"GENETIC ASSOCIATION OF CONSANGUINITY AND FAMILY HISTORY IN CONGENITAL HEARING LOSS"

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LIST OF ABBREVIATIONS

GLOSSARY	ABBREVIATIONS
DFNA	AUTOSOMAL DOMINANT NON-SYNDROMIC HEARING LOSS
CHL	CONDUCTIVE HEARING LOSS
HL	HEARING LOSS
YLD	YEARS LIVING IN DISABILITY
DNA	DEOXYRIBONUCLEIC ACID
WGS	WHOLE GENOME SEQUENCING
NGS	NEXT GENERATION SEQUENCING
GJB2	GAP JUNCTION PROTEIN BETA -2
NSHL	NONSYNDROMIC HEARING LOSS
BERA	BRAINSTEM EVOKED RESPONSE AUDIOMETRY
CMV	CYTOMEGALOVIRUS
NICU	NEONATAL INTENSIVE CARE UNIT
OAE	OTOACOUSTIC EMISSION
PCR	POLYMERASE CHAIN REACTION
SNV	SINGLE NUCLEOTIDE VARIANT

ABSTRACT

Title - Genetic association of consanguinity and family history in congenital hearing loss

Background: Understanding how consanguinity and family history relate to congenital hearing loss is essential to preventing this morbidity from occurring in subsequent generations. The study's goal was to determine how strongly consanguinity and family history relate to congenital hearing loss and the genetic prevalence of GJB2 gene in our study .

Objective: To evaluate the genetic association of consanguinity and family history of congenital hearing loss .

Material and Methods: The case control study was done using 32 consanguineous congenital hearing loss and 32 non-consanguineous nonsyndromic congenital hearing loss. Detailed history was taken from the parents with the regard to family history of hearing loss and consanguinity.2ml blood samples were collected and DNA was extracted and looked for GJB2 gene in both groups.

Statistical analysis: Comparative analysis between case and control groups using a chi-square test or fisher extract test and independence *t*-test. Statistical analysis was performed with SPSS 24.0 software. The correlation was considered as significant statistically if p-value < 0.05.

Results: In our study, among consanguineous marriages, the breakdown of the degree of consanguinity showed that 37.5% of cases first-degree relatives, 37.5% involved second-degree relatives and 25% involved third-degree relatives . These results indicated statistically a very significant correlation among consanguineous

marriages and degree of consanguinity ($p<0.05$). Among children with consanguineous parents, 21.88% had a familial history of hearing loss, whereas 18.75% of those with non-consanguineous parents had a family history. This finding showed no significant association between consanguinity and family history suggesting that family history alone may not be a strong predictor of congenital hearing impairment when compared to the impact of consanguinity.

The genotype and allele frequencies were identical in compared two groups with Ref/Ref and C/C variants being the most prevalent. *GJB2* gene specifically the rs104894396 variant (chr13:20189511, GRCh38) which involves a C>T single nucleotide variation is present in both consanguineous and non-consanguineous group .

Conclusion: In conclusion, based on the results, a very significant correlation among consanguineous marriages and degree of consanguinity ($p<0.05$). The incidence of congenital hearing loss is our study is 64 (100%). *GJB2* gene specifically the rs104894396 variant (chr13:20189511, GRCh38) which involves a C>T single nucleotide variation is present in both consanguineous and non-consanguineous group indicating that mutation in GBJ2 gene is one of the various cause leading to non-syndromic congenital deafness in our study .

Keywords: Congenital hearing loss, consanguineous and non-consanguineous marriages , GJB2 gene , Family history

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INTRODUCTION

The most prevalent sensory neuropathy in developing countries is hearing loss with prevalence between 1-3 children per 1,000 newborns,of which more than half caused by hereditary.[1] According to the 2005 estimates of WHO, 278 million people have disabling hearing impairment. The prevalence of deafness in Southeast Asia ranges from 4.6% to 8.8 .The second most frequent factor causing years living in disability (YLD), accounting for 4.7% of all YLD is hearing loss.[2]

Children's speech, language, and schooling are all impacted by hearing impairment, which frequently results in less opportunities as people with deafness isolate from society. Hearing loss is often neglected as it cannot be seen and also not enough knowledge of parents regarding hearing programmes diagnosis is also delayed. Early recognization of deafness in child enables an effective application of medical, rehabilitative, and preventive interventions.[3]

Persistent deafness of childhood is divided into hereditary in 50%, acquired in 25% and unknown in 25%.[4.] According to the phenotype, hearing loss is classified either nonsyndromic or syndromic. Mostly 70% of HHL is non-syndromic while 30% is syndromic [5,] 70 genes linked to nonsyndromic types, as well as more than 400 hearing loss syndromes. There are approximately 100 loci associated with congenital deafness, one of which is the gap junction Beta2 which is present at chromosome 13q12 encoding for protein connexin 26 that's mainly found in cochlea and in the epidermis [6]. Severe to profound autosomal recessive nonsyndromic deafness most commonly carried by beta 2 gene (GJB2) mutation .The sensorineural hearing function depends on Connexin 26, a tiny molecule that is a gap junction protein which is essential in potassium recycling in the cochlea.[7] This gene was originally associated

with an autosomal recessive kind of deafness.[8] Mutations in connexin 26 account for at least 20% of all hereditary hearing loss and 10% of all deafness. Connexin 26 gene (pW24X) mutations in India accounts for more than 80% of non-syndromic recessive impairment patients.[9]

Half of sensorineural deafness cases are caused by inherited hearing loss. Numerous documented genes, environmental or a mix of factors that contribute to deafness making it an etiologically diverse feature. Significant new understandings of the pathophysiology of hearing have been made possible by the discovery of over 125 distinct genes linked to deafness.[10] Neonatal hearing testing facilities are offered for the universal disease in the majority of developed countries. The goal of these programs is to screen every baby within a month after birth. Better developmental outcomes later in early childhood are supported by early diagnosis, intervention, and therapy. Whole Genome Sequencing and Whole Exome Sequencing (WGS) has become commonplace because of the establishment of Next Generation Sequencing and capacity to sequence numerous base pairs simultaneously changing the approach to disease-gene discovery.[11]

Consanguinity is the connection among the people who are descended from a shared ancestor. The name originates from latin terms "con" indicating “with" and "sanguis" indicating “blood” indicating blood relationships. A consanguineous marriage is the relationship among two people who are connected as close relatives or second cousin.[12] In certain parts like the Middle East, Asia, Africa, and Latin America, consanguineous marriage are especially common.[13] Consanguineous marriages make up 20% to more than 50% of the general population in the majority of South Asia.[14]

Siblings of consanguineous couples are much more likely to suffer from inherited illnesses such as hearing loss. Despite these deeply ingrained cultural and social imperatives consanguineous marriage are associated with increased genetic risks including congenital hearing loss, underscoring a critical public health issue linked to this long standing tradition.[15]. The majority of consanguineous marriages are inbetween first cousin of mother's aswell as father's side this tradition is especially common in the southern states of Andhra Pradesh, Telangana, Tamilnadu as well as Karnataka. In addition to other backward sections, the lower middle class, and those with higher wealth indices.[16]

A child who has a family history of deafness is six times most probably like to develop permanent hearing loss. In the southern region of India, family history remains a significant risk factor. [17] Female baby mortality rates were substantially more than male and consanguineous group had noticeably higher stillbirth rates. Mothers of consanguineous marriages reported a considerably greater rate of congenital abnormalities than mothers in non-consanguineous marriages [18]. Ocular genetic abnormalities are more likely to occur in consanguineous individuals.[19] Couples married inside biological relationships had a much greater rate of intellectual handicap.[20] Children born into non-consanguineous marriages are less likely to have low birth weights [21]. The odds of their children and grandchildren suffering mental illnesses, cardiac disease, hypertension, stroke, diabetes and stroke were shown to be 0.85%, 0.84%, 1.57%, 0.43%, 0.34%, and 0.14% respectively, in consanguineous marriages.[22]

In the Indian literature, to our knowledge there aren't much research linking hearing impairment to family history. The degree to which consanguinity and family history are linked to hearing impairment in India is not well known. Planning

nationwide initiatives like the Prevention of Deafness will benefit from this data. Furthermore, there is a dearth of literature in a region like south India where consanguinity is quite prevalent. This study purpose is to know the prevalence of congenital deafness in children from both consanguineous and non-consanguineous marriages and identifying genes involved in non-syndromic hearing loss which will aid in prevention, proper diagnosis and understanding of the underlying mechanism.

AIMS AND OBJECTIVES

- **Primary objective -**To identify the genetic association for congenital deafness in children with consanguineous marriage and family history
- **Secondary objective -**To find the incidence of congenital hearing loss in study population

REVIEW OF LITERATURE

Many genes have been linked to non-syndromic congenital deafness. Seminal study on the genetic basis of hearing loss identified about 100 genes including GJB2 (connexin 26), SLC26A4 (pendred syndrome) and MYO15A. GJB2 mutations are the leading cause of autosomal recessive non-syndromic hearing loss accounting for around half of cases in some populations [23]

One of the major reason for congenital nonsyndromic recessive hearing impairement is W24X gene mutation . It would be easier to diagnose deafness early if this mutation could be found early in life. This will assist the subjects in putting the intervention strategies into practice such as learning sign language and using hearing aids to improve their quality of life and obtain formal education. 5 out of 20 subjects were found to have 25 % mutation in the W24X gene. [24]

Usher, Pendred, Treacher Collins and Alport syndromes, Waardenburg syndrome, branchio-oto-renalsyndrome , biotinidase deficiency as well as Norrie disease are a few of the most prevalent syndromes related to congenital syndromic hearing loss.[25]

Gap junctions are a key intercellular communication mechanism that is crucial for exchanging metabolites as well as electrolytes. A class of membrane proteins known as connexins forms gap-junction channels with connexons that are homologous in neighboring cells. By regulating the clonal growth of cell clusters, connexins play a crucial function in development. By establishing the foundation for cortical circuits that form during synaptic growth connexin expression may direct the creation of complex functional structures in the growing brain [26]

Understanding these genetics behind non-syndromic deafness is needed for both patients and physicians. First, the patient might be informed about the NSHL's aetiology. Family history, audiologic tests, temporal bone CT and blood examinations, thyroid function assays , in certain situations an ECG to rule out cardiac abnormalities in syndromic hearing loss .

Even so, it could be challenging to distinguish between NSHL or syndromic deafness[27]. This is the point at which genetic testing becomes crucial because it can often reveal the reason for hearing impairment. The main types of deafness - mixed, sensorineural and conductive.[28]Patients with a strong genetic background may be examined for mutations prior to six months of age. Before the age of six months has been shown to dramatically improve language development with early intervention, rehabilitation can therefore begin right away.[29]

Second, both diagnosis and prognosis can be achieved by identifying the particular mutations. Since certain mutations are linked to particular auditory characteristics, prognostication can be achieved by identifying these variants.Tests such as BERA were accessible and crucial for the workup but administering subjective assessments to children is challenging. Genetic tests can be used to anticipate auditory features which gives the doctor important information when scheduling hearing aid placements and follow-up.[30]

Thirdly, those who are genetically susceptible should not take certain medications or engage in certain activities. Aminoglycosides could cause or worsen SHNL in people having the A1555G mitochondrial mutations.[31] Even when there is no contact to aminoglycosides it has been demonstrated that patients with the A1555G mutation had a relatively high frequency of SNHL[32].

Fourth, syndromic hearing loss may be suspected of owning related disorders if causal mutations are found in them. Diabetes mellitus is also present in people with the mitochondrial DNA mutation A3243G. Patients with the SLC26A4 mutation get goiters in addition to SNHL.[33] Within certain situations, doctors can anticipate related illnesses and check the baby for them. Fifth, prognostication following surgery may potentially benefit from genetic testing. Despite the fact that all patients are regularly offered cochlear implant surgery, those who have mitochondrial abnormalities recover considerably better from the procedure. Even though mitochondrial mutations that cause SNHL are extremely uncommon, cochlear implant surgery has been shown to be quite helpful in these situations, indicating that the cochlea is the primary organ affected by mitochondrial DNA alterations.[34] At last, determining the hereditary origin of hearing loss might assist the physician in offering appropriate genetic counselling.

