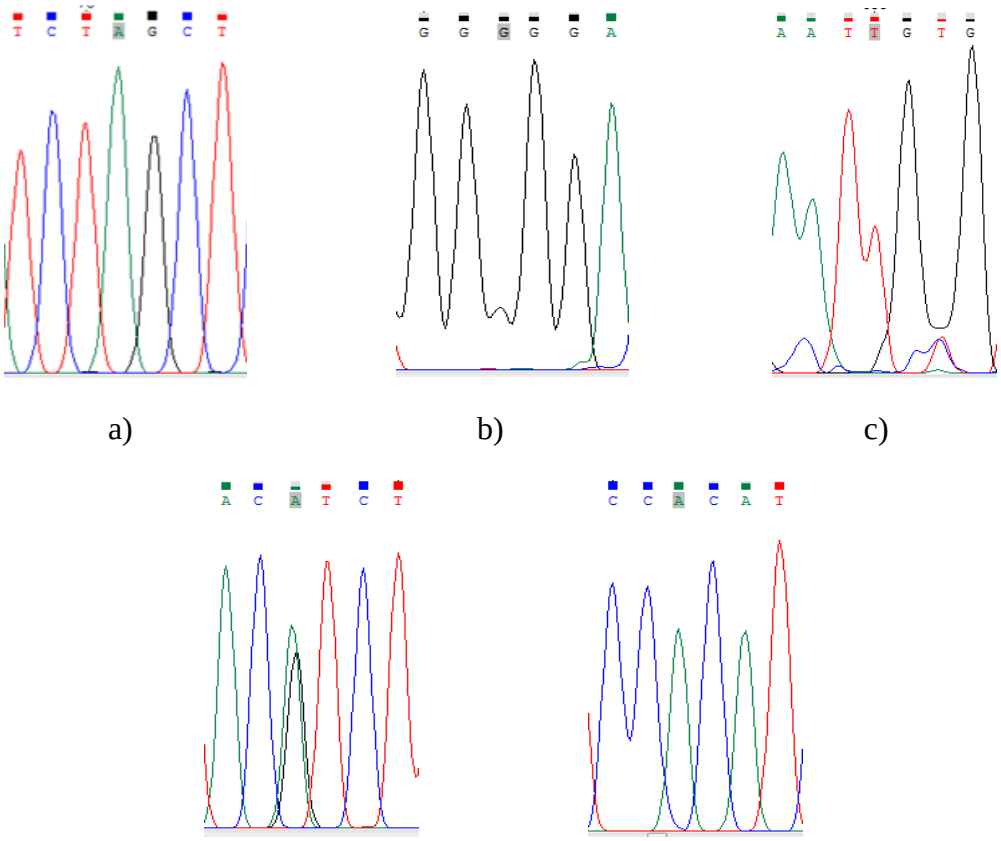
26173390 The genomic DNA is structured by long chains of nucleotides comprising Adenine, Guanine, Cytosine and Thiamine. A single nucleotide polymorphism is defined as a change of single nucleotide at a point in a given population. A change in the DNA sequence observed at specific point among more than 1% of the individuals is referred as SNPs. Such changes occurring at coding part of the functional gene, then the gene is said to be allele. The SNPs are also noticed at the noncoding

regions of the gene. However, the SNPs may or may not associated with the causative effect of certain disease or defects.

In contrary, a change in the DNA sequence due to insertion, deletion, duplication of single nucleotide or nucleotides fetching large change in the functional proteins leading to quantifiable adverse effect in a disease or defects, called as mutation. Mutations are estimated to be very rare at lesser than 1% of the given population.

VIII.6.2) SNPs / Mutations at exon region:

All the identified SNPs and mutations in the present research are discussed under separate headings.



a) b) c)

d) e)

Figure.24 : Sequence chromatograms showing mutations

a) p.W24X b) p.W134G c) p.C213WfsX1 d) p.V153I e) p.R127H

A) p.W24X :

It is a nonsense mutation caused due to nucleotide substitution of G>A at 71 position leading to change in the codon for amino acid tryptophan to stop codon. As discussed wide supra and from the table: 4 it is clearly evident that, there is wide ethnic diversity for the p.W24X mutation. It is most prevalent among the Asian population and is of nonsignificant among the populations of Belgian, Italian, Spanish, American, Syrian, Chinese and Magnolia.

Till date among all the mutations reported in Indian population p.W24X is most prevalent and allelic frequencies accounts for 19.5% and 6.5%(42) among Hyderabad region, 19.4% among the Maharastra and Tamil Nadu population,(45) 13.33% at three different states of India(31) and all hypothesized that the mutation has pathogenic effect for causing the HI.

In our study p.W24X accounts for 52% among all the mutations identified. Out of 42 subjects 5 cases were homozygous and 3 were heterozygous in condition. Allelic frequency of p.W24X is calculated to 15.5% which is at similar percentage compared to the previous study conducted in Indian population. The overall homozygous mutations ranges from 7- 17% among Indian population and in our study it is estimated to 11.9% which supports the previous research data available.

B) Novel mutations:

p.W134G and p.C213WfsX1 are the novel mutations identified in our research work which are not listed in the dbSNP database and also nowhere in the research articles published till date as per our knowledge. We have identified these homozygous mutation in two separate cases with profound hearing loss with congenital hearing impairment and

none among the control samples. The **p.W134G** is a the missense mutation described as c.400T>G leading to Trp134 to Gly134 and the **p.C213WfsX1** is a frameshift mutation described as a result of deletion of T at 213 codon leading to frame shift and truncation at 215 position leading to Cys213 to Trp213, denoted as c.639delT. As there are no information regarding pathogenicity of these mutations predicting its functional effect is difficult. These mutations will be submitted for the dbSNP database for the further functional analysis and authentication.

C) p.V153I:

This is a missense mutation and describes as Val153 to Ile153 denoted as c.457G>R. there are arguable findings and opinions regarding the functional implications of the mutation. This variant has been reported as both a benign polymorphism as well as a pathogenic variant.(34) Many studies shows p.V153I mutation has observed at almost equal frequency among cases and controls.(36) It was also identified in 5.6% of South Asian population by the Exome Aggregation Consortium(65) Functional studies have demonstrated no impact to synthesis and localization of the connexin 26 protein. It has also been reported in a homozygous state in 3 individuals with normal hearing as well as identified as a compound heterozygote in a normal hearing individual.(36)

In our study we have identified this homozygous mutation in one subject with profound hearing loss with congenital hearing impairment and none among the control samples. Considering occurrence of the mutation in both cases and controls this mutation is counted to be non-pathogenic.

D) p.R127H:

This is a missense mutation and describes as Arg172 to His172 denoted as c.380G>A. In our present study the allelic frequency is estimated to 27% among the mutations identified including two homozygous and two heterozygous conditions.

Given its frequent incidence rate of 17.5% in Asia and 9.5% in the Indian control group, this mutation is regarded as benign. In people with normal hearing, there were homozygous and compound heterozygous identifications.(16,45) Additionally, it has been documented 0.23% in general population in two significant population studies: the 1000 Genomes Project and the NHLBI Exome Sequencing Project.

It is not anticipated that this variation would have any clinical relevance. Despite some debate in the literature regarding the relevance of this variant, we have arrived at the conclusion that the Arg127His variant is not an explanation of hearing loss after examining the findings of many investigation.(28,36,42,58,66,67)

E) SNPs:

Apart from the above discussed mutations among subject samples the only in one control sample number 23H there were two SNPs identified.

NM_004004.6:G95C described as, at 95th position there is a change in a nucleotide Cytosine to Guanine in the DNA sequence. Normally the CCC a 32nd codon of the mRNA transcribed by GJB2 gene codes for Proline. Due to nucleotide change from Cytosine to Guanine at 95th position which is the 2nd nucleotide of the CCC codon now is read as CGC which codes for Arginine (Pro32Arg). Hence it is a missense SNP leading to misinterpretation of the codon.

Similarly, NM_004004.6:A160C, described as, at 106th position there is a change in a nucleotide Adenosine to Cytosine. Normally the AAC a 54th codon of the mRNA transcribed by GJB2 gene codes for Asparagine. Due to nucleotide change from Cytosine to Adenine at 106th position which is the 1st nucleotide of the AAC codon now is read as CAC which codes for Histidine (Asn54His). Hence it is a missense SNP leading to misinterpretation of the codon.

As in this study the functional implications of the nucleotide changes were not assessed, at this movement we consider this as SNP only.

VIII.6.3) SNPs / Mutations at Basal Promoter region:

Promoter region mutations has direct implications on gene expression and its functional efficacy of the protein functions. The promoter region analysis is not been a regular consideration in many gene functional analysis. Promoter region analysis is very complex and demands standardization of the procedures and protocols which makes a tough job for a researchers. But it comprehend of the role of promoter region among gene-promoter defects and diseases.