A child born into a consanguineous marriage has a threefold increased risk of permanent hearing impairment, the second degree of consanguinity has a threefold increased risk and the third degree has a twofold increased risk.[35] Consanguineous marriage is a significant topic for scientists because of its effects on reproduction .Research indicates that children of consanguineous couples are more likely to experience pregnancy loss and health defects.[36]

Consanguineous marriage in India is mostly determined by religion and the north-south regional split in culture rather than socioeconomic status. Consanguineous marriage was 9.9% common overall, the largest and lowest prevalences were seen in the South (23%) and North-East (3.1%) regions, respectively. The percentage of Muslims was 15% higher than that of Hindus (9%). The likelihood of women marrying their cousins was lower for those who lived in cities, were part of nuclear

families, had more education and were from wealthy families ($p < 0.01$). Darbhangia Khotta Muslims in the West Bengal marriages were between first cousins with incidence of 92.0%.[37]Among rural women in Karnataka's Belgaum District, around 52.0% of weddings were negotiated unions between first cousins. [38]

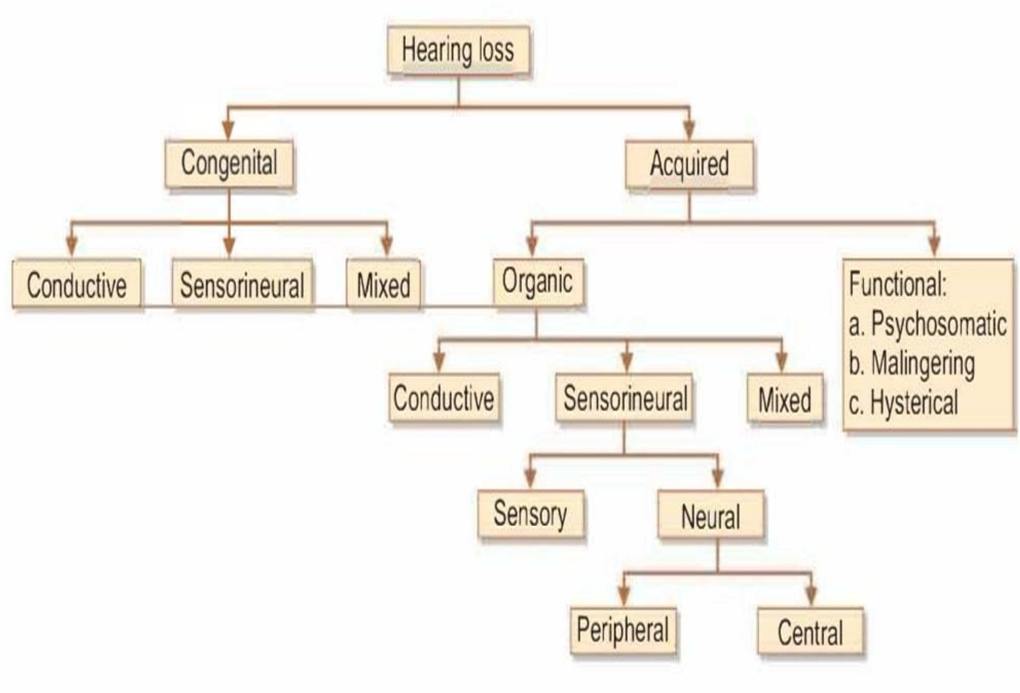
In India, consanguineous marriage is common, and studies have identified a number of contributing factors. According to earlier research, respondents who had previously been married, had lower educational attainment, lived in rural regions, and had a lower economic standing were far more likely to be consanguineous [39]

In the Northern part of the Indian state of Bihar noted that due to the less dowry, consanguinity is more likely to be supported by households with blood relatives. In these kinds of unions, the newlyweds rather than only the groom's family can profit from the bride's parents having some influence over the dowry's utilization [40] Additionally, it has been noted that in order to preserve cultural identity, people in communities marry their cousins because there are no members of the same cultural group in the migrated area [41] . The main justifications for choosing consanguineous marriage in areas with high consanguinity rates are preserving the family's lineage solidarity, making it easier for partners to find a compatible spouse, supporting female status and better relationships with in-laws, reducing the cost of marriage, increasing the possibility of receiving better care for elderly individuals and most importantly improving the stability of the marriage .Many large human populations highly favor consanguineous marriage. Consanguinity's impact on hereditary deafness has been thoroughly investigated and recorded. Numerous writers have proposed that genetic factors account for about half of children's sensorineural hearing loss in both Indian and western literatures .

Family history is a significant component in determining the likelihood of congenital deafness. Their findings emphasised the importance of complete family history assessments in clinical settings which can guide genetic testing and counselling.[42]

The presence of affected relatives increased the likelihood of discovering genetic variants linked to hearing loss. This research emphasises the relavance of family history in determining the genetics landscape of congenital deafness.[43] They discovered that 40% of the subjects had a positive family history indicating a significant genetic component in this community.[44]

CLASSIFICATION OF HEARING LOSS DETERMINED BY THE LESION SITE



1. Conductive deafness affecting the external / middle ear
2. Sensori-neural deafness that impacts the central auditory pathway, cochlear nerve or inner ear.
3. Mixed deafness

BASED ON AETIOLOGY

Sensorineural deafness is categorised into three types: Acquired, genetic and unknown

1.**Acquired HL**- Teratogens including viral as well as pharmacological factors can cause hearing loss. HL could be caused by biochemical, physiological or viral causes. But genetic heritage has a significant impact on its prevalence. The following risk indicators could affect the hearing process :

a. Prenatal causes such as congenital illnesses (CMV, herpes virus, smallpox, syphilis, measles and toxoplasmosis) and ear abnormalities since birth
b. Birth factors such as preterm, low weight at birth (less than 1500gm) as well as increase bilirubin levels in blood
c. Post-delivery complications such as blood, otitis media, mumps, and bacterial meningitis, infections as well as autotoxin medicines like aminoglycosides as well as head injuries or skull fractures .

2. **Genetic HL**: The inheritance of HL was described by Irish physician William Wild in the early 1800s. He makes a distinction between recessive and dominant inheritance in his idea. He also discovered that X-linked transmission was more common in men.

SEVERITY - Decibels is the unit of sound (dB), which are the logarithm of the intensity of the sound wave divided by 10 -15 dB is the typical hearing threshold. The normal level for a conversation is between 45 and 60 dB.

DURATION - HL is divided into the following kinds according to the age of onset:

a. Prelingual: Hearing loss develops prior to learning to speak. Congenital hearing loss prevents a child from speaking normally.
b. Post-lingual: Hearing loss arises after the development of speech.
c. Presbycusis: More than 50% of people aged 80 and more than 25% of people aged 60 have presbycusis

Table 1 – Classification based on etiology

CRITERION	CLASS	DEFINITION AND EXAMPLES
Age of onset	Prelingual deafness	Deafness develops prior to learning to speak
	Postlingual deafness	Deafness arises following the emergence of speech
	Presbycusis	Age related deafness
Etiology	Acquired	Due to environmental agents
	Genetic	Due to genetic mutations
	Idiopathic	Unknown etiology
Clinical phenotypes	Syndromic	In conjunction with additional symptoms
	Non syndromic	The only shortcoming is deafness
Position of damage	Conductive HL	Affecting the external / middle ear
	Sensorineural HL	Impacts the central auditory pathway, cochlear nerve or inner ear
	Mixed HL	Combination of sensorineural as well as conductive hearing loss
Severity	Mild	26-40dB
	Moderate	41-55dB

	Moderately severe	56-70 dB
	Severe	71-90 dB
	Profound	>90 dB
Mode of inheritance	Autosomal dominant	DFNA loci
	Autosomal recessive	DFNB loci
	Sex linked	DFNX loci
	Y - linked	DFNY loci
	Mitochondrial	12SrRNA, tRNASer

EPIDEMIOLOGY

WHO estimates that approximately 7.5 million people have deafness.The incidence of deafness is 2.83 per 1,000 children and 3.5 per 1,000 in adolescents .[45]

RISK FACTORS

The Joint Committee on Infant Hearing have recognized these risk factors for hearing impairment such as positive family history , premature babies , infants 750–1500 g in weight, use of aminoglycosides, venous access and assisted ventilation in the NICU.[46] It has also been discovered that a hospital stay longer than 12 days as well as receiving high-frequency breathing therapy are independent hazards leading

to deafness. There are chances of possibility of delayed maturation of auditory system among newborns admitted in this setting.[47] Particular gene mutation or a combination of gene mutations can cause deafness; environmental factors like trauma, drugs, illnesses or both environmental factors and genetics can interact to cause deafness.

Hearing loss caused by genetics can be classified into syndromic or non-syndromic. Eighty percent of hereditary instances of autosomal recessive nonsyndromic deafness are congenital. Twenty percent of deafness are autosomal dominant non-syndromic. Modes of inheritance connected to maternal mitochondrial DNA or X-linked DNA are rare .[48.] Severe to profound autosomal recessive nonsyndromic deafness most commonly carried by beta 2 gene (GJB2) mutation .[49] Early palliative care and special education are essential and important in child life.

Syndromic cause of congenital deafness can be made by clinical examination and morphological characteristics such heterochromia iridis, branchial cysts or fistulae, dystopia canthorum, pre-auricular pits and tags and pigmentary anomalies.[50] Over 400 of these syndromes have been described.[51]

The gene for GJB2 is located on chromosome 13q12 and is one of around 100 loci associated with congenital deafness. This gene produces connexin 26 a protein mainly in the epidermis and cochlea. The substance known as Connexin 26 is essential for cochlea's recycling of potassium and for the sensorineural hearing function. It works by facilitating the transfer of small molecules from cell to cell. This is the initial gene linked with the autosomal recessive form. At least 20% of all genetic hearing loss and 10% of remaining deafness in children are caused by mutations in connexin 26.[52]

Consanguinity can affect the overall incidence of deafness in a society by affecting how recessive genes are expressed. Therefore, consanguinity-based genetic inheritance will boost the gene for hearing impairment's endurance and dissemination.

Mechanisms/pathophysiology

Forms of congenital hearing deafness are 1- Genetic 2- Acquired. Pathophysiology and processes are different from each other. All the components of the hearing pathway (auditory function or pathophysiological mechanisms that may impair it) are susceptible to genetic alterations. However, the majority of genes connected to syndromic hearing loss are also linked to the same-named disease.

Homeostasis of inner ear – Endolymph and stria vascularis maintain inner ear equilibrium. Endolymph is necessary for the sound transfer and surrounds the inner ear hair cells . The basal, marginal and middle layers make up the stria vascularis. The intermediate and basal cells below are attached to fibrocytes which is underlying the spiral ligament and to each other through gap junctions, while the marginal cells view the endolymph. This gap junction network which also allows ion transport connects the cells electrically. The genes GJB-2 and GJB-6 encode its proteins.

Mutations in either of the two MARVEL domain-containing protein 2 (MARVELD2) or Claudin-14 (tricellulin) resulting in human autosomal recessive non-syndromic deafness .[53,54] Inner ear homeostasis also depends on the endolymph's pH being maintained at the proper level . Although the precise source of these oscillations and the sudden loss of hearing is unknown, it is likely that they are connected to endolymphatic hydrops a condition marked by a surplus of endolymph buildup in the vestibular system as well as cochlea.[55]

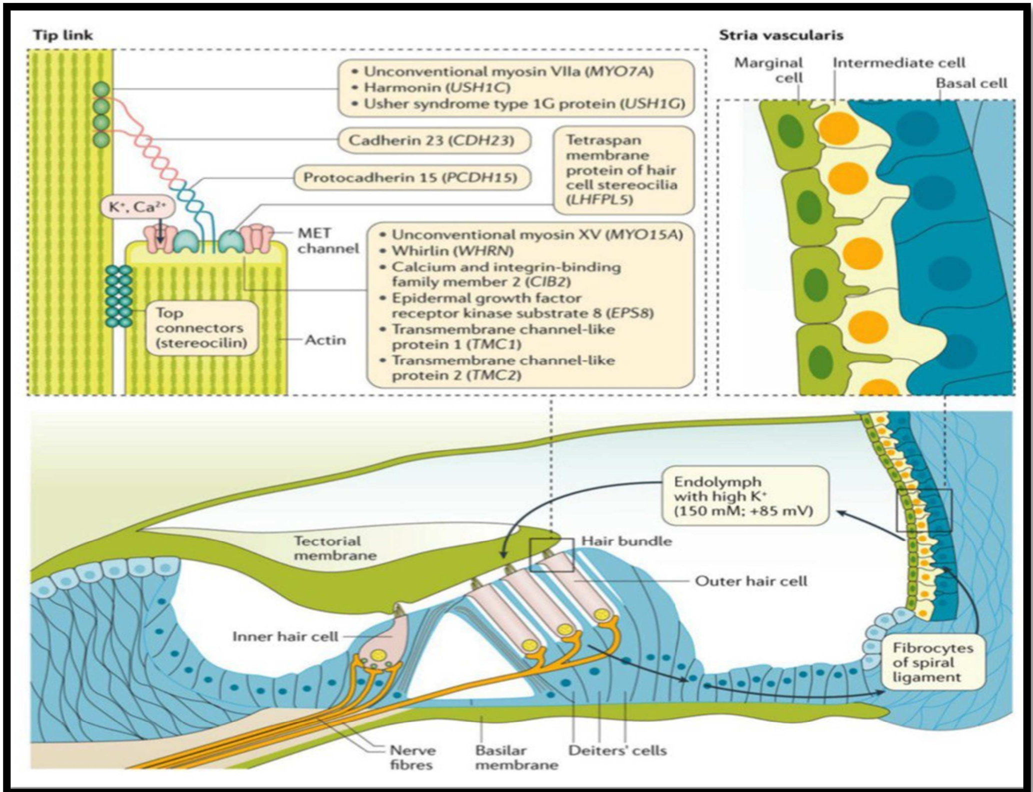


Figure 1: Homeostasis of inner ear

Electrical-mechanical transduction: On the hair cells apical surface, stereocilia are actin-rich projections that resemble staircases and are connected by proteins. As a result, the stereocilia anchored in the basilar membrane shifts in reaction to sound towards tectorial membrane creating a shearing motion by pressing of hair cells. One rather common kind of autosomal dominant non-syndromic deafness is by mutations in KCNQ4. Longitudinal actin fibres within the stereocilia are cross-linked by many proteins including Espin encoding the ESPN gene to provide strength and stiffness. ESPN mutations can result in either autosomal recessive non-syndromic or autosomal dominant non syndromic deafness. Otoferlin (encoded by OTOF) plays a key role in this exocytosis process.[56]

ACQUIRED CAUSES - The most frequent prenatal infectious agent that causes deafness is Herpesvirida, which is a member of the Cytomegalovirus family. For months the viral DNA has been detected in body fluids primarily in urine and saliva. Pregnancy-related vertical transfer of congenital CMV is 32% likely. There is 1.4% chance of vertical transmission in moms who are seropositive after reactivation or reinfection. 10% of neonates with CMV infection exhibit symptoms since childhood and their features is critical when the mother contracts the virus during the first trimester of pregnancy or around conception .[57]

Rubella— Congenital heart disease, cataracts and hearing loss are the classic trifecta of congenital rubella syndrome symptoms[58,59] Direct cochlear injury, cell mortality in Corti organ as well as stria vascularis , changes of endolymph's makeup after strial damage can be caused by viruses.[60]

ASSESSMENT OF HEARING STATUS

If the screening is not successful, the infants should ideally be evaluated medically and audiologically to determine whether they have a hearing loss before they turn three months old. Applying the level of deafness to the better ear and averaging it across frequencies of 500, 1,000, 2,000 as well as 4,000 Hz is advised.

Evoked Oto-acoustic emissions -A physiological test specifically gauges how outer hair cells react when a click stimulus is presented.[61]A small, flexible plug is used by OAAE and placed in baby's ear. Through the plug, sounds are transmitted. A recording device in the plug records the OAE results of a normal ear to stimuli. Emissions are absent in a baby with deafness.

AUTOMATED AUDITORY BRAINSTEM RESPONSE - Are measurements of electrical activity produced in the brainstem pathway responsible for hearing. These tests are triggered by fast, multifrequency clicks to evaluate the function of brainstem at various auditory pathway stages. The baby's scalp is covered with tiny metal discs with thin electrodes, which send impulses to a computer so that the results may be recorded. Uses a broadband click stimulus in the ear to measure the cochlear response in the 1-4 kHz range.[62]

PURE TONE AUDIOMETRY - Once a child reaches 4 years old standard audiometry is usually performed.

ASSESSMENT OF CONGENITAL DEAFNESS

It guides these parents to understand their child's hearing loss, offer personalised genetic counselling, alleviate guilt and helps in treatment like cochlear implantation, adapted educational needs or hearing aids. It may also aid in detecting concurrent medical issues that should be treated or monitored. Finally, determining the aetiology may help identify avoidable hazards for deafness involving in future deterioration like head trauma or the use of aminoglycosides, as well as partially predict the deterioration of deafness. For unilateral deafness, the aetiological work-up may consist of comprehensive clinical assessment to determine whether hearing loss has a syndromic cause .[63] Further research may be required in the future depending on the anticipated diagnosis and the outcomes of extensive genetic testing.

Genetic diagnosis -The creation of a pedigree and family history are always the first steps. Autosomal recessive inheritance may be present in a patient who has no other afflicted family members but environmental factors causing deafness is taken into consideration. For non-syndromic hearing loss, DNA testing is mandatory as they

include multiple putative causal gene and fewer markers making it tough to detect with DNA.[64] Consequently, the diagnostic application of identifying genes linked to hearing loss has far behind scientific advancement. Advancements in technology, such Next-generation DNA sequencing succeeded in analysing several genes at once. Genetics play a crucial role in enabling personalised patient diagnosis and management [65]. Drawback with extensive genetic testing is the difficulty in interpreting a large number of variations which give important details regarding the potential pathogenicity of genetic variations.

Diagnostics in acquired congenital hearing loss - Amniotic fluid in CMV PCR can be used to prove CMV infection in gestation. Following delivery, the newborn's samples of saliva, urine, or throat swabs should be obtained and examined. Sample of blood is frequently drawn within the first week of birth in many developed nations in order to check for endocrine, metabolic and other problems.[66]

On desiccated blood sites the residual blood is kept. However, local storage regulations may limit the samples availability. Furthermore, the diagnostic sensitivity of these bloodspots is inferior to that of samples of saliva or urine taken in "real-time."MRI, visual function evaluation and hearing examination is necessary in case of suspected congenital CMV infection. [67]

MANAGEMENT

Cytomegalovirus: Antiviral therapy is necessary for clinically evident congenital CMV infection involving the brain. An ideal course of treatment was initially thought to involve six weeks of intravenous ganciclovir or oral valganciclovir while neutropenia was acknowledged as a potential side effect. Several more treatment

strategies that are presently being investigated are immunoglobulin therapy, prophylactic vaccination and antiviral treatment in pregnancy.[68]

Congenital rubella infection - Prevented by taking a prepubertal immunisation, along with other illnesses. Immunisation against haemagglutination inhibition antibody is advised for women of reproductive age and has a negative test result for the antibody, as well as for children aged 12 to 15 months, with a booster shot at age 4-5 years.

HEARING RESTORATION

Conventional hearing aid provides the mainstay treatment in auditory rehabilitation for sensorineural deafness patients. Hearing aids are the primary management for conductive deafness patients when medical or surgery is contraindicated. Notwithstanding these benefits, there are a number of drawbacks to wearing hearing aids such as little perceived benefit, high cost, problems (including blockage of the external auditory canal) and aesthetic issues.[69] Consequently, barely one out of every five persons who qualify does so.[70]. Compared to conventional aid, implants provide greater gains and less distortion. However, its widespread acceptance has been hampered by the requirement for surgical treatment, exorbitant prices and no insurance.

BONE ANCHORED HEARING AIDS - Useful for child with external auditory canal complete bony atresia. Drawbacks - 2.4-38.1 percent of cases reported skin reactions, 18% had failure of osseo integration , 44% in second surgery .[71] Bone-anchored hearing implants are beneficial for people while they are intermediately beneficial for those with typical hearing levels of 45–60 dB . The contralateral routeing of the offside signal aid will wirelessly transmit that sound to a hearing aid

worn in the good ear, while the processor on the side of the deaf ear functions as a microphone, sending sound straight to the hearing ear through the skull bone.

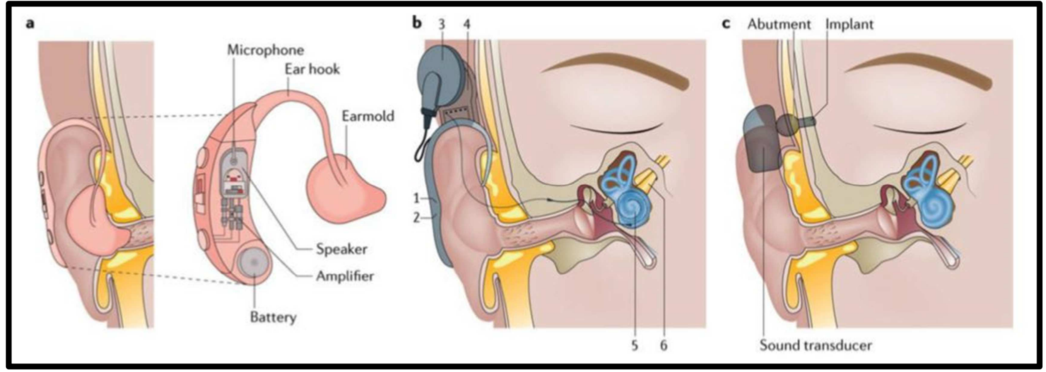


Figure 2 – Bone anchored hearing aids

COCHLEAR IMPLANTS - The best treatment for severe to profound deafness is cochlear implantation which gives sufficient sound quality for meaningful speech understanding. Electrode arrays are introduced in basal turn of cochlea. Nowadays, parents who prefer oral communication for their kid with substantial congenital hearing loss should consider cochlear implantation as the standard of treatment.

THE GENETIC FOUNDATION OF NON-SYNDROMIC HEARING LOSS

The inner ear is highly susceptible to genetic locus alterations, and the function of the inner ear is influenced by several genes because of the inner ear's distinct physiology and structure, which set it apart from other anatomical regions. Cochlea and inner ear problems can result from mutations in genes that regulate hair cell adhesion, ionic homeostasis, hair cell cytoskeletons, intracellular transport, and neurotransmitter release.

GJB2/GJB6 - Globally, Connexin 26 is major reason for hearing loss in a wide range of ethnic groups. It is thought that the cochlea's gap junction protein, GJB2 is

necessary for the recycling of potassium ions that transduce into sensory hair cells. Mutations in GJB2 which impact DFNB3 are the primary reason of hereditary hearing loss. Over 100 GJB2 mutation being discovered in the non-syndromic deafness patients and amount and distribution of these mutations varies by population. However, for the majority of these genotypes, some phenotypic diversity was still seen particularly in 35delG homozygous patients.

In 1999, it was first proposed that GJB6, the gene on chromosome 13 next to GJB2 had a role in nonsyndromic congenital hearing loss . The cochlea expresses the GJB2 and GJB6 genes, which work together to create cell membrane's hemichannels and are essential for the inner ear's potassium control.[72] Allelic variability may exist at the GJB2 locus, as evidenced by documented phenotypic variances among families. GBJ2 is a small gene with 5.5 kilobases. A 4.2Kb mRNA as well as 226 amino acid protein are produced by the gene's two exons. The GJB2 gene's coding region has a six-repeat G at locations 30 to 35.[73] 50 % of patients with GJB2 gene mutations get profound HL with 30% experiencing severe, moderate 20% , 2% mild [74] The 261 amino acid protein connexin 30 expresses the GJB6 gene . The brain, thyroid as well as cochlea are among the bodily tissues that generate connexin 30 [75]. GBJ genes encode connexins which is related to a transmembrane protein family with 20 people in humans [76].Connexins share a similar architecture despite variances in size and primary amino acid makeup. Two exterior and one intracellular loop connect the proteins four transmembrane domain. The cytoplasmic side is where amino as well as carboxyl terminals are present. Connexons are hemichannels created by the oligomerization of six connexins. The gap junction channels are then created by connexons moving through the membrane. Connexins preferentially interact with one another to generate both homomeric and heteromeric channels.