Around 1% of single base-pair change become pathogenic in genetic disease due to mutations at gene promoter regions, which hamper the normal pathway of gene activation and initiation of transcription. This will also lead to downregulation or upregulation of the amount of mRNA and protein.(53)

There are many diseases, defects caused by promoter mutations, which include – β-thalassemia(68), Bernard- Soulier syndrome(69), pyruvate kinase deficiency,(70) familial hypercholesterolemia, and hemophilia(71)

In 2007, Study conducted among Portuguese patients by T D Matos et.al. first time came up with the hypothesis of being mutational changes at promoter region and identified -3438 C to T mutation in the promoter region which could lead to loss of the GC box function, so impairing or abolishing the GJB2 transcription in the cochlea.(55)

Followed by which in 2016 A Pollak et.al. carried out similar study among Polish population to presence and prevalence of mutations in the promoter region and found no such mutation that is -3438 C to T at the promoter region of GJB2 gene. It was also emphasized that study may be conducted in larger samples to ascertain the pathogenicity of the mutation at promoter region.(56)

Considering the importance of promoter region the present study was taken to identify the possible mutations in gene regulating promoter sites. GJB2 gene including promoter region was sequenced and analyzed in 42 profound hearing loss and 43 normal hearing children. In the current population of Karnataka, India may be generalized as South Indian population the promoter region shown five SNPs which are described as follows.

A. NG_008358.1:7059 G>C:

This variant is described as change in a nucleotide (SNP) at 7059 position from Guanine to Cytosine in basal promotor region. It was observed only in 44K (Case Sample ID) in homozygous condition not in the controls

B. NG_008358.1:7170 A>G :

This variant is described as change in a nucleotide (SNP) at 7170 position from Adenine to Guanine in basal promotor region. It was noticed that except 17K and

14H (sample ID) all the subject and control samples this type of nucleotide variation in homozygous condition.

C. NG_008358.1:7175 G>A :

This variant is described as change in a nucleotide at 7175 position from Guanine Adenine to in basal promotor region. It was noticed that except 17K and 14H (sample ID) all the subject and control samples this type of nucleotide variation in homozygous and heterozygous conditions.

D. NG_008358.1:7220 G>R:

This variant is described as change in a nucleotide at 7220 position from Guanine Adenine to in basal promotor region. It was observed only in 3H control sample with heterozygous presentation. According to the IUPAC coding system having Adenosine and Guanine at the place of Guanine it is denoted as G>R.

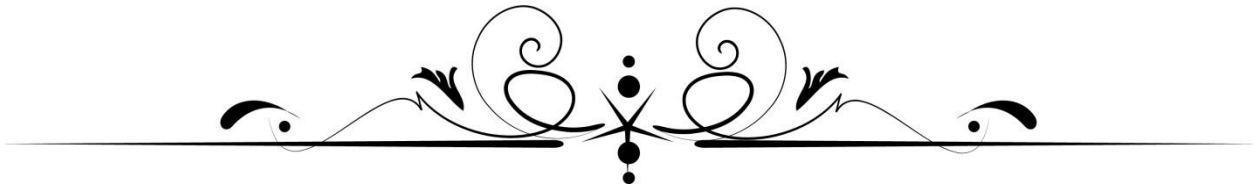
E. NG_008358.1:7249 G>K:

This variant is described as change in a nucleotide at 7249 position from Guanine to Thymine to in basal promotor region. Only control sample number 18H presents this SNP with heterozygous presentation. According to the IUPAC coding system having Thymine and Guanine at the place of Guanine it is denoted as G>K.

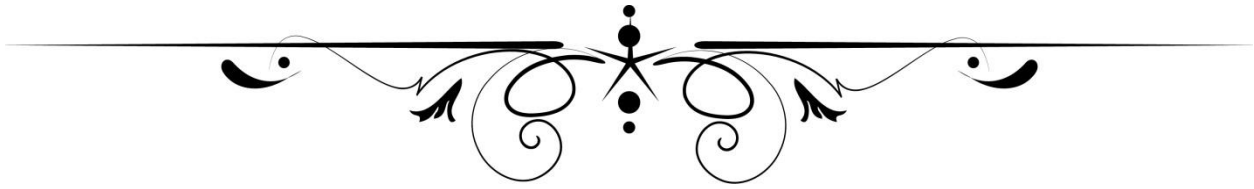
Among above described NSPs of basal promotor region NG_008358.1:7170 A>G and NG_008358.1:7175 G>A are document by the similar study conducted involving Northern part of Karnataka, India as mutations at regulatory region with 100% and 25% of frequency respectively among control samples.(72) In the present study these changes are noticed in both cases and control with whopping 98%. Even though the

researcher listed them in the mutation category the functional implications of this variations are yet to be established.

Rest three SNPs identified in this research work NG_008358.1:7059 G>C, NG_008358.1:7220 G>R and NG_008358.1:7249 G>K novel SNPs. Former SNP presented with homozygous and later two were heterozygous conditions.



CONCLUSION



VIII.7) CONCLUSION:

In this study 50 cases and 48 controls were enrolled and subjected to mutational analysis of exons and promoter region. By adopting RFLP and Sequencing methods the mutations and SNPs were identified in the exon region and also at basal promoter region.

Overall five different types of mutations were identified in the exon region of cases and two SNPs were identified in the control samples. The mutations p.W24X, p.R127H, p.W134G, and p.613delT exhibits homozygous conditions and p.R127H and p.V153I were heterozygous. The mutations p.R127H, p.W134G and p.V153I are of no clinical significance as they are found to non-pathogenic. The p.W24X mutation frequency is found to be pathogenic and is at higher levels compared earlier studies from Indian population. In our research work we present a novel mutation p.613delT in homozygous condition. The functional implications of the mutation is yet to be identified with functional correlation studies.

The study emphasizes on the possible association of the SNPs / mutations at the promoter region as there were five different nucleotide changes were identified in the basal promoter region. The functional implications of these changes would suggest the gene upregulation or down regulations of connexin26 protein by the GJB2 gene. The studies comprising quantification assessment of the connexin26 among these nucleotide variations would be beneficial in this cases to affirm the pathogenic or causative effect in the probands of nonsyndromic hearing impairment.

The greatest preference for identifying molecular abnormalities in patients for genotype, phenotype correlation, and illness management is to use more advanced and cutting-edge technology like next generation sequencing (NGS) approaches.(73)

There is significant evidences of enhanced speech perception among the recipients of cochlear implants and the prognosis of the hearing and acquiring speech skills is very much promising. Among mild to moderate affected children oral communication, sign language, visual cued speech, assistive listening and telecommunication devices definitely make the affected child's life better.(74)

In concurrent to genetic testing other diagnostic assessments would help in strategic approach to treatment. As in a study, MRI scans were used to examine the gross anatomical abnormalities in Heschle's gyrus, which is connected to the primary auditory cortex, in congenitally deaf children using sign language versus acquired spoken language skills. In comparison, there were statistically significant shifts in the volume of the brain tissue. This shows the importance of clinical and other diagnostic findings prior to the treatment plans.(75–77)

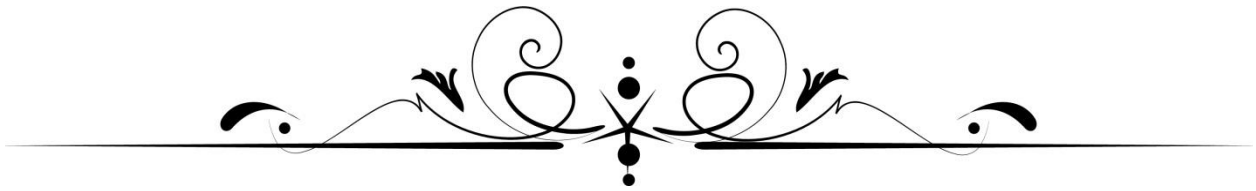
Based on the research outcomes of the animal experiments the human gene therapy may be foresighted not far away than the decade. There is new ray of hope generated by possible gene therapeutic measures among the hearing impairment cases. Given that fact that hearing impairment poses various gene involvement the gene therapy could be only possible for the monogenic hearing loss cases. Thus identifying the putative gene causing hearing impairment is need of the hour. It is also required to assess the conditions of other key factors like number of hair cells, spiral ganglion neurons and supporting cells. Among profound hearing loss cases the degenerative changes were observed in the cochlea.(78)

Currently there are few humans trials aimed at triggering the supporting cells to trans differentiate to hair cells so that there is increased number of functional hair cells.

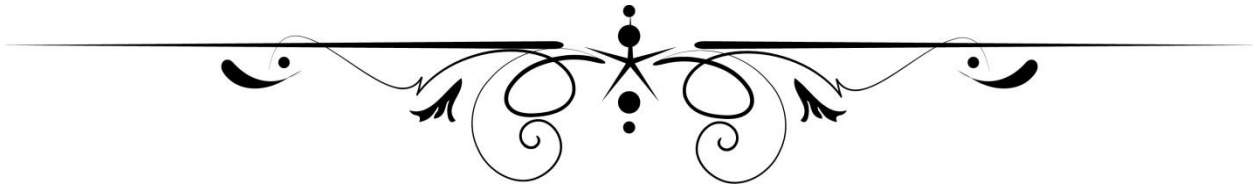
The controlled adenoviruses were employed to forceful expression of TFs in the supporting cells.(79)

On the positive note Innovations in the genetic engineering, induced pluripotent stem cell (iPSC), and organoid development bid bright future for regenerative therapy. (80)

In this direction it is very important and high time to pile up the significant data with respect to prime pathogenic mutations. It is also necessary to generate prevalence of the mutations which are population specific. The study's findings highlights the higher prevalence of pathogenic p.W24X mutation followed by homozygous novel mutations p.613delT and p.W134G. This draw a special attention on probability of co-implication of the autosomal dominant, codominant, or another connexin gene, indicating a multigene and multifactorial origin of the hearing loss that may be connected to the putative creation of heteromeric connexons or heterotypic channels.

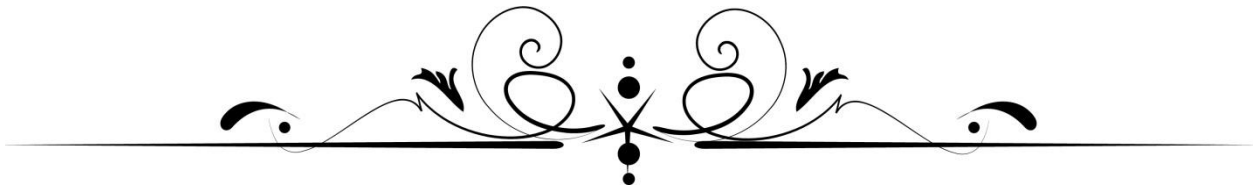


STRENGTH AND WEAKNESS

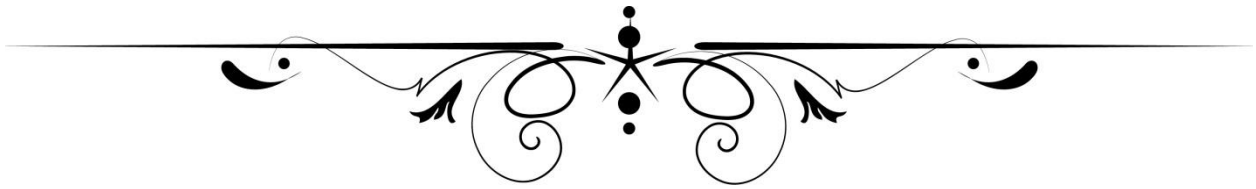


IX) STRENGTH AND WEAKNESS:

- Concept of considering the GJB2 gene promoter region for assessing the mutations is a unique work in the Indian population
- Promoter regions dose shows certain novel nucleotide variations making researchers to work in this section of the gene.
- Considering the sample size of present study it is suggested to carry out the further research with larger sample to make impactful and generalized statements with respect to prevalence and significance of the SNPs and Mutations identified.



REFERENCES



XI) REFERENCES:

1. Sheffield AM, Smith RJH. The Epidemiology of Deafness. Cold Spring Harb Perspect Med. 2019 Sep 3;9(9):a033258.

2. Kral A, O'Donoghue GM. Profound deafness in childhood. N Engl J Med. 2010 Oct 7;363(15):1438–50.

3. Fekete DM. Cell fate specification in the inner ear. Curr Opin Neurobiol. 1996 Aug;6(4):533–41.

4. Torres M, Giráldez F. The development of the vertebrate inner ear. Mech Dev. 1998 Feb;71(1–2):5–21.

5. Petit C, Levilliers J, Hardelin JP. Molecular genetics of hearing loss. Annu Rev Genet. 2001;35:589–646.

6. Kikuchi T, Kimura RS, Paul DL, Adams JC. Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. Anat Embryol (Berl). 1995 Feb;191(2):101–18.

7. Takeuchi S, Ando M, Kakigi A. Mechanism generating endocochlear potential: role played by intermediate cells in stria vascularis. Biophys J. 2000 Nov;79(5):2572–82.

8. Assad JA, Shepherd GM, Corey DP. Tip-link integrity and mechanical transduction in vertebrate hair cells. Neuron. 1991 Dec;7(6):985–94.

9. Markin VS, Hudspeth AJ. Gating-spring models of mechanoelectrical transduction by hair cells of the internal ear. Annu Rev Biophys Biomol Struct. 1995;24:59–83.

10. Ryan A, Dallos P. Effect of absence of cochlear outer hair cells on behavioural auditory threshold. Nature. 1975 Jan 3;253(5486):44–6.

11. Frank G, Hemmert W, Gummer AW. Limiting dynamics of high-frequency electromechanical transduction of outer hair cells. Proc Natl Acad Sci U S A. 1999 Apr 13;96(8):4420–5.

12. Frolenkov GI, Atzori M, Kalinec F, Mammano F, Kachar B. The membrane-based mechanism of cell motility in cochlear outer hair cells. Mol Biol Cell. 1998 Aug;9(8):1961–8.

13. Mehl AL, Thomson V. Newborn hearing screening: the great omission. Pediatrics. 1998 Jan;101(1):E4.

14. Mehl AL, Thomson V. The Colorado newborn hearing screening project, 1992-1999: on the threshold of effective population-based universal newborn hearing screening. Pediatrics. 2002 Jan;109(1):E7.

15. Morton NE. Genetic epidemiology of hearing impairment. Ann N Y Acad Sci. 1991;630:16–31.

16. Cryns K, Orzan E, Murgia A, Huygen PLM, Moreno F, del Castillo I, et al. A genotype-phenotype correlation for GJB2 (connexin 26) deafness. J Med Genet. 2004 Mar;41(3):147–54.

17. Bitner-Glindzicz M. Hereditary deafness and phenotyping in humans. Br Med Bull. 2002;63:73–94.

18. Shearer AE, Hildebrand MS, Schaefer AM, Smith RJ. Genetic Hearing Loss Overview. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, Gripp KW,

et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993 [cited 2023 Jun 6]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1434/

19. Keats BJ, Nouri N, Huang JM, Money M, Webster DB, Berlin CI. The deafness locus (dn) maps to mouse chromosome 19. Mamm Genome. 1995 Jan;6(1):8–10.

20. Kikuchi T, Kimura RS, Paul DL, Takasaka T, Adams JC. Gap junction systems in the mammalian cochlea. Brain Res Brain Res Rev. 2000 Apr;32(1):163–6.

21. Bruzzone R, White TW, Paul DL. Connections with connexins: the molecular basis of direct intercellular signaling. Eur J Biochem. 1996 May 15;238(1):1–27.

22. Sáez JC, Nairn AC, Czernik AJ, Spray DC, Hertzberg EL, Greengard P, et al. Phosphorylation of connexin 32, a hepatocyte gap-junction protein, by cAMP-dependent protein kinase, protein kinase C and Ca2+/calmodulin-dependent protein kinase II. Eur J Biochem. 1990 Sep 11;192(2):263–73.

23. Rubin JB, Verselis VK, Bennett MV, Bargiello TA. Molecular analysis of voltage dependence of heterotypic gap junctions formed by connexins 26 and 32. Biophys J. 1992 Apr;62(1):183–93; discussion 193-195.

24. Gabriel HD, Jung D, Bützler C, Temme A, Traub O, Winterhager E, et al. Transplacental uptake of glucose is decreased in embryonic lethal connexin26-deficient mice. J Cell Biol. 1998 Mar 23;140(6):1453–61.

25. Guilford P, Ben Arab S, Blanchard S, Levilliers J, Weissenbach J, Belkahia A, et al. A non-syndrome form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. Nat Genet. 1994 Jan;6(1):24–8.

26. Kelsell DP, Di WL, Houseman MJ. Connexin mutations in skin disease and hearing loss. Am J Hum Genet. 2001 Mar;68(3):559–68.

27. Gasparini P, Estivill X, Volpini V, Totaro A, Castellvi-Bel S, Govea N, et al. Linkage of DFNB1 to non-syndromic neurosensory autosomal-recessive deafness in Mediterranean families. Eur J Hum Genet. 1997;5(2):83–8.

28. Estivill X, Fortina P, Surrey S, Rabionet R, Melchionda S, D'Agruma L, et al. Connexin-26 mutations in sporadic and inherited sensorineural deafness. Lancet. 1998 Feb 7;351(9100):394–8.

29. Löffler J, Nekahm D, Hirst-Stadlmann A, Günther B, Menzel HJ, Utermann G, et al. Sensorineural hearing loss and the incidence of Cx26 mutations in Austria. Eur J Hum Genet. 2001 Mar;9(3):226–30.

30. Kudo T, Ikeda K, Kure S, Matsubara Y, Oshima T, Watanabe K i, et al. Novel mutations in the connexin 26 gene (GJB2) responsible for childhood deafness in the Japanese population. Am J Med Genet. 2000 Jan 17;90(2):141–5.

31. Ramchander PV, Nandur VU, Dwarakanath K, Vishnupriya S, Padma T. Prevalence of Cx26 (GJB2) Gene Mutations Causing Recessive Nonsyndromic Hearing Impairment in India. International Journal of Human Genetics. 2005 Dec 1;5(4):241–6.

32. Dai P, Yu F, Han B, Liu X, Wang G, Li Q, et al. GJB2 mutation spectrum in 2,063 Chinese patients with nonsyndromic hearing impairment. J Transl Med. 2009 Apr 14;7:26.

33. Naghavi A, Nishimura C, Kahrizi K, Riazalhosseini Y, Bazazzadegan N, Mohseni M, et al. GJB2 mutations in Baluchi population. J Genet. 2008 Aug;87(2):195–7.

34. Snoeckx RL, Huygen PLM, Feldmann D, Marlin S, Denoyelle F, Waligora J, et al. GJB2 mutations and degree of hearing loss: a multicenter study. Am J Hum Genet. 2005 Dec;77(6):945–57.