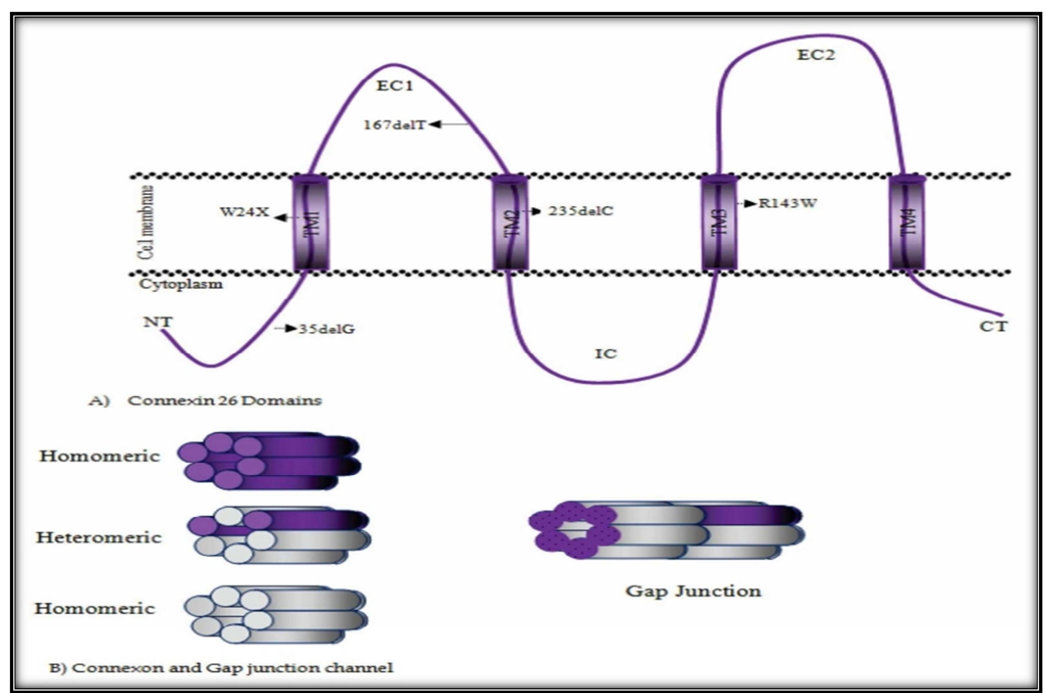


Figure 3 : Schematic structure and domains of connexin 26 protein, Connexon and Gap junction channel

DFNB2 (MYO7A) - Usher syndrome Type 1B is linked with MYO7A mutations, which is located at 11q13 and over a hundred distinct MYO7A mutant alleles have been discovered as of now. [77]

DFNB4(SLC26A4 gene) – On chromosome 7q31, SLC26A4 gene is located and can have recessive mutations that cause sensorineural deafness with [78]or without goitre.[79] This gene is associated with pendred syndrome which caused by cochlear defects such as dilated sacs and ducts of endolymph and swollen vestibular ducts.Goitre usually happens in early adolescence or adult. The majority of those impacted have vestibular impairment.

DFNB7 - Located on chromosome 9q13-q21, HL was discovered to be caused by DFNB7 . Eleven DFNB7/11 families and one DFNA36 family are associated with eight mutations in this gene.[80]

DFNB67 (TMHS gene) - LHFPL5 encoding the 2162 nucleotide mRNA and has four exons. is equivalent to a 219 amino acid protein. A transmembrane domain with four passes is the anticipated protein structure.[81]

NON-SYNDROMIC AUTOSOMAL DOMINANT DEAFNESS

DFNA2 (GJB3) – Encodes connexin 31 gene which is found on chromosomes 1p33–p35 generates the 270 amino acid protein and identified as the reason for DFNA as well as DFNB.

DFNA5 – Located on chromosome 7p15.The DFNA5 genes have been found to contain five known mutations.

DFNA6 - With eight exons, 33.4 kb length ,size of 3.6 kb and located in the DFNA6 locus on chromosome 4p16 and encoding the 890 amino acid polypeptides[82] Wolframin, an endoplasmic plasmareticulum protein have a role in processing, membrane trafficking as well as calcium homeostasis regulation.[83]

DFNA9(COCH gene) - On chromosome 14q12-q13, the DFNA9 gene is linked with vestibular dysfunction and deafness. The extracellular matrix protein cochlin which has Von Willebrand factor A-like domains and the LCCL domain is encoded by COCH.[84]

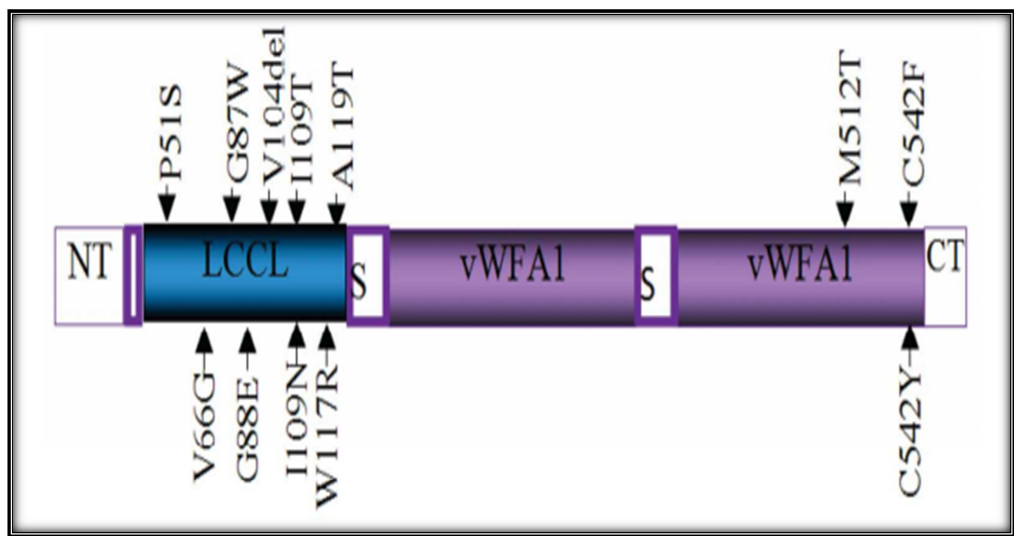


Figure 4 : Cochlin's schematic structure and the locations of mutations across its domains. Two vWF domains and an LCCL domain come after the NT signal peptide. Several cysteine residues are indicated by S, the amino (NH2) and carboxyl (COOH) termini are indicated by NT and CT.

X and Y linked HL - The causative gene is PCDHY which codes for protocadherin.[85]

MITOCHONDRIAL HEARING LOSS – Several genes contribute to mitochondrial HL.[86] Mutations in mitochondrial DNA which produce energy through oxidative phosphorylation, can lead to systemic neurological diseases. Mitochondrial DNA mutations, whether inherited or acquired can lead to various clinical symptoms including myopathy, neuropathy, hyperglycemia, and sensorineural HL.[164] In the homoplasmic state the multiorganic mitochondrial disorders can be fatal. Mitochondrial homoplasmy occurs in LHON (Leber Hereditary Optic Neuropathy) and maternally inherited HL[87]

TABLE 2 - Identified mitochondrial DNA mutations in Deafness

GENE	MUTATION	PHENOTYPE
12SrRNA	1555A->C	Non syndromic deafness /Aminoglycoside induced/ worsened
	1494C->T	Non syndromic deafness /Aminoglycoside induced/worsened
	961	Non syndromic deafness/Aminoglycoside induced/worsened
	1095T>C	Non syndromic deafness /Aminoglycoside induced/parkinsonism and neuropathy
	827A>G	Non syndromic deafness /Aminoglycoside induced
tRNA^{Ser}	7444G>A	Non syndromic deafness /Aminoglycoside induced
	7445A->G	Non syndromic deafness /Palmoplantar Keratoderma
	7472insC	Non syndromic /Neurological dysfunction, including ataxia, dysarthria and myoclonus
	7510T->C	Nonsyndromic deafness / no other manifestations
	7511T->C	Non syndromic deafness / no other manifestations
	7512T>C	Deafness /Progressive myoclonic epilepsy and ataxia
Trna^{Leu}	3243A>G	Maternally inherited diabetes and deafness /MELAS
tRNA^{Lys}	8296A>G	Maternally inherited diabetes and deafness
	8332A>G	Dystonia, stroke like episodes and deafness
tRNA^{Glu}	14709T>C	Maternally inherited diabetes and deafness

Syndromic genetic deafness -

Usher Syndrome - Boughman et al. (1983) state that Usher syndrome which bears the name of British ophthalmologist Charles Usher (1914), accounting 3–5% of autosomal recessive deafness among general people.Yan and Liu (2010) identified clinical subgroups based on HL severity and retinitis pigmentosa onset .[88]

TABLE 3 - Genes linked to Usher syndrome

LOCUS	GENES
11q13.5	MYO7A
10q22.2	CDH23
10q21-22	PCDH15
17q24-25	SANS
USH2A (1q41)	USH2A
USH2D (9q32)	WHRN
USH3 (3q21-q25)	USH3A
10q24.31	PDZD7

Pendred syndrome: Coined by British physician Vaughan Pendred (1896) is the prevalent syndromic form of HL linked to goitre. About 4–10% of deaf people have this autosomal recessive disorder. SLC26A4 is located on chromosome 7q31 and codes for the pendrin protein. [89]

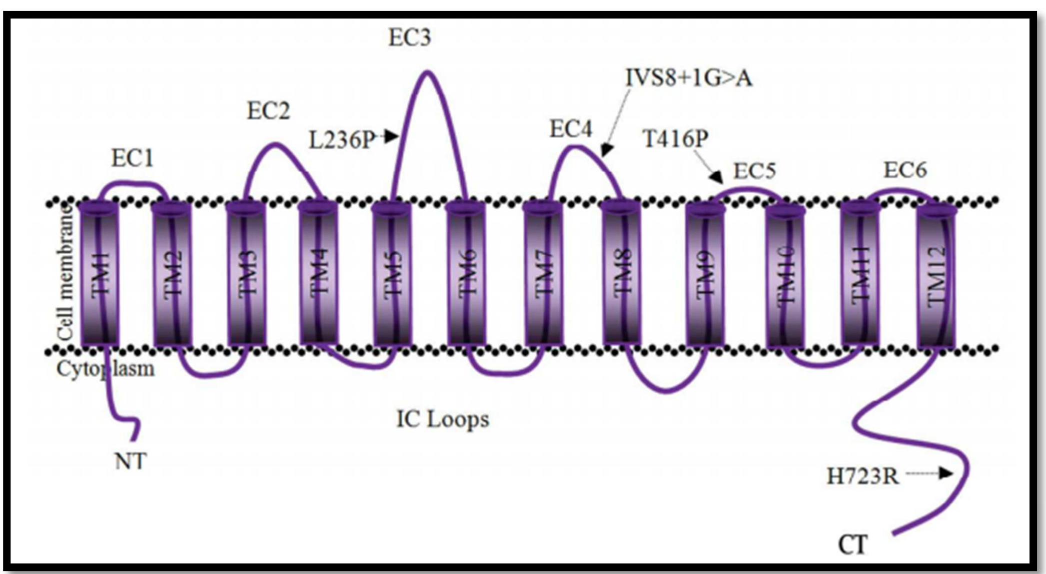


Figure 5 : Hypothetic structure and domains of pendrin protein. The leading mutations which make up 60% of the entire PS genetic load are displayed: L236P, IVS8+1G>A, T416P and H723R. Transmembrane domains are indicated by TM1–TM12, extracellular domains by ECI-6, transmembrane domains by IC, amino (NH2) terminus by NT and carboxyl (COOH) terminus by CT.