35. Naghavi A, Nishimura C, Kahrizi K, Riazalhosseini Y, Bazazzadegan N, Mohseni M, et al. GJB2 mutations in Baluchi population. J Genet. 2008 Aug;87(2):195–7.

36. RamShankar M, Girirajan S, Dagan O, Ravi Shankar HM, Jalvi R, Rangasayee R, et al. Contribution of connexin26 (GJB2) mutations and founder effect to non-syndromic hearing loss in India. J Med Genet. 2003 May;40(5):e68.

37. Park HJ, Hahn SH, Chun YM, Park K, Kim HN. Connexin26 mutations associated with nonsyndromic hearing loss. Laryngoscope. 2000 Sep;110(9):1535–8.

38. Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ. Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet. 2000 Jan;37(1):41–3.

39. Ohtsuka A, Yuge I, Kimura S, Namba A, Abe S, Van Laer L, et al. GJB2 deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation. Hum Genet. 2003 Apr;112(4):329–33.

40. Batissoco AC, Abreu-Silva RS, Braga MCC, Lezirovitz K, Della-Rosa V, Alfredo T, et al. Prevalence of GJB2 (connexin-26) and GJB6 (connexin-30) mutations in a cohort of 300 Brazilian hearing-impaired individuals: implications for diagnosis and genetic counseling. Ear Hear. 2009 Feb;30(1):1–7.

41. Mani RS, Ganapathy A, Jalvi R, Srikumari Srisailapathy CR, Malhotra V, Chadha S, et al. Functional consequences of novel connexin 26 mutations associated with hereditary hearing loss. Eur J Hum Genet. 2009 Apr;17(4):502–9.

42. Padma G, Ramchander PV, Nandur UV, Padma T. GJB2 and GJB6 gene mutations found in Indian probands with congenital hearing impairment. J Genet. 2009 Dec;88(3):267–72.

43. Tekin M, Xia XJ, Erdenetungalag R, Cengiz FB, White TW, Radnaabazar J, et al. GJB2 mutations in Mongolia: complex alleles, low frequency, and reduced fitness of the deaf. Ann Hum Genet. 2010 Mar;74(2):155–64.

44. Al-Achkar W, Moassass F, Al-Halabi B, Al-Ablog A. Mutations of the Connexin 26 gene in families with non-syndromic hearing loss. Mol Med Rep. 2011;4(2):331–5.

45. Godbole K, Hemavathi J, Vaid N, Pandit AN, Sandeep MN, Chandak GR. Low prevalence of GJB2 mutations in non-syndromic hearing loss in Western India. Indian J Otolaryngol Head Neck Surg. 2010 Jan;62(1):60–3.

46. Masmoudi S, Elgaied-Boulila A, Kassab I, Ben Arab S, Blanchard S, Bouzouita JE, et al. Determination of the frequency of connexin26 mutations in inherited sensorineural deafness and carrier rates in the Tunisian population using DGGE. J Med Genet. 2000 Nov;37(11):E39.

47. Minárik G, Ferák V, Feráková E, Ficek A, Poláková H, Kádasi L. High frequency of GJB2 mutation W24X among Slovak Romany (Gypsy) patients with non-syndromic hearing loss (NSHL). Gen Physiol Biophys. 2003 Dec;22(4):549–56.

48. Posukh O, Pallares-Ruiz N, Tadinova V, Osipova L, Claustres M, Roux AF. First molecular screening of deafness in the Altai Republic population. BMC Med Genet. 2005 Mar 24;6:12.

49. Danilenko N, Merkulava E, Siniauskaya M, Olejnik O, Levaya-Smaliak A, Kushniarevich A, et al. Spectrum of genetic changes in patients with non-syndromic hearing impairment and extremely high carrier frequency of 35delG GJB2 mutation in Belarus. PLoS One. 2012;7(5):e36354.

50. Schimmenti LA, Martinez A, Telatar M, Lai CH, Shapiro N, Fox M, et al. Infant hearing loss and connexin testing in a diverse population. Genet Med. 2008 Jul;10(7):517–24.

51. Chen D, Chen X, Cao K, Zuo J, Jin X, Wei C, et al. High prevalence of the connexin 26 (GJB2) mutation in Chinese cochlear implant recipients. ORL J Otorhinolaryngol Relat Spec. 2009;71(4):212–5.

52. Maheshwari M, Vijaya R, Ghosh M, Shastri S, Kabra M, Menon PSN. Screening of families with autosomal recessive non-syndromic hearing impairment (ARNSHI) for mutations in GJB2 gene: Indian scenario. Am J Med Genet A. 2003 Jul 15;120A(2):180–4.

53. Cooper DN. Human gene mutation in pathology and evolution. J Inherit Metab Dis. 2002 May;25(3):157–82.

54. Maston GA, Evans SK, Green MR. Transcriptional regulatory elements in the human genome. Annu Rev Genomics Hum Genet. 2006;7:29–59.

55. Matos TD, Caria H, Simões-Teixeira H, Aasen T, Nickel R, Jagger DJ, et al. A novel hearing-loss-related mutation occurring in the GJB2 basal promoter. J Med Genet. 2007 Nov;44(11):721–5.

56. Pollak A, Mueller-Malesinska M, Skórka A, Kostrzewa G, Ołdak M, Korniszewski L, et al. GJB2 and hearing impairment: promoter defects do not explain the excess of monoallelic mutations. J Med Genet. 2008 Sep;45(9):607–8.

57. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988 Feb 11;16(3):1215.

58. Dahl HHM, Tobin SE, Poulakis Z, Rickards FW, Xu X, Gillam L, et al. The contribution of GJB2 mutations to slight or mild hearing loss in Australian elementary school children. J Med Genet. 2006 Nov;43(11):850–5.

59. Scott DA, Kraft ML, Carmi R, Ramesh A, Elbedour K, Yairi Y, et al. Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss. Hum Mutat. 1998;11(5):387–94.

60. Rickard S, Kelsell DP, Sirimana T, Rajput K, MacArdle B, Bitner-Glindzicz M. Recurrent mutations in the deafness gene GJB2 (connexin 26) in British Asian families. J Med Genet. 2001 Aug;38(8):530–3.

61. Jacob F, Monod J. Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol. 1961 Jun;3:318–56.

62. Lee TI, Young RA. Transcriptional regulation and its misregulation in disease. Cell. 2013 Mar 14;152(6):1237–51.

63. Oyamada M, Takebe K, Oyamada Y. Regulation of connexin expression by transcription factors and epigenetic mechanisms. Biochim Biophys Acta. 2013 Jan;1828(1):118–33.

64. Tan L wai, Bianco T, Dobrovic A. Variable promoter region CpG island methylation of the putative tumor suppressor gene Connexin 26 in breast cancer. Carcinogenesis. 2002 Feb;23(2):231–6.

65. Seeman P, Malíková M, Rasková D, Bendová O, Groh D, Kubálková M, et al. Spectrum and frequencies of mutations in the GJB2 (Cx26) gene among 156 Czech patients with pre-lingual deafness. Clin Genet. 2004 Aug;66(2):152–7.

66. Marlin S, Garabédian EN, Roger G, Moatti L, Matha N, Lewin P, et al. Connexin 26 gene mutations in congenitally deaf children: pitfalls for genetic counseling. Arch Otolaryngol Head Neck Surg. 2001 Aug;127(8):927–33.

67. Chaleshtori MH, Farrokhi E, Shahrani M, Kheiri S, Dolati M, Rad LH, et al. High carrier frequency of the GJB2 mutation (35delG) in the north of Iran. Int J Pediatr Otorhinolaryngol. 2007 Jun;71(6):863–7.

68. Hardison RC, Chui DHK, Giardine B, Riemer C, Patrinos GP, Anagnou N, et al. HbVar: A relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. Hum Mutat. 2002 Mar;19(3):225–33.

69. Ludlow LB, Schick BP, Budarf ML, Driscoll DA, Zackai EH, Cohen A, et al. Identification of a mutation in a GATA binding site of the platelet glycoprotein Ibbeta promoter resulting in the Bernard-Soulier syndrome. J Biol Chem. 1996 Sep 6;271(36):22076–80.

70. van Wijk R, van Solinge WW, Nerlov C, Beutler E, Gelbart T, Rijksen G, et al. Disruption of a novel regulatory element in the erythroid-specific promoter of the human PKLR gene causes severe pyruvate kinase deficiency. Blood. 2003 Feb 15;101(4):1596–602.

71. Crossley M, Brownlee GG. Disruption of a C/EBP binding site in the factor IX promoter is associated with haemophilia B. Nature. 1990 May 31;345(6274):444–6.

72. Hegde S, Hegde R, Kulkarni SS, Das KK, Gai PB, Bulgouda R. Molecular alteration in the Gap Junction Beta 2 (GJB2) gene associated with non-syndromic sensorineural hearing impairment. Intractable Rare Dis Res. 2021 Feb;10(1):31–6.

73. Tafazoli A, Eshraghi P, Pantaleoni F, Vakili R, Moghaddassian M, Ghahraman M, et al. Novel mutations and their genotype-phenotype correlations in patients with Noonan syndrome, using next-generation sequencing. Adv Med Sci. 2018 Mar;63(1):87–93.

74. Green GE, Scott DA, McDonald JM, Teagle HFB, Tomblin BJ, Spencer LJ, et al. Performance of cochlear implant recipients with GJB2-related deafness. Am J Med Genet. 2002 May 1;109(3):167–70.

75. Smith KM, Mecoli MD, Altaye M, Komlos M, Maitra R, Eaton KP, et al. Morphometric differences in the Heschl's gyrus of hearing impaired and normal hearing infants. Cereb Cortex. 2011 May;21(5):991–8.

76. Shibata DK. Differences in brain structure in deaf persons on MR imaging studied with voxel-based morphometry. AJNR Am J Neuroradiol. 2007 Feb;28(2):243–9.

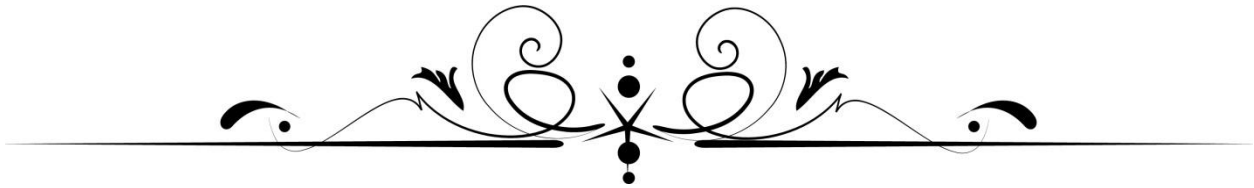
77. Olulade OA, Koo DS, LaSasso CJ, Eden GF. Neuroanatomical profiles of deafness in the context of native language experience. J Neurosci. 2014 Apr 16;34(16):5613–20.

78. Kleinlogel S, Vogl C, Jeschke M, Neef J, Moser T. Emerging Approaches for Restoration of Hearing and Vision. Physiol Rev. 2020 Oct 1;100(4):1467–525.

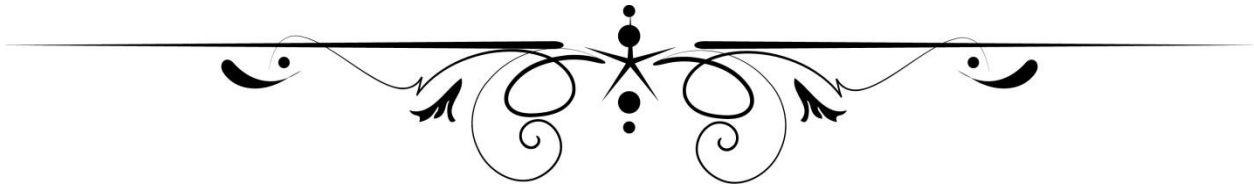
79. Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, et al. Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nat Med. 2005 Mar;11(3):271–6.

80. Koehler KR, Nie J, Longworth-Mills E, Liu XP, Lee J, Holt JR, et al. Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. Nat Biotechnol. 2017 Jun;35(6):583–9.

81. Hayes D. State programs for universal newborn hearing screening. Pediatr Clin North Am. 1999 Feb;46(1):89–94.



ANNEXURE



ANNEXURE-1

SDUAHER

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

SRI DEVARAJ URS MEDICAL COLLEGE

Tamaka, Kolar

INSTITUTIONAL ETHICS COMMITTEE

Members

1. **Sri K. Prahallad Rao, Editor, Kolar Patrike, Kolar. (Chairman)**
2. **Dr. Jagadamba. A** Assoc. Prof of Physiology, SDUMC (Member Secretary)
3. Dr. D.E.Gangadhar Rao, Prof. of Zoology, Govt. Boys College, Kolar.
4. Sri M.G.Venkata Reddy, Advocate & Notary, Kolar
5. Dr. S.R. Prasad, Prof of Microbiology, & Director, PG. Studies, SDUMC
6. Dr. Mohan Kumar.K, Prof of Surgery & Medical Superintendent, R.L. Jalappa Hospital &, R.C
7. Dr. Ranganath.B.G, Prof. & HOD of Comm. Medicine, SDUMC
8. Dr. C.S.B. Rajendra Prasad, Prof. & HOD, of Pathology, SDUMC
9. Dr. Sudha Reddy.V.R Prof of Peadiatrics, SDUMC
10. Dr. Anand Tippanna Talikoti Prof. of Anaesthesia, SDUMC
11. Dr. Srinivasa Reddy.P Prof. of Forensic Medicine, SDUMC
12. Dr. Sumathi.M.E Assoc. Prof of Biochemistry, SDUMC
13. Dr. Bhuvana.K Assoc. Prof of Pharmacology, SDUMC
14. Dr. Pavan B K Asst. Prof. of Surgery, SDUMC
15. Dr.Hariprasad S Asst. Prof. of Orthopedics, SDUMC

No. DMC/ KLR/ IEC/ 26 / 2017-18 Date: 29-06-2017

PRIOR PERMISSION TO START OF STUDY

The Institutional Ethics Committee Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the study entitled **"Spectrum of GJB2 gene with its promotor region in congenital non syndromic hearing impairment"** being investigated by Dr. Kumaraswamy Revanakimath[1], Dr Venkatesh KV[1], Dr. S M Azeem Mohiyuddin[2] and Dr. AVM Kutty[3] in the Department of Anatomy[1], ENT[2] and Biochemistry[3] at and Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.** However final report has to be submitted to the Ethics Committee after completion of the study for presentation in conference or for publication.

Member Secretary
Institutional Ethics Committee
SDUMC, Tamaka Kolar
Member Secretary
Ethical Committee
SDUMC, Kolar.

Chairman
Institutional Ethics Committee
SDUMC, Tamaka Kolar
CHAIRMAN
Institutional Ethics Committee
Sri Devaraj Urs Medical College.
Tamaka, Kolar

ANNEXURE-2

PATIENT INFORMATION SHEET

Date:

A person who is not able to hear as well as someone with normal hearing is said have hearing loss when normal hearing thresholds of 25dB or better (in both ears) is not heard. Hearing loss may be mild, moderate, severe or profound. It can affect one ear or both ears, and leads to difficulty in hearing conversational speech or loud sounds.

Hearing impairment is the most frequent sensory disorder. Approximately one child in 500–650 is born deaf. In infants and young children with hearing loss, early identification and management through infant hearing screening programmes can improve the linguistic and educational outcomes for the child. Children with deafness should be given the opportunity to learn sign language along with their families.

People with hearing loss can benefit from the use of hearing devices, such as hearing aids, assistive listening devices and cochlear implants. They may also benefit from speech therapy, aural rehabilitation and other related services. People who develop hearing loss can learn to communicate through development of lip-reading skills, use of written or printed text, and sign language.

By keeping above things in mind we have taken up research which focus on occurrence of *GJB2 gene* mutations. Now I would like to invite you to participate in our study. Your participation dose not induce any expenditure or investment by any means. The decision to participate in this study is based on your whole hearted consent. You have got all the rights to withdraw from the study at any given point without any explanation.

Study requires 3ml of venous blood. This will be drawn by the experts. Procedure does not involve any risk except a minimal discomfort while drawing blood sample. The blood sample is only used for the research purpose. We will not be sharing the identity of those participating in the research with anyone. The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will not be identified by your name but by a number.

ANNEXURE-3

PATIENT INFORM CONSENT FORM

Patient identification number:

Title of research project: Mutational spectrum of GJB2 gene with its promotor region in congenital nonsyndromic hearing impairment.

Principal investigator: Dr. Kumaraswamy Revanakimath.

Contact number : 9880465500

Research taken at: Department Anatomy, Sri Devaraj Urs academy of Higher Education and Research, Tamaka, Kolar-563 103.

The contents in the information sheet dated_____ that was provided have been read carefully by me/ explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions and I got the satisfactory clarification of the same.

The nature and purpose of the study and its risks/ benefits and applications and expected duration of the study and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care and legal rights being affected.

I understand that the information collected about me from my participation in this research and sections of any my medical notes are kept confidential. I give the permission for researcher (principal investigator) to have access to my records.

I agree to take part in the above mentioned research project.

_____ Date:

(signature / left thumb impression) Place:

Name of the participant:
Son/ daughter of:
Complete postal address:

This is to certify that the above consent has been obtained in my presence.