TABLE 4 - Summary of syndromic genes

Mode of Inheritance	Syndrome	Locus/Gene
Autosomal dominant	Neurofibromatosis 2	NF2
		EYA2,3
		SIX1,5
	Treacher collins	TCOF
	Stickler syndrome	COL2A1
		STL2COL11A2
		STL3/COL11A1
		STL4/COL9A1
		STL5/COL9A2
	Waardenburg syndrome	PAX3
		MITF
		SNA12
		EDN3
		EDNRB
Autosomal recessive	Pendred syndrome	PDS/SLC26A4
	Jervell and Lange Nielson syndrome	JLNS1/KCNQ1
		JLNS2/KCNE1
	Usher syndrome	MYO7A
		USHC,1D,1E,1F
		SANS
		CIB2
		2A,2C,2D,3A,3B
	Refsum disease	PHYH/PAHX
		PEX7
X-Linked dominant	Alport syndrome	COL4A5,A3,A4
Mitochondrial	MELAS	MTTL
	MERRF	MTTK

Next generation sequencing and hearing loss: unlocking genetic mysteries

Tailored diagnostic procedures are necessary for genetically connected disorders such as hearing impairment to identify their molecular causes. Frederick Sanger developed the DNA sequencing technique in 1975, which was a watershed moment in this field of study.[90] Direct sequencing or Sanger sequencing can identify the disease-causing genes in a small number. During the last decade, next-generation sequencing (NGS) has transformed genetic research and diagnostics by reducing DNA sequencing costs and time. The complete genome or hundreds of genes might be sequenced in a few days which can analyze more than a million base pairs in a single experiment. [91]

The most popular NGS techniques for finding pathogenic variants linked to deafness are whole exome sequencing and multigene panels. The WES offers the advantage of sequencing the entire coding DNA sequence (about 20,000 protein-coding genes). Because of this, WES may be used to diagnose almost every known genetic condition. Multigene panel sequencing provides a cost-effective diagnostic solution. Panel sequencing analyses the known genes related with a certain disease.

Targeted NGS offers benefits but whole-genome sequencing (WGS) remains the most advanced method, despite its high cost. In the area of genetics linked HL unique genes have been found using NGS technology for all of the patterns of inheritance listed above. NGS technology has successfully identified genes that were wrongly categorised as pathogenic.[92]

There are numerous commercial NGS tests available for clinical diagnostics, each with its own set of technology and gene counts. In the past, 20 commercially available test for genetically related hearing loss performed by NGS technology. [93]

Due to the ongoing decrease in prices and accessibility NGS-based diagnostic approaches will become the norm in the near future considerably improving the level of patient care.

MATERIALS AND METHODS

Methodology:

Material and Methods

Source of data – Children who came to ENT and HNS OPD with complaints of congenital hearing loss

Study design – Case and Control group

Study period – One year

Sample size - As per formula

$$n = \frac{\gamma+1}{\gamma} \frac{\left(z\frac{\alpha}{2} + z-\frac{\beta}{2}\right)^2 \; p*(1-p*)}{(\overline{p1}-\overline{p2})^2}$$

$\gamma = ratio$ of control to cases
P1=proportion in cases
P2= proportion in control
P*=average proportion in exposed

$$n = \frac{1+1}{1} \frac{(2+1.037)2 \; (12.5)(87.5)}{(25)^2}$$

$$n = \frac{1+1}{1} \frac{(3.037)2 \; (12.5)(87.5)}{(25)^2}$$

$$n = \frac{1+1}{1} \frac{(9.223 \; x \; 1093.75)}{(625)^2} = \frac{(2x10087.65)}{(625)}$$

$n=32.28$

So Minimum Required sample size= nx2= 32 case group + 32 control group =64

Inclusion criteria : **1-** Patients with congenital deafness

2-Age group from 2-18years

Exclusion criteria: **1-** Patients who had acquired deafness due to environmental factors like infections, trauma, ototoxic medications .

2. Patient who didn't give consent for study

Identifying Genetic Markers for Non-Syndromic Deafness

The present study aimed to determine genetic markers related with congenital deafness among two groups: deaf children born to consanguineous marriages (n=32) and deaf children born to non-consanguineous marriages (n=32). The genetic focus was on the *GJB2* gene , specifically the rs104894396 variant (chr13:20189511, GRCh38.p14), which involves a C>T single nucleotide variation (SNV).Involvement of this gene mutation is one of the responsible cause for congenital deafness so our genetic focus was on GJB2 gene .Samples of blood were taken from consenting children for extraction of DNA, amplification, and genotyping.

1. **Sample Collection:** Blood samples were collected from 64 deaf children, divided into two groups: those born to consanguineous marriages (n=32) and those born to non-consanguineous marriages (n=32). EDTA tubes were used to ensure that the blood samples remained unclotted and the genetic material preserved for downstream processing.
2. **DNA Isolation:** The QIAamp DNA Blood Mini Kit (Cat. No. 51106) is employed to extract genomic DNA from blood samples. Proteinase K was added to facilitate protein digestion, and Buffer AL was used to lyse cells, releasing the DNA into solution. Ethanol was then added to encourage the

silica membrane's DNA attachment in the QIAamp Mini spin columns. Sequential washing with Buffer AW and Buffer AW2 removed impurities, including proteins and other cellular components. Finally, DNA was eluted using Buffer AE, ensuring the recovery of high-purity genomic DNA for further analysis.

3. **DNA Quality Check (QC):** Quality as well as concentration of the isolated DNA were assessed using a NanoPhotometer (N-60 touch, Implen, Germany). The A260/A280 ratio was checked to confirm DNA purity, ensuring it was free from significant contamination by RNA or proteins. DNA concentration measurements ensured sufficient template availability for PCR amplification.
4. **PCR Amplification:** The region of the *GJB2* gene containing the rs104894396 variant was amplified using specific primers. The forward primer (TCTTTTCCAGAGCAAAC CGC) and reverse primer (GACACGAAGATCAGCTGCAG) were used in a reaction mixture that included Taq DNA Polymerase 2x Master Mix RED, template DNA (50 ng), and water of PCR quality. A 286 bp product was produced for analysis by denaturation-related thermal cycling circumstances, annealing at 54°C and extension.
5. **Restriction Digestion:** The PCR result was broken down with the enzyme AluI, which recognizes and cuts specific sequences at the variant site. To guarantee thorough digestion, the reaction mixture was incubated for one to two hours at 37°C. Digested fragments of varying sizes indicated the genotype (C/C, C/T or T/T) based on the presence of specific nucleotide changes.
6. **Gel Electrophoresis:** Ethidium bromide-stained 3% agarose gel was used to assess the PCR results. The gel was run under standard conditions, and DNA

fragments were visualized under UV light. Fragment sizes were used to determine the genotype of each sample.

7. **Data Analysis:** Genotype distributions and the two groups' allele frequencies were evaluated to assess the association between the variant and non-syndromic deafness. The role of consanguinity was evaluated to identify its contribution to genetic susceptibility.

Explanation in detail:

1. **Sample Collection**

Patient histories were collected by reviewing via conversations with parents. Demographics, onset, pre- and postnatal history and related ENT clinical examination findings, including pure tone audiometry, tympanometry, auditory brainstem response, otoacoustic emissions, and computed tomography and magnetic resonance imaging were all obtained from medical records. Patients' medical records were also examined for other potential causes of SNHL. Patients having an acquired aetiology (such as preterm, meningitis, hyperbilirubinemia, trauma or ototoxic medication exposure) were excluded, as were those with confirmed congenital illnesses. Auditory neuropathy was detected using otoacoustic emission and auditory brainstem response, which are routine tests at our center.

After obtaining consent, parents were asked if they were blood relatives (first cousins who "shared the same grandparent," or second cousins who "shared the same great grandparent," or the same tribe), had a family history of hearing loss (paternal or maternal), had a number of children with congenital SNHL, had education and socioeconomic status, and were aware of the impact of consanguineous marriage on offspring hearing prior to marriage.

Blood samples were collected from 64 deaf children of nonsyndromic congenital hearing loss These participants were divided into two groups: 32 children born to consanguineous marriages and 32 children born to non-consanguineous marriages. The two groups (consanguineous vs nonconsanguineous) was analysed on various factors, including (1) Degree of consanguinity (2) The percentage of child with congenital SNHL (3) Family history (4) Type of genotype (5) Type of allele

The blood samples were drawn using sterile venipuncture techniques and exchanged right away into tubes coated with EDTA to maintain the stability of the genetic material and avoid clotting. Participants were recruited with informed consent obtained from their guardians. To preserve samples integrity, all samples were shipped on ice and kept at -20°C until DNA isolation.

2. DNA Isolation

The QIAamp DNA Blood Mini Kit (Cat. No. 51106) was used to obtain genomic DNA from the blood samples that were collected. The following protocol was followed to ensure high-quality DNA extraction:

a. Proteinase K Treatment:

- A microcentrifuge tube of 1.5mL was prepared with Proteinase K of 20 µL to digest proteins in the blood sample.
- 200 µL volume of blood was put into the tubes ensuring sufficient sample availability for DNA extraction.
- The sample and enzyme were mixed thoroughly.

b. Cell Lysis:

- To guarantee thorough mixing, 200 µL of Buffer AL was added to the tube and vortexed for 15 seconds.
- To lyse cells the mixture was incubating them for 10 minutes at 56°C and release the DNA into the solution.

c. DNA Binding Preparation:

- Ethanol (200 µL, 96–100%) were incorporated into lysate and mixed thoroughly to promote attachment of DNA onto the spin column's silica membrane.

d. **DNA Binding to Spin Column:**

- QIAamp mini spin column was carefully positioned in a 2 ml containing tube with produced lysate
- For one minute, centrifugation was carried out in 6000x g (8000 rpm) to enable the DNA binding to the column membrane

e. **Washing Steps**:

- To eliminate contaminants, 500 µL of buffer AW was introduced to column and centrifuged for one minute at 6000 x g.
- To guarantee complete washing, 500 µL of buffer AW2 was introduced, and the column was centrifuged for three minutes at full speed (20,000 x g).

f. **Elution:**

To elute the DNA, 200 µL of buffer AE have been introduced into spin column after it had been moved to a sterile 1.5 mL microcentrifuge tubes .

- The pure DNA was collected by centrifuging the column at 6000 x g for one minute following a one-minute incubation period at room

temperature. For additional examinations, the extracted DNA is maintained at -20°C.

3. **DNA Quality Check (QC)**

The isolated DNA was of good quality and quantity was assessed by a NanoPhotometer (N-60 touch, Implen, Germany). Absorbance was measured at 260nm and 280nm to evaluate the quality of DNA. The A260/A280 ratio was calculated, with a worth around 1.8 indicating purity of DNA. Concentration of DNA was recorded to ensure that sufficient amounts were available for PCR amplification.

4. **PCR Amplification**

The *GJB2* gene region containing the rs104894396 variant was magnified using PCR .Steps were performed as follows:

1. **Primer Design:**
    - Forward primer: TCTTTTCCAGAGCAAACCGC.
    - Reverse primer: GACACGAAGATCAGCTGCAG.
    - These primers targeted the specific 286 bp region of the *GJB2* gene containing the variant.
2. **Reaction Preparation:**
    - PCR was set up in a total volume of 25 µL containing:
        - ♣ Taq DNA Polymerase 2x Master Mix RED (Ampliqon, 5200300) – 12.5 µL.
        - ♣ Forward primer (1.0 µM) – 0.5 µL.
        - ♣ Reverse primer (1.0 µM) – 0.5 µL.
        - ♣ Template DNA (50 ng) – 1 µL.
        - ♣ PCR-grade water – 10.5 µL.

3. **Thermal Cycling Conditions:**
    - Denaturation at 95°C in 3 minutes.
    - 35 cycles of:
        - ♣ Denaturation at 95°C for 30 seconds.
        - ♣ Annealing at 54°C for 30 seconds.
        - ♣ Extension at 72°C for 30 seconds.
    - Finally 72°C for 5 minutes. The amplified PCR products are stored at 4°C.
4. **Restriction Digestion -** The restriction enzyme AluI was used to analyze the PCR results for restriction fragment length polymorphism.

The digestion process included:

1. **Reaction Setup:**
    - A mixture containing the PCR product, **AluI** enzyme, and the appropriate buffer was prepared.
    - To guarantee full digestion, the reaction has been incubated for one to two hours at 37°C.
2. **Expected Fragment Sizes:**
    - **C/C (wild type):** 274 bp and 14 bp.
    - **C/T (heterozygous):** 247 bp, 168 bp, 104 bp, and 14 bp.
    - **T/T (homozygous mutant):** 168 bp, 104 bp, and 14 bp.