Date:

Place: (Signature of principal investigator)

<u>ಅಧ್ಯಯನ ವಿಷಯದ ಬಗ್ಗೆ ರೋಗಿಯ ವಿವರಣಾ ಪತ್ರ</u>

ಒಬ್ಬ ವ್ಯಕ್ತಿ 25 ಡೆಸಿಬಲ್ ಅಥವಾ ಅದಕ್ಕಿಂತ ಹೆಚ್ಚಿನ ಧ್ವನಿಯನ್ನು ಗ್ರಹಣ ಮಾಡಲು ವಿಫಲವಾದರೆ ಅಂತಹ ವ್ಯಕ್ತಿಯನ್ನು ಕಿವುಡಿತನದಿಂದ ಬಳಲುತ್ತಿರುವವರೆಂದು ಹೇಳಲಾಗುವುದು. ಕಿವುಡುತನವು ಲಘು, ಮಧ್ಯಮ, ವಿಪರೀತ ಸ್ವರೂಪದ್ದಾಗಿರುತ್ತದೆ. ಒಂದು ಅಥವಾ ಎರಡು ಕಿವಿಗಳಲ್ಲಿ ನ್ಯೂನ್ಯತೆಯನ್ನು ಒಳಗೊಂಡಿರಬಹುದು. ಇದರಿಂದ ಸಂಭಾಷಣೆ ಅಥವಾ ಧ್ವನಿಯನ್ನು ಆಲಿಸುವಲ್ಲಿ ತೊಂದರೆ ಅಥವಾ ವಿಫಲತೆ ಉಂಟಾಗುವುದು. ಕಿವಿಡುತನವು ಸಾಮಾನ್ಯವಾಗಿ ಕಂಡುಬರುವ ತೊಂದರೆಯಾಗಿದ್ದು ಹುಟ್ಟುವ 500ರಿಂದ 650 ಮಕ್ಕಳಲ್ಲಿ ಒಂದು ಮಗುವು ಕಿವುಡುತನದಿಂದ ಬಳಲುತ್ತದೆ.

ನವಜಾತ ಶಿಶು ಮತ್ತು ಚಿಕ್ಕ ಮಕ್ಕಳಲ್ಲಿ ಕಿವುಡುತನವನ್ನು ಗುರುತಿಸುವುದು ತುಂಬಾ ಮಹತ್ತರವಾಗಿರುತ್ತದೆ. ಇದರಿಂದ ಮಕ್ಕಳಿಗೆ ಯಾವ ರೀತಿಯ ಚಿಕಿತ್ಸೆಗಳ ಉಪಲಬ್ದಿ ಮತ್ತು ಅದರ ಸಾಧ್ಯ ಅಸಾಧ್ಯತೆಗಳ ಬಗ್ಗೆ ಅರಿವಾಗುತ್ತದೆ. ಆಧುನಿಕ ಶ್ರವಣ ಸಾಧನಗಳ ಸಹಾಯದಿಂದ ಸ್ವಲ್ಪ ಮಟ್ಟಿಗೆ ಶ್ರವಣ ಶಕ್ತಿಯನ್ನು ಹೆಚ್ಚಿಸಬಹುದಾಗಿದೆ.ಅದರ ಜೊತೆಗೆ ಲಿಪ್ ರೀಡಿಂಗ್, ಸ್ಪೀಚ್ ತೆರಪಿಯು ಸಹ ಸಹಾಯಕವಾಗಬಲ್ಲವು.

ಈ ಮೇಲಿನ ವಿಷಯಗಳನ್ನು ಸಾಧಿಸುವ ದಿಕ್ಕಿನಲ್ಲಿ ನಾವು ಡಿ.ಎನ್.ಎ ದಲ್ಲಾಗುವ ಲೋಪದೋಷಗಳಿಂದ ಉಂಟಾಗುವ ಜನ್ಮಜಾತ ಕಿವುಡುತನದ ಬಗ್ಗೆ ಸಂಶೋಧನಾ ಅಧ್ಯನವನ್ನು ತೆಗೆದುಕೊಂಡಿರುತ್ತೇವೆ. ಆದುದರಿಂದ ನಾವು ನಿಮ್ಮನ್ನು ಇದರಲ್ಲಿ ಭಾಗವಹಿಸಲು ಆಹ್ವಾನಿಸುತ್ತಿದ್ದೇವೆ. ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆಯಿಂದ ನಿಮಗೆ ಯಾವುದೇ ತರಹದ ಆರ್ಥಿಕ ಹಾಗೂ ಸಾಮಾಜಿಕ ತೊಂದರೆಗಳನ್ನು ತರುವುದಿಲ್ಲ. ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಇಚ್ಚೆಯಿಂದ ಕೂಡಿರುತ್ತದೆ. ಈ ಅಧ್ಯನವು ಅನುವಂಶಿಕ ಪರೀಕ್ಷೆಗಳನ್ನು ಒಳಗೊಂಡಿದ್ದು, ನಿಮ್ಮ ಡಿ.ಎನ್.ಎ ಯನ್ನು ಪರೀಕ್ಷೆಗೆ ಒಳಪಡಿಸಲಾಗುವುದು. ಅದಕ್ಕಾ ಗಿ ನೀವು ನಿಮ್ಮ ಅಥವಾ ಮಗ/ಮಗಳ ಮೂರು ಮಿಲಿ ರಕ್ತವನ್ನು ಪರೀಕ್ಷಾರ್ತವಾಗಿ ಕೊಡಬೇಕಾಗುತ್ತದೆ. ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಲ್ಪಟ್ಟ ಮಾಹಿತಿ ಹಾಗೂ ರಕ್ತದ ಪರೀಕ್ಷೆಯ ಫಲಿತಾಂಶಗಳನ್ನು ನಿಮ್ಮ ಹೆಸರಿನಲ್ಲಿ ಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲವೆಂದು ಮತ್ತು ಗುರುತನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗುವುದೆಂದು ಭರವಸೆ ನೀಡುತ್ತೇವೆ.

ಸಮ್ಮತಿ ಪತ್ರ

ರೋಗಿಯ ಗುರುತಿನ ಸಂಖ್ಯೆ:

ಸಂಶೋಧನೆಯ ಶೀರ್ಷಿಕೆ: "ಡಿ.ಎನ್.ಎ. ದಲ್ಲಾಗುವ ಲೋಪದೋಷಗಳಿಂದಾಗುವ ಜನ್ಮಜಾತ ಕಿವುಡುತನದ ಕಾರಣಗಳ ಬಗೆಗಿನ ಅಧ್ಯಯನ**".**

ಮುಖ್ಯ ಸಂಶೋದನಾಕಾರರು: ಡಾ॥.ಕುಮಾರಸ್ವಾಮಿ

ದೂರವಾಣಿ ಸಂಖ್ಯೆ: 09880465500

ಸಂಶೋಧನಾ ಸ್ಥಳ: ಅನುವಂಶಿಕ ಪರೀಕ್ಷಣಾ ಕೇಂದ್ರ, ವೈದ್ಯಕೋತ್ತರ ಆರೋಗ್ಯ ವಿಜ್ಞಾನಗಳ ವಿಭಾಗ, ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಹಾಗೂ ಸಂಶೋಧನಾ ಸಂಸ್ಥೆ.

ಮಾಹಿತಿ ಪತ್ರದಲ್ಲಿರುವ ಎಲ್ಲಾ ವಿಷಯಗಳನ್ನು ನಾನು ಓದಿ ತಿಳಿದಿರುತ್ತೇನೆ/ನನಗೆ ಅರ್ಥವಾಗುವ ಭಾಷೆಯಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ. ಸಂಶೋಧನಾ ಪ್ರಕ್ರಿಯೆ ಬಗೆಗೆ ವಿವರಿಸುವಾಗ ನನ್ನಲ್ಲಿ ಉದ್ಭವಿಸಿದ ಪ್ರಶ್ನೆಗಳಿಗೆ ಸರಿಯಾದ ಮತ್ತು ಸಮಾದಾನಕರವಾದ ಉತ್ತರಗಳು ದೊರೆತಿವೆ.

ಸಂಶೋಧನಾ ರೀತಿ, ಅವಶ್ಯಕತೆ ಮತ್ತು ಅದರಲ್ಲಿರುವ ಅಪಾಯಗಳು ಹಾಗೂ ಉಪಯೋಗಗಳು ಮತ್ತು ಇತರ ಅವಶ್ಯಕ ಮಾಹಿತಿಗಳನ್ನು ವಿವರವಾಗಿ ತಿಳಿಯಪಡಿಸಲಾಗಿದೆ.ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ನನ್ನ ಬಾಗಿತ್ವವು ಸ್ವಇಚ್ಚೆಯಿಂದ ಕೂಡಿದ್ದು ಮತ್ತು ಈ ಸಂಶೋಧನೆಯಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹೊರಬರುವ ಹಕ್ಕನ್ನು ಹೊಂದಿರುವ ಬಗ್ಗೆ ಮತ್ತು ಇದರಿಂದ ನನಗೆ ಸಿಗುತ್ತಿರುವ ಯಾವುದೇ ತರಹದ ವೈದ್ಯಕೀಯ ಚಿಕಿತ್ಸೆ ಹಾಗೂ ಸಹಾಯಗಳಲ್ಲಿ ತೊಂದರೆಗಳು ಉಂಟಾಗುವುದಿಲ್ಲವೆಂದು ತಿಳಿದಿರುತ್ತೇನೆ.

ನಾನು ನನ್ನ ಸ್ವಇಚ್ಚೆಯಿಂದ, ನನ್ನ ಮಗ/ಮಗಳ ರಕ್ತದ ಮಾದರಿಯನ್ನು ,ಭಾವ ಚಿತ್ರಗಳನ್ನು , ಕೌಟುಂಬಿಕ ಮಾಹಿತಿಯನ್ನು ಸಂಶೋಧನಾಕಾರರಿಗೆ ಸಂಶೋಧನೆ ಹಾಗೂ ವೈದ್ಯಕೀಯ ಅಧ್ಯಯನಕ್ಕಾಗಿ ಬಳಸಲು ಸಮ್ಮತಿಯನ್ನು ಕೊಟ್ಟಿರುತ್ತೇನೆ. ಈ ಮಾಹಿತಿಯನ್ನು ನನ್ನ ಗುರುತು ಬಹಿರಂಗ ಪಡಿಸದ ರೀತಿಯಲ್ಲಿ ಬಳಸಲಾಗುವುದೆಂದು ತಿಳಿದಿರುತ್ತೇನೆ.

ನಾನು ಈ ಮಾಹಿತಿ ಪತ್ರದಲ್ಲಿರುವ ಅಂಶಗಳನ್ನು ಸಂಪೂರ್ಣವಾಗಿ ಅರ್ಥೈಸಿಕೊಂಡಿರುತ್ತೇನೆ ಮತ್ತು ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಂಡು ಅನುವಂಶಿಕ ಪರೀಕ್ಷೆಗೆ ಒಳಪಡಲು ಸಮ್ಮತಿ ನೀಡಿರುತ್ತೇನೆ.

_____

(ಸಹಿ ಅಥವಾ ಹೆಬ್ಬೆರಳಿನ ಗುರುತು) **ದಿನಾಂಕ:**

ರೋಗಿಯ ಹೆಸರು:

ತಂದೆಯ, ತಾಯಿ/ಪೋಷಕರ ಹೆಸರು:

ಮನೆಯ ವಿಳಾಸ:

ದೂರವಾಣಿ ಸಂಖ್ಯೆ:

ಈ ಸಮ್ಮತಿ ಪತ್ರವನ್ನು ನನ್ನ ಸಮ್ಮುಖದಲ್ಲಿ ತೆಗೆದುಕೊಳ್ಳಲಾಗಿದೆ.

_____

(ಮುಖ್ಯ ಸಂಶೋಧನಾಕಾರರು) **ದಿನಾಂಕ:**

ಸಾಕ್ಷಿಗಳು: **ಸ್ಥಳ:**

1. **2.**

_____ _____

ಹೆಸರು: **ಹೆಸರು:**

ವಿಳಾಸ: **ವಿಳಾಸ:**

ANNEXURE-4

Sri Devaraj Urs Medical College, Kolar
Department of Anatomy

Ph D Thesis:

Mutational spectrum of GJB2 gene with its promoter region in congenital nonsyndromic hearing impairment.

Principal Investigator: Dr Kumaraswamy Revanakimath

Register No: Ph.d/ANAT/FM/102/2015-16

****

GENERAL INFORMATION OF THE PARTICIPANT

Enrollment as Subject / Control

Participant ID: _____ Date: _____

Name: _____

First Name Last Name

Date of Birth: / / Age :

Gender: M / F/ Unknown

Postal Address:

Contact number:

INCLUSION / EXCLUSION CRITERIA CHECKLIST

1. Congenital hearing loss – yes / no
2. Order of birth –
3. At what age it is detected –
4. Any infectious diseases during pregnancy – yes / no
    a. If yes details:
5. Siblings are affected – yes / no
    a. If yes details:
6. Parents are affected – father / mother / both / none
    a. Details of affected:
7. Parents had consanguineous marriage – yes/ no
8. Father's relatives affected – yes / no
    a. If yes details:

9. Mother's relatives affected – yes /no
    a. If yes details:
10. Phenotypical abnormalities – present / absent
    a. If present, details:
11. System / organ malfunction/malformation – yes / no
    a. If yes, details :
12. Otoscopic examination – normal / abnormal
    a. Details of abnormality:
13. Audiologist examination and findings
    a. Conductive deafness / Sensory- neural deafness
    b. Severity with range of hearing loss :

The above information is provided by the parents / guardian as per their current knowledge.

Participant's medical information and family history has been reviewed by the principal investigator and it has been determined that participant has met the eligibility criteria of the above-mentioned study.

Signature / Thumb impression of the informer

Witness:

Information received and reviewed by Dr Kumaraswamy R.

Signature & Date: _____

ANNEXURE-5

DEMOGRAPHIC DATA OF SUBJECT SAMPLES

Subject ID	Age	Gender	Severity of hearing loss	Family History					
				Affected		Consanguinity	Siblings		Heredity
				Father	Mother		Affected	Non-affected	
1k	13	M	Profound	No	No	Yes	1	1	Yes
2k	8	F	Profound	No	No	Yes	1	1	Yes
3k	8	M	Profound	No	No	No	1	3	Yes
4k	7	M	Profound	No	No	Yes	1	1	Yes
5k	7	M	Profound	No	Yes	Yes	2	0	Yes
6k	6	M	Profound	No	No	Yes	1	1	Yes
7k	9	M	Profound	Yes	No	No	2	1	Yes
8k	9	M	Profound	No	No	Yes	1	0	Yes
9k	8	M	Profound	No	Yes	Yes	1	2	Yes
10k	8	M	Profound	No	No	Yes	1	1	No
11k	8	M	Profound	No	No	No	1	5	No
12k	8	F	Profound	No	No	Yes	1	0	No
13k	10	M	Profound	No	No	No	0	1	No
14k	9	M	Profound	No	No	Yes	1	1	Yes
15k	10	M	Profound	No	No	Yes	0	2	Yes
16k	6	M	Profound	No	No	No	0	3	No
17k	10	M	Profound	No	No	Yes	1	1	No
18k	7	M	Profound	No	No	Yes	0	1	Yes
19k	7	F	Profound	No	No	No	0	1	Yes
20k	7	F	Profound	No	No	Yes	1	2	No
21k	6	F	Profound	No	No	Yes	1	1	No
22k	6	F	Profound	No	No	Yes	1	1	Yes
23k	7	M	Profound	No	No	Yes	1	1	No
24k	7	M	Profound	Yes	No	Yes	1	0	Yes
25k	8	M	Profound	Yes	No	Yes	1	1	Yes

Subject ID	Age	Gender	Severity of hearing loss	Family History					
				Affected		Consanguinity	Siblings		Heredity
				Father	Mother		Affected	Non-affected	
26k	9	M	Profound	No	No	Yes	1	1	No
27k	8	M	Profound	No	No	No	0	2	No
28k	9	F	Profound	No	No	Yes	1	2	Yes
29k	9	F	Profound	Yes	No	Yes	2	1	Yes
30k	10	M	Profound	No	Yes	Yes	2	1	Yes
31k	13	F	Profound	No	No	Yes	1	3	Yes
32k	15	M	Profound	No	No	No	1	4	No
33k	15	F	Profound	No	No	Yes	1	1	No
34k	15	F	Profound	No	No	Yes	1	1	Yes
35k	14	F	Profound	No	Yes	Yes	3	0	Yes
36k	6	F	Profound	Yes	No	Yes	3	1	Yes
37k	16	M	Profound	Yes	No	Yes	2	0	Yes
38k	16	M	Profound	No	No	No	1	2	No
39k	14	F	Profound	No	No	No	1	0	No
40k	16	M	Profound	No	No	Yes	1	1	Yes
41k	13	M	Profound	No	No	Yes	1	1	No
42k	9	F	Profound	No	No	Yes	2	1	Yes
43k	7	F	Profound	No	No	Yes	1	1	No
44k	7	M	Profound	No	Yes	Yes	3	0	No
45k	11	M	Profound	No	No	Yes	1	1	No
46k	11	M	Profound	No	No	No	1	1	No
47k	13	F	Profound	Yes	No	Yes	3	1	Yes
48k	13	M	Profound	No	No	No	2	2	Yes
49k	13	M	Profound	No	No	Yes	2	2	Yes
50k	14	F	Profound	No	No	Yes	2	3	Yes

DEMOGRAPHIC DATA OF CONTROL SAMPLES

Subject ID	Age	Gender	Severity of hearing loss	Family History					
				Affected		Consanguinity	Siblings		Heredity
				Father	Mother		Affected	Non-affected	
1H	9	F	Normal	No	No	No	0	1	No
2H	11	F	Normal	No	No	No	0	3	No
3H	7	M	Normal	No	No	No	0	2	No
4H	8	F	Normal	No	No	Yes	0	0	No
5H	6	M	Normal	No	No	No	0	3	No
6H	8	F	Normal	No	No	No	0	2	No
7H	7	M	Normal	No	No	No	0	1	No
8H	9	M	Normal	No	No	No	0	2	No
9H	7	M	Normal	No	No	No	0	3	No
10H	8	F	Normal	No	No	No	0	2	No
11H	8	M	Normal	No	No	No	0	2	No
12H	9	M	Normal	No	No	Yes	0	1	No
13H	6	M	Normal	No	No	No	0	1	No
14H	10	F	Normal	No	No	Yes	0	2	No
15H	8	M	Normal	No	No	No	0	1	No
16H	7	M	Normal	No	No	No	0	3	No
17H	9	F	Normal	No	No	Yes	0	2	No
18H	6	M	Normal	No	No	No	0	1	No
19H	10	F	Normal	No	No	No	0	1	No
20H	8	M	Normal	No	No	No	0	0	No
21H	6	M	Normal	No	No	Yes	0	1	No
22H	7	M	Normal	No	No	No	0	0	No
23H	9	M	Normal	No	No	No	0	1	No
24H	8	M	Normal	No	No	Yes	0	0	No
25H	8	M	Normal	No	No	No	0	1	No