6. Gel Electrophoresis

Digested PCR products was examined utilizing 3% agarose gel electrophoresis. Ethidium bromide is put to prepare gel so that DNA could be seen under a UV lamp

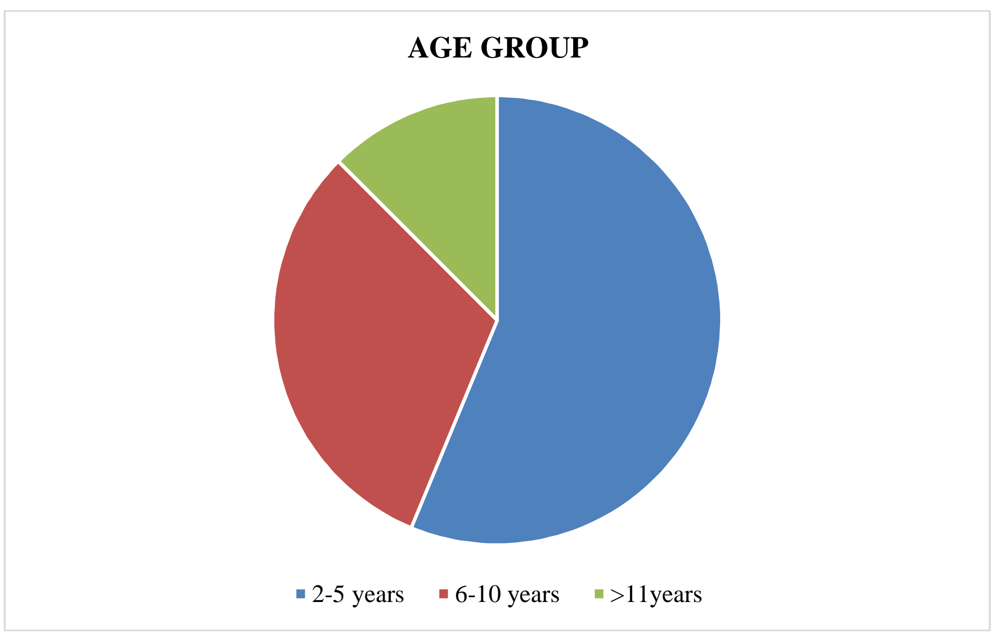
RESULTS

A total of 64 samples were analyzed. 32 cases of Consanguineous marriage and 32 cases of nonconsanguinous marriage were considered. All patients who presented to the OPD of Department of Otorhinolaryngology and Head & Neck surgery, at Jawaharlal Nehru Medical College, KAHER.

All results are studied and described under following headings: Age and Sex Distribution: Out of a total of 64 cases , the distribution of data by age group looked like this: Thiry six patients were identified in the age range of 2-5 years, Twenty cases were observed within the age bracket of 6-10 years, and the remaining eight cases were reported among individuals aged >11 years. This distribution provides insight into the prevalence of congenital hearing loss in consanguineous and nonconsanguineous marriage across various age demographics, indicating a notable representation of cases in the 2-5 years age range, followed by those in early teenage years the late childhood with mean 21.33 and standard deviation of 11.47

Table 5 – According to age distribution

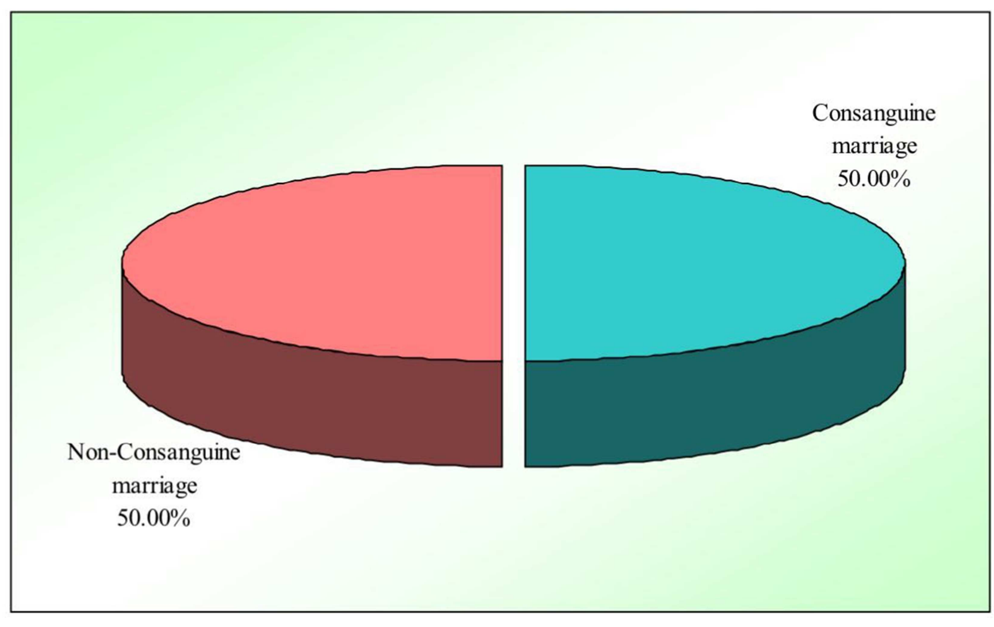
Age group	Number	Percentage
Two to 5 years	36	56.25
6 to ten years	20	31.25
More 11 years	8	12.50
Total	64	100.00
Mean	21.33	
SD	11.47	



GRAPH 1 : Age wise distribution

Table 6 : Type of parental marriage wise distribution and incidence of congenital hearing loss

Type of parental marriage	Number	Percentage
Consanguineous marriage	32	50.00
Non-Consanguineous marriage	32	50.00
Total	64	100.00

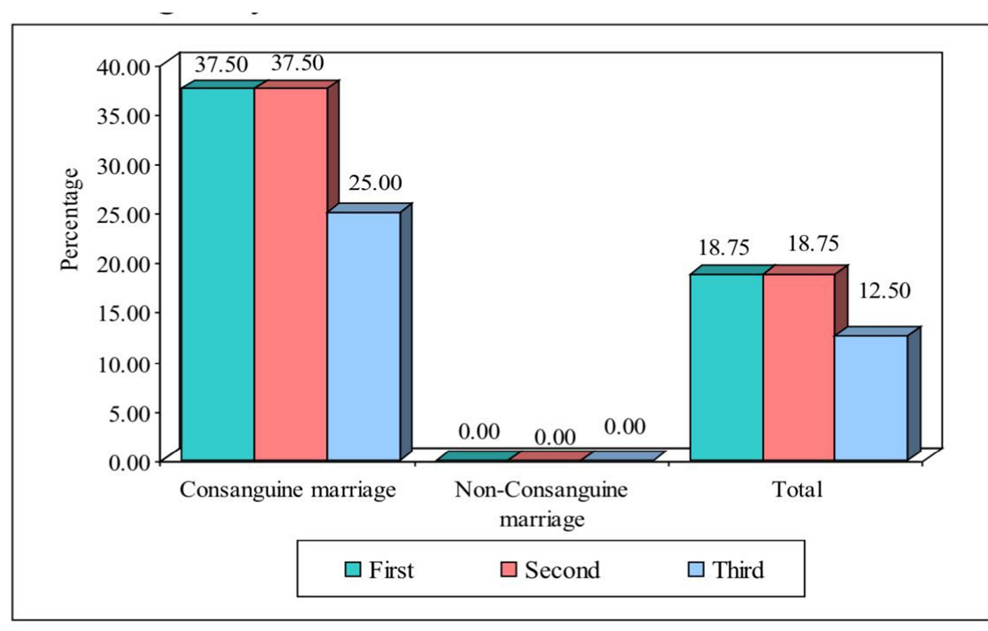


GRAPH 2: Type of parental marriage wise distribution

Table 7 : Comparison of type of parental marriage with degree of consanguinity

Degree of Consanguinity	Consanguineous marriage	%	Non-Consanguineous marriage	%	Total	%
Nil	0	0.00	32	100.00	32	50.00
First	12	37.50	0	0.00	12	18.75
Second	12	37.50	0	0.00	12	18.75
Third	8	25.00	0	0.00	8	12.50
Total	32	100.00	32	100.00	64	100.00
Chi-square=64.0000, p=0.0001*						

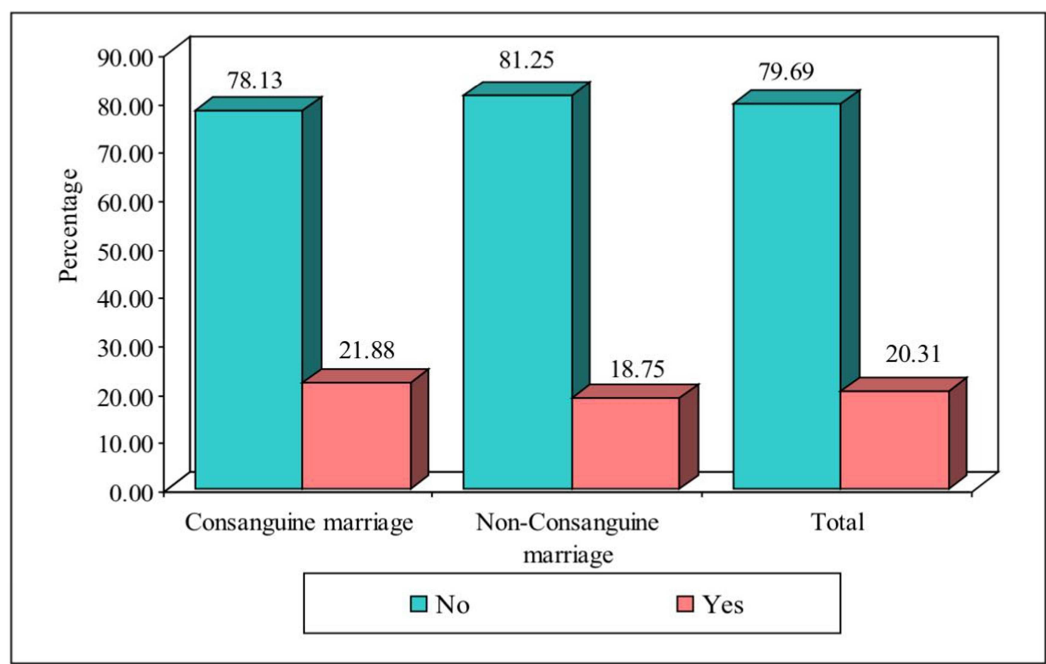
*p<0.05



GRAPH 3 : Comparison of type of parents marriage with degree of consanguinity

Table 8 : Comparison of type of parental marriage with Family history

Family history	Consanguineous marriage	%	Non-Consanguineous marriage	%	Total	%
No	25	78.13	26	81.25	51	79.69
Yes	7	21.88	6	18.75	13	20.31
Total	32	100.00	32	100.00	64	100.00
Chi-square=0.0970, p=0.7560						



GRAPH 4 : Comparison of type of parental marriage with Family history

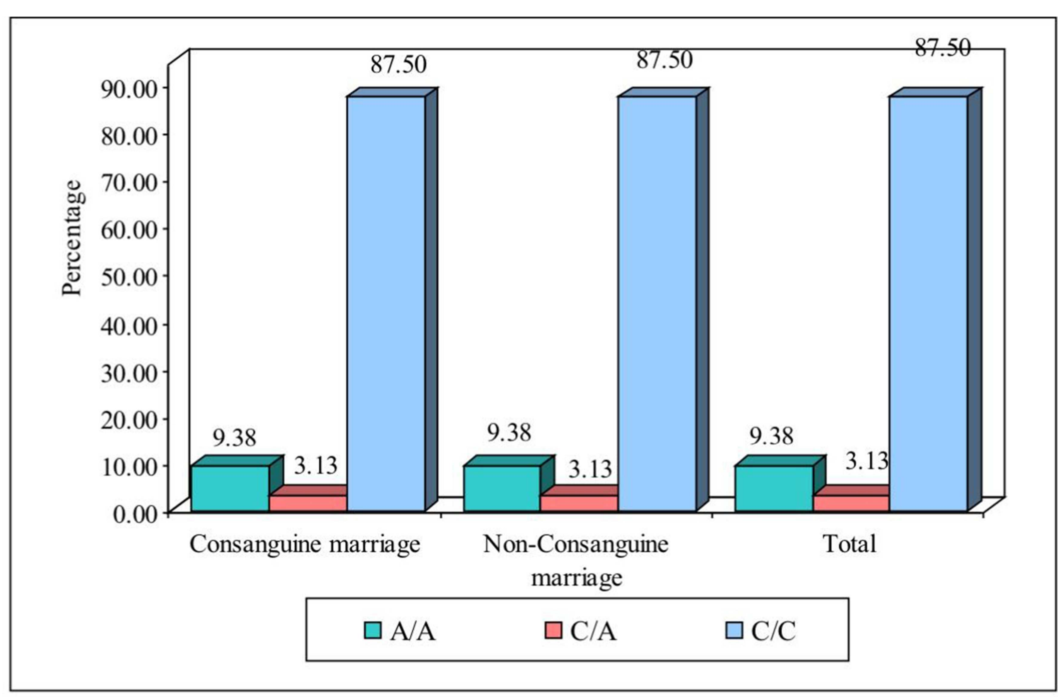
Table 9 : Comparison of type of parental marriage with Genotype Type

Genotype Type	Consanguinous marriage	%	Non-Consanguinous marriage	%	Total	%
Alt/Alt	3	9.38	3	9.38	6	9.38
Ref/Alt	1	3.13	1	3.13	2	3.13
Ref/Ref	28	87.50	28	87.50	56	87.50
Total	32	100.00	32	100.00	64	100.00
Chi-square=0.0001, p=1.0000						

GRAPH 5 : Comparison of type of parental marriage with Genotype Type

Table 10 : Comparison of type of parental marriage with Alleles

Alleles	Consanguinous marriage	%	Non-Consanguinous marriage	%	Total	%
A/A	3	9.38	3	9.38	6	9.38
C/A	1	3.13	1	3.13	2	3.13
C/C	28	87.50	28	87.50	56	87.50
Total	32	100.00	32	100.00	64	100.00
Chi-square=0.0001, p=1.0000						



GRAPH 6 : Comparison of type of parental marriage with Alleles

1. Descriptive Statistics

- **Sample Size:**
  - Total subjects: 64
  - **Consanguineous Group:** 32 subjects
  - **Non-consanguineous Group:** 32 subjects
  - The incidence of congenital hearing loss is 100% (64 patients)
- **Age Distribution:**
  - **Consanguineous:**
    - Age varied between 2 to 16 years
    - Mean age ≈ 10.3 years
  - **Non-consanguineous:**
    - Ages varied between 2 to 15 years
    - Mean age ≈ 8.7 years
  - *Note:* A formal t-test (or Mann–Whitney U test, given possible non-normality) can be used to assess if this age difference is statistically significant.

2. Genotype Distribution (rs104894396)

Genotypes were determined by PCR-RFLP and are noted as:

- **C/C (Wild-type homozygote)**
- **C/A (Heterozygote)**
- **A/A (Mutant homozygote)**

Overall (n = 64):

- C/C: 56 subjects (87.7%)
- C/A: 2 subjects (3.1%)
- A/A: 6 subjects (9.2%)

By Group:

Group	C/C	C/A	A/A	Total
Consanguineous (32)	28 (87.5%)	1 (3.1%)	3 (9.4%)	32
Non-consanguineous (32)	28 (87.9%)	1 (3.0%)	3 (9.1%)	32

- **Analysis:**

  A chi-square or Fisher's exact test among the both shows virtually identical genotype frequencies ($p \approx 1.0$), indicating significant difference between children born of both groups regarding this SNV is nil .

3. Allele Frequencies

Since each subject carries two alleles, total alleles = $64 \times 2 = 128$.

- **Calculation:**
  - *C Allele:*
    - From C/C: $57 \times 2 = 114$
    - From C/A: $2 \times 1 = 2$
    - Total C = $114 + 2 = 116$
  - *A Allele:*
    - From C/A: $2 \times 1 = 2$
    - From A/A: $6 \times 2 = 12$
    - Total A = $2 + 12 = 14$
- **Frequencies:**
  - Frequency of C $\approx 116/130 = 89.2\%$
  - Frequency of A $\approx 14/130 = 10.8\%$

4. Hardy–Weinberg Equilibrium (HWE)

Using the observed allele frequencies (p = 0.1077 for A and q = 0.8923 for C), the expected genotype frequencies would be:

- **Expected Proportions:**
  - A/A (p^2): $(0.1077)^2 \approx 0.0116$
  - C/A (2pq): $2 \times 0.1077 \times 0.8923 \approx 0.1920$
  - C/C (q^2): $(0.8923)^2 \approx 0.7962$
- **Expected Counts (n = 64):**
  - Expected A/A ≈ 0.0116 × 64 ≈ 0.74
  - Expected C/A ≈ 0.1920 × 64 ≈ 12.2
  - Expected C/C ≈ 0.7962 × 64 ≈ 50.9
- **Observed Counts:**
  - A/A = 6, C/A = 2, C/C = 57
- **Chi-square Calculation:**

χ2=(57–51.75)251.75+(2–12.5)212.5+(6–0.75)20.75\chi^2 = \frac{(57 - 51.75)^2}{51.75} + \frac{(2 - 12.5)^2}{12.5} + \frac{(6 - 0.75)^2}{0.75}χ2=51.75(57–51.75)2+12.5(2–12.5)2+0.75(6–0.75)2

  - For C/C: (57–51.75)2/51.75≈(5.25)2/51.75≈0.53(57 - 51.75)^2/51.75 \approx (5.25)^2/51.75 \approx 0.53(57–51.75)2/51.75≈(5.25)2/51.75≈0.53
  - For C/A: (2–12.5)2/12.5≈(10.5)2/12.5≈8.82(2 - 12.5)^2/12.5 \approx (10.5)^2/12.5 \approx 8.82(2–12.5)2/12.5≈(10.5)2/12.5≈8.82
  - For A/A: (6–0.75)2/0.75≈(5.25)2/0.75≈36.75(6 - 0.75)^2/0.75 \approx (5.25)^2/0.75 \approx 36.75(6–0.75)2/0.75≈(5.25)2/0.75≈36.75
  - **Total $\chi^2 \approx 0.53 + 8.82 + 36.75 = 46.1$**

- **Degrees of Freedom:** 1 (for a biallelic marker)
- **Interpretation:**

  The very high chi-square value ($\chi^2 \approx 46.1$) with one degree of freedom provides a p-value < 0.001, showing a notable variation from Hardy–Weinberg equilibrium.

  Possible reasons include:

  - The study population is not a random sample from the general population , All samples have congenital deafness.
  - There may be selection bias or association of the SNV with the disease phenotype.

5. Association with History of family hearing loss

- **Family History (Yes/No):**
  - In both groups, all children with a reported family history (7 in consanguineous, 6 in non-consanguineous) had the C/C genotype.
  - In contrast, the few individuals with variant alleles (C/A or A/A) were found only among those without a positive family history.
- **Interpretation:**

  While the numbers are very small there does not appear to be an connection between the existence of the A allele and family history of deafness in this sample.

DISCUSSION

This study has found a substantial link between consanguineous marriages and deafness. Information about the risk variables of deafness will be of significant therapeutic importance since it can give families and medical professionals important information about the etiologic, other related health issues and the likelihood of recurrence in a subsequent pregnancy.[94] Consanguinity and family history suggest that genetic aetiology may be involved. Two Latin terms, "con0" (common) and "sanguineus" (blood) are the roots of the word "consanguinity." Two close relatives have a marriage relationship[95].

Approximately 20% of people globally currently reside in societies that favor consanguineous marriage.Over 50% of congenital deafness is hereditary with autosomal recessive inheritance being the commonest cause. Autosomal recessive inheritance is strongly linked to consanguinity. It raises the likelihood that the offspring will share the faulty gene which raises the likelihood that hearing loss will run in the family. In 2nd and 3rd degree consanguinity, the percentage of gene sharing is 25% and 12.5% respectively [96,97]. The present study is done to ascertain the genetic association of consanguinity as well as family history in congenital hearing loss. Our study population consisted of 64 individuals with nonsyndromic congenital hearing loss were half of the affected children (50%) were born to consanguineous parents, while the other half had non-consanguineous parents. Among consanguineous marriages, the breakdown of the degree of consanguinity showed that 37.5% of cases involved first-degree relatives, 37.5% involved second-degree relatives, and 25% involved third-degree relatives. These results indicated a statistically a very significant correlation among consanguineous marriages and degree of consanguinity

($p<0.05$). This strong correlation suggests that the closeness association among parents, the higher chance of likelihood of congenital hearing loss in their offspring.

Comparatively, Selvarajan et al.[98] studied 210 infants with permanent hearing impairment and examined the genetic relationship between consanguinity and family history and congenital hearing impairment. They found a very significant correlation ($p=0.000$) and an odds ratio of 3.7 indicating that consanguineous marriage almost quadruples the risk of hearing impairment. They also examined the risk associated with varying degrees of consanguinity demonstrating that second-degree consanguinity carries a higher risk than third-degree. Similar to our study, in the first and second Saudi Arabian population surveys between 45 and 47 percent of respondents were consanguineous, where the prevalence of inherited sensorineural hearing impairment in that same cohort ranged as 66% and 37% respectively [99]. According to comprehensive nationwide research conducted in Oman[100], 70% of deaf children had parents who were consanguineous.

According to a recent Iranian study, 61.4% of the population with hearing impairments was consanguineous[101]. Additionally, they discovered that consanguineous marriages were common among 77.1% of people with more than one disabled child in the family. According to a school-based study conducted in South India 41% of students with hearing impairments had consanguineous parents[102]. Almazroua et al. in another larger trial where 189 children with congenital hearing deafness participated[103] revealed that 69.3% of the impacted children's parents were direct cousins and 83.1% had parents who were blood related. With an odds ratio of 4.8 their study also shows that consanguineous parents had a considerably increased chance of having numerous children with congenital hearing loss ($p=0.005$). The

thorough analysis of consanguinity levels in their work was one of its main advantages.

According to their findings, first cousin marriages were 3.5 several times more probable to result in many children among CHL than non-consanguineous unions. The risk persisted even when second and third cousins were eliminated (RR: 1.4, OR: 1.6). Examining several impacted children in the same family was a significant difference in Almazroua et al.[104] study. They observed that compared to 6.25% of non-consanguineous parents, 31.4% of parents with consanguineous had presented congenital hearing loss in several children (p=0.005). In addition to consanguinity, hearing impairment can be directly influenced by a family history of early hearing impairment. This specific medical history can help lead the diagnosis and determine whether further testing is necessary. Family history is always the first piece of information gathered for genetic testing.

Understanding the pattern of inheritance of hearing impairment is beneficial. When analyzing the relationship between parental marriage type and family history of congenital hearing impairment in our study, the data indicated that 79.69% of affected individuals had no reported family history, while 20.31% had a positive family history. Among children with consanguineous parents, 21.88% had a familial history of hearing loss whereas 18.75% of those with non-consanguineous parents had a family history. This finding showed no significant association between consanguinity and family history suggesting that family history alone may not be a strong predictor of congenital hearing impairment when compared to the impact of consanguinity. Similar to present results, Almazroua et al.reported that 20.6 percent and 22.8 percent of affected children had familial history of hearing loss on the father and mother sides.[105].

Selvarajan et al carried out a study[106] found that 6.5 odds ratio which indicates that a positive family history raises the risk by 6.5 times and a very significant correlation (p=0.000) between hearing impairment and family history. Additionally, they found that with an odds ratio of 21, newborns that have both consanguinity and family history are at the highest risk. Additionally, examining the degree of deafness, they observed that family history had a modest correlation (r=0.68) in their study. This risk factor was present in about 30% of children with hearing impairment, which led to the strongest correlation between it and other risk factors such infections, low birth weight, and premature birth. After extraction, the risk estimate seems to be 67 in their study. According to a Karnataka study [107], 25% of infants with deafness having a familial history of the condition. 38% of hearing-impaired children in Kerala had a familial background of the ailment [108]. These statistics show how important it is to raise awareness of a treatable cause of deafness within India. In the present study, the genotype distribution among affected individuals was consistent across both consanguineous and non-consanguineous marriage groups. The majority of individuals (87.50%) had the Ref/Ref genotype, while 9.38% had the Alt/Alt genotype, and only 3.13% had the Ref/Alt genotype, which indicated that GJB2 gene allele is present in both groups and has no statistically significant association between parental marriage type and genotype distribution.

Given the small numbers of heterozygotes and mutant homozygotes, larger research is warranted to ascertain the possible involvement in congenital deafness and to explore whether the observed HWE deviation is driven by disease association or other factors. The mutant A allele is relatively rare (≈10.8%) in this study sample. The marked deviation from Hardy–Weinberg equilibrium ($p < 0.001$) likely reflects the

fact that the study sample is a selected group of congenitally deaf children rather than a random sample of the general population.

Genotype and allele frequencies were nearly identical between children born to consanguineous and non-consanguineous parents suggesting that parental consanguinity does not influence the distribution of this SNV among the deaf children in this study. Hence no consanguinity effect on genotype and GJB2 genetic mutation is present in both groups and is one of the cause of nonsyndromic congenital hearing loss .This also suggest that genes related to congenital hearing loss is complex and may involve various genes as well as environmental variables beyond those analyzed in this study.

LIMITATIONS

Our study had few limitations. The study size (n=64) is comparatively modest, could restrict the findings that are generalizable to larger groups. The study focuses primarily on consanguinity and family history without considering other potential environment and genes variables that influence to congenital hearing loss. Additionally, genetic analysis is limited to a specific set of genotypes and alleles of connexin 26 , potentially overlooking other relevant genetic mutations. The study also relies on reported family history which could be impacted by recollection bias. Finally, the lack of detailed environmental exposure data limits thorough comprehension of the multifactorial nature of congenital hearing loss. Future research with larger cohorts and broader genetic screening is needed to validate these findings.

CONCLUSION

The study we conducted highlights the significant connection among consanguineous marriages and congenital deafness demonstrating that a higher degree of parental consanguinity correlates with an increased likelihood of the condition. Incidence of congenital hearing loss in our study was 100% (64 patients) who are nonsyndromic congenital hearing loss .The statistical analysis shows that among congenital hearing loss in children, 50% were born to parents in consanguineous marriages, with a significant proportion being first- and second-degree relatives. These finding underscores genetic hazards connected to consanguinity, as it increases the likelihood of acquiring recessive genes mutations that contribute to hearing impairment. However, despite the strong link between consanguinity and congenital hearing loss our study finds no significant association between parental marriage type and family history of hearing impairment.

The majority of affected individuals (79.69%) lacked a reported families with hearing problems suggesting that many cases may arise due to spontaneous genetic mutations or unrecognized hereditary patterns.

Additionally, the study finds no significant relationship between consanguinity and specific genotype types or allele distributions. The genotype and allele frequencies were nearly identical in compared two groups with Ref/Ref and C/C variants being the most prevalent. *GJB2* gene specifically the rs104894396 variant (chr13:20189511, GRCh38.p14), which involves a C>T single nucleotide variation (SNV) is present in both consanguineous and non-consanguineous group indicating that mutation in GBJ2 gene is one of cause responsible for nonsyndromic congenital deafness in our study as same as other studies which had been published in literatures

by other authors .This implies that while consanguinity increases the overall genetic risk, it does not necessarily determine the presence of specific genetic variants responsible for the condition.

Overall, the results of this analysis highlight the significance of antenatal screening, genetic counselling as well as public awareness, especially in communities where consanguineous marriages are common. Early genetic screening and audiological assessments for children born to consanguineous parents could help in the initial identification as well as management in congenital deafness . Furthermore, universal neonatal hearing screening must be made a necessary component of the National Health Mission, which should cover factors such as family history of deafness and consanguinity.

SUMMARY

Hearing loss is a critical public health concern particularly in developing countries where its prevalence can range from 1-3 per 1,000 newborns. Hereditary factors account for over half of these congenital cases making it essential to understand the genetic association and risk factors associated with hearing impairment. This study investigates the prevalence of congenital deafness in children from both consanguineous and non-consanguineous marriages with a specific focus on the genetic associations linked to the GJB2 gene (connexin 26) which is known to play a significant role in hereditary hearing loss.

Objectives is to identify genetic association for congenital deafness in children born to consanguineous marriages and those with a family history of hearing loss and also to determine the incidence of congenital hearing loss within the study population.A case-control study was conducted over one year involving 64 children aged 2-18 years who presented with nonsyndromic congenital hearing loss at the ENT and HNS outpatient department. The sample comprised 32 children from consanguineous marriages and 32 from non-consanguineous marriages.Blood samples were collected for DNA extraction and genotyping specifically targeting the rs104894396 variant of the GJB2 gene, which is associated with non-syndromic hearing loss.Results stated that the mean age of participants was 21.33 years with a significant proportion (56.25%) aged between 2-5 years indicating that congenital hearing loss is most prevalent in early childhood. Half of the children (50%) were born to consanguineous parents, revealing a significant correlation between consanguinity and congenital hearing loss ($p < 0.05$). This finding underscores the genetic risks associated with consanguineous marriages where the likelihood of inheriting recessive genetic traits increases.

A family history of hearing loss was reported in 20.31% of the cases however, no significant association was found between family history and consanguinity suggesting that many cases of congenital hearing loss may arise from spontaneous genetic mutations rather than inherited patterns.

Genotype analysis revealed that the majority of children (87.5%) had the Ref/Ref genotype, with no significant difference in genotype distribution between the two groups ($p = 1.000$). This indicates that the presence of the GJB2 gene variant does not differ significantly between children from consanguineous and non-consanguineous marriages. The allele frequency analysis showed that the C allele was present in approximately 89.2% of the samples, while the A allele was found in 10.8%. A significant deviation from Hardy-Weinberg equilibrium ($p < 0.001$) was observed, suggesting that the study population may not represent a random sample of the general population potentially due to selection bias.

The findings of this study highlight a significant association between consanguineous marriages and congenital deafness emphasizing the need for increased awareness and early intervention strategies in communities where consanguinity is prevalent. While consanguinity raises the overall genetic risk for hearing loss, it does not necessarily predict the presence of specific genetic variants associated with the condition. The study underscores the importance of genetic counselling and early screening for hearing loss, particularly for children born to consanguineous parents. Future research should focus on larger cohorts and broader genetic screening to further elucidate the complex genetic landscape of congenital hearing loss, as well as the potential environmental factors that may contribute to this condition.

BIBLIOGRAPHY

1. Morton NE. Genetic epidemiology of hearing impairment. Ann N Y Acad Sci. 1991;630:16-31.
2. World Health Organization. Deafness and hearing loss [Internet]. Fact sheet. 2021 [cited 2025 Mar 24].
3. Selvarajan HG. Association of family history and consanguinity with permanent hearing impairment. Indian J Otol. 2013;19.
4. Alberti PW. Anatomy and physiology of ear and hearing. In: Occupational exposure to noise: Evaluation, prevention and control. 2001:51-63.
5. Sheffield AM, Smith RJH. The epidemiology of deafness. Cold Spring Harb Perspect Med. 2019 Sep.
6. Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, et al. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature. 1997;387:80-3.
7. Kikuchi T, Adams JC, Paul DL, Kimura RS. Gap junction systems in the rat vestibular labyrinth: Immunohistochemical and ultrastructural analysis. Acta Otolaryngol. 1994;114:520-8.
8. Maheswari 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. 2003;120:180-4.
9. Stelma F, Bhutta MF. Non-syndromic hereditary sensorineural hearing loss: Review of the genes involved. J Laryngol Otol. 2014;128(1):13-21. doi: 10.1017/s0022215113003265.

10. Goodwin S, McPherson JD, McCombie WR. Coming of age: Ten years of next-generation sequencing technologies. Nat Rev Genet. 2016;17:333-51. doi: 10.1038/nrg.2016.

11. Hamamy H. Consanguineous marriages. J Community Genet. 2011;3(3):185-92. doi: 10.1007/s12687-011-0072-y. PMC 3419292. PMID 22109912.

12. Tadmouri GO, Nair P, Obeid T, Al Ali MT, Al Khaja N, Hamamy HA. Consanguinity and reproductive health among Arabs. Reprod Health. 2009;6:17.

13. Modell B, Darr A. Science and society: Genetic counselling and customary consanguineous marriage. Nat Rev Genet. 2002;3:225-9.

14. Bittles AH. The role and significance of consanguinity as a demographic variable. Popul Dev Rev. 1994;20:561-84.

15. Jaber L, Halpern GJ, Shobat M. The impact of consanguinity worldwide. Public Health Genomics. 1998;1:12-7.

16. Bener A, et al. Consanguineous marriage and their effects on common adult diseases: Studies from an endogamous population. Med Princ Pract. 2007;16(4):262-7.

17. Tuncbilek E. Clinical outcomes of consanguineous marriages in Turkey. Turk J Pediatr. 2001;43(4):277-9.

18. Kumaramanickavel G, Joseph B, Vidhya A, Arokiasamy T, Shridhara SN. Consanguinity and ocular genetic diseases in South India: Analysis of a five-year study. Community Genet. 2002;5(3):182-5.

19. Bellad M, Goudar S, Edlavitch S, Mahantshetti N, Naik V, Hemingway-Foday J, et al. Consanguinity, prematurity, birth weight, and pregnancy loss: A prospective cohort study at four primary health center areas of Karnataka, India. J Perinatol. 2012;32:431-7.

20. Kundu S, Jana A. Consanguineous marriage and associated diseases among their children and grandchildren in India: Evidence from large-scale data. J Biosoc Sci. 2024;1-13.
21. Mahdieh N, Rabbani B, Wiley S, Akabari MT, Zeinali S. Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations. J Hum Genet. 2010;55(10):639-48.
22. Tekin M, Arnos KS, Pandya A. Advances in hereditary deafness. Lancet. 2001;358(9287):1082-90.
23. Fortnum H, Davis A. Epidemiology of permanent childhood hearing impairment in Trent Region, 1985-1993. Br J Audiol. 1997;31:409-46.
24. Fortnum HM, Summerfield AQ, Marshall DH, Davis AC, Bamford JM. Prevalence of permanent childhood hearing impairment in the United Kingdom and implications for universal neonatal hearing screening: Questionnaire-based ascertainment study. BMJ. 2001;323:536-40.
25. Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, et al. Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature. 1995;374:60-1.
26. Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, et al. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat Genet. 1997;17:411-22.
27. Yoshinaga-Itano C, Sedey AL, Coulter DK, Mehl AL. Language of early- and later-identified children with hearing loss. Pediatrics. 1998;102:1161-71.
28. Smith RJH, Shearer AE, Hildebrand MS, et al. Deafness and hereditary hearing loss overview. In: Pagon RA, Adam MP, Ardinger HH, editors. GeneReviews [Internet]. Seattle: University of Washington; 1999 Feb 14:1993-2015.

29. Matsunaga T, Hirota E, Bito S, Niimi S, Usami S. Clinical course of hearing and language development in GJB2 and non-GJB2 deafness following habilitation with hearing aids. Audiol Neurootol. 2006;11:59-68.
30. Sinnathuray AR, Raut V, Awa A, Magee A, Toner JG. A review of cochlear implantation in mitochondrial sensorineural hearing loss. Otol Neurotol. 2003;24:418-26.
31. Usami S, Abe S, Kasai M, et al. Genetic and clinical features of sensorineural hearing loss associated with the 1555 mitochondrial mutation. Laryngoscope. 1997;107:483–90.
32. Matsunaga T, Kumanomido H, Shiroma M, Ohtsuka A, Asamura K, Usami S. Deafness due to A1555G mitochondrial mutation without use of aminoglycoside. Laryngoscope. 2004;114:1085–91.
33. Matsunaga T. Value of genetic testing in the otological approach for sensorineural hearing loss. Keio J Med. 2009;58:216–22.
34. Sinnathuray AR, Raut V, Awa A, Magee A, Toner JG. A review of cochlear implantation in mitochondrial sensorineural hearing loss. Otol Neurotol. 2003;24:418–26.
35. Hussain et al. 2001; Banerjee & Roy, 2002; Saggar et al. 2008; Mumtaz et al. 2010; Bellad et al. 2011; Kuntla et al. 2013; Fareed et al. 2017.
36. Banerjee & Roy 2002; Kuntla et al. 2013; Fareed et al. 2017; Islam 2013; Teeuw et al. 2014; Oniya et al. 2019; Anwar et al. 2020.
37. Sharma RK, Golub JS, Patel VM, Lin FR. Prevalence and severity of hearing loss in the older old population. JAMA Otolaryngol Head Neck Surg. 2020;146(8):762-764. doi:10.1001/jamaoto.2020.0888.

38. Metgud CS, Naik VA, Mallapur MD. Factors affecting birth weight of a newborn – A community-based study in rural Karnataka, India. PLoS ONE. 2012;7(7):e40040.

39. Banerjee & Roy 2002; Kuntla et al. 2013; Fareed et al. 2017; Islam 2013; Teeuw et al. 2014; Oniya et al. 2019; Anwar et al. 2020.

40. Srinivasan & Lee. The dowry system in Northern India: women's attitudes and social change. J Marriage Fam. 2004.

41. Kalam MA, Ghosh MDG. Marital practices among a group of Muslim community living in coastal Odisha. EBIOS. 