Subject ID	Age	Gender	Severity of hearing loss	Family History					
				Affected		Consanguinity	Siblings		Heredity
				Father	Mother		Affected	Non-affected	
26H	10	F	Normal	No	No	Yes	0	1	No
27H	9	M	Normal	No	No	No	0	2	No
28H	9	M	Normal	No	No	No	0	2	No
29H	8	F	Normal	No	No	No	0	1	No
30H	11	M	Normal	No	No	No	0	1	No
31H	9	M	Normal	No	No	No	0	3	No
32H	13	M	Normal	No	No	No	0	4	No
33H	12	F	Normal	No	No	Yes	0	1	No
34H	9	M	Normal	No	No	No	0	1	No
35H	8	F	Normal	No	No	No	0	0	No
36H	6	F	Normal	No	No	No	0	1	No
37H	7	M	Normal	No	No	No	0	0	No
38H	11	F	Normal	No	No	No	0	2	No
39H	10	F	Normal	No	No	No	0	0	No
40H	9	M	Normal	No	No	No	0	1	No
41k	11	F	Normal	No	No	No	0	1	No
42k	12	F	Normal	No	No	No	0	1	No
43k	8	M	Normal	No	No	No	0	1	No
44k	7	M	Normal	No	No	No	0	0	No
45k	10	M	Normal	No	No	No	0	1	No
46k	7	F	Normal	No	No	No	0	1	No
47k	9	F	Normal	No	No	Yes	0	1	No
48k	10	M	Normal	No	No	No	0	2	No

ANNEXURE-6

Certificates of Presentations and Publication

NATCON 67
Anatomy Sans Frontiers

67TH NATIONAL CONFERENCE OF ANATOMICAL SOCIETY OF INDIA

18TH - 20TH NOVEMBER 2019
ORGANIZED BY : DEPARTMENT OF ANATOMY
VMMC & SAFDARJUNG HOSPITAL, NEW DELHI

Certificate of Participation

Prof./Dr./Mr./Mrs **Kumaraswamy R**

Presented Scientific Research Paper Entitled *Highly Prevalent GJB2 Gene Mutations among Congenital Nonsyndromic Hearing Impairment in South India Population*

at Annual Conference of Anatomical Society of India, 67th NATCON held at Dr. Ambedkar International Center, New Delhi on 18th - 20th November 2019.

Dr. Chintamani
Chief Co-ordinator &
Chairman of Scientific Committee

Dr. Mangala Kohli
Congress Chairperson

Dr. Reeha Mahajan
Organizing Secretary
& Treasurer

22nd KCACON 2022-HASSAN

Evolving Anatomy towards Clinical Perspective

Organized by

Karnataka Chapter of Anatomists

In association with

Department of Anatomy HIMS, Hassan

This is to certify that Dr/Sri.....Dr Kumaraswamy Revanakimath.....

bearing Reg No .....A-21215..... Registered with.....AYUSH..... Medical Council,

has participated as Delegate/Faculty/Chairperson/Speaker

has presented Oral titled

Promotor and coding region sequence analysis of GJB2 Gene for Mutations among Congenital Nonsyndromic Hearing Impairment in South Indian Population.

in State level Pre Conference CME and Conference held on 16th to 18th September 2022.

The Karnataka Medical Council has granted "5 hours" of Accreditation for participating in this Conference, Vide letter no KMC/CME/301/2022 dated 05/09/2022

Dr. Nagaraj Annegowda
Zonal Chairman

Dr. Kantha S Jadhav
Founder President
KCA

Dr. Priya Ranganath
President
KCA

Dr. N.M. Suresh
Secretary
KCA

Dr. Prakash B.S.
Organizing Secretary
22KCACON

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Highly Prevalent GJB2 Gene Mutations among Congenital Nonsyndromic Hearing Impairment in a South Indian Population

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Abstract: Hearing impairment (HI) is due to genetic (autosomal recessive) having a frequency of one in 1000 children. Hearing impairment has a widespread gene involvement of various chromosomes. Mutations in the GJB2 gene on chromosome 13 were frequent findings for congenital non-syndromic hearing impairment. To make it more complicated, it has been observed that these mutations show population variations. Objective: To determine the prevalence of the most common mutations in the GJB2 gene among the Indian population. Methods: In this study, 38 subjects (22 cases and 16 controls) enrolled, and the degree of hearing loss was assessed. To identify the involvement of GJB2 gene mutations p.W24X and c.235delC among the populations, PCR Technique and RFLP were adopted. Results: The mutations under investigation were at a higher prevalence rate, i.e., 45.45% for c.235delC and a lower prevalence rate of 18.18% for p.W24X, and both the mutations had heterozygous natures. Conclusion: This paper is the first of this kind that tries to identify a prevalent mutation in the GJB2 gene in the population of Karnataka state. The results are supported to some extent by previous Indian studies involving other than the current population. It is also noteworthy that the mutations identified are heterozygous and, therefore, are not pathogenic. This implies either the existence of potential mutations in the gene's unexplored region or the possible co-implication of another connexin gene, i.e., the digenic origin of the hearing loss, which could be related to the putative formation of heteromeric connexons or heterotypic channels.

Keywords: congenital nonsyndromic hearing loss, p.W24X, c.235delC mutations.

南印度人群先天性非綜合徵性聽力障礙中高度流行的国杰宝2基因突變

摘要：聽力障礙是由於遺傳（常染色體隱性遺傳）導致的，頻率為千分之一。聽力障礙廣泛涉及各種染色體的基因。13號染色體上国杰宝2基因的突變是先天性非綜合徵性聽力障礙的常見發現。更複雜的是，已經觀察到這些突變顯示出種群變異。目的：確定印度人群中国杰宝2基因最常見突變的流行程度。方法：在本研究中，納入了38名受試者（22名病例和16名對照組），並評估了聽力損失的程度。為了確定国杰宝2基因突變p.W24X和c.235刪除C在人群中的參與，採用聚合酶链反应技術和RFLP。結果：被調查的突變具有較高的流行率，即c.235delC為45.45%，p.W24X的流行率為18.18%，且兩種突變均具有雜合性。結論：本文是此類嘗試確定卡納塔克邦人群中国杰宝2基因中普遍存在的突變的首篇論文。這些結果在一定程度上得到了之前印度研究的支持，這些研究涉及的不是當前人口。還值得注意的是，鑑定出的突變是雜合的，因此不是致病性的。這意味著該基因的未探索區域存在潛在突變或另一個連接蛋白基因可能共同暗示，即聽力損失的雙基因起源，這可能與異聚連接子或異型通道的假定形成有關。

关键词：先天性非綜合徵性聽力損失，p.W24X，c.235delC突變。

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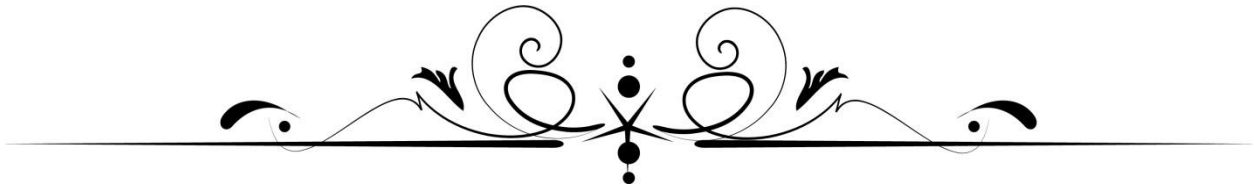
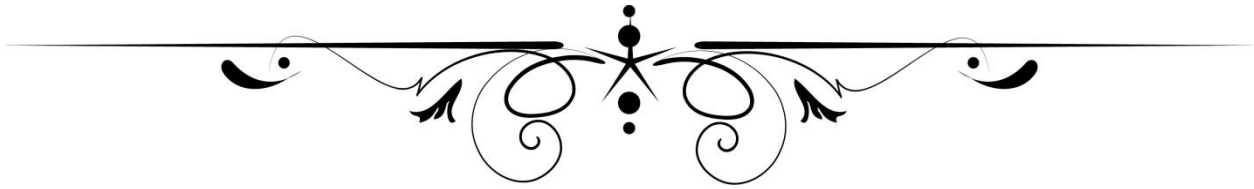


RECOMMENDATIONS



X) RECOMMENDATIONS:

Considering the incident and its social, economic burden on individual, family and society the screening programs at large scale are achievable and are found to be advantageous. Population based identifying the pathogenic mutations and adopting reliable and specific advanced screening tests at the early stage could offer best available treatment strategies.

The United States of America has effective screening formula where in a child is screened for hearing impairment within first month. On confirmation of hearing loss the specific hearing aids are supplemented within three months. Before the child attains six months intervention strategies should be calculated. This modus operandi may be implemented for high risk children if not possible for all the newborn.(81)

India has robust health care system. The district hospitals may be designated to collect the data and identify the potential high risk cases and families. It may not be possible to identify the deficit at birth. It may be noticeable only after few months. Such cases may be subjected to re-investigate at regular intervals. The screening should be extended to children born to alleged history of pregnancy susceptible to cause congenital hearing impairment.

Once the affected individuals are identified it is necessary to document the basic data so that awareness and extend the available services to support them to live at par with normal children. Counselling to the family and relatives could be provided to facilitate and assist the affected child to ease with social challenges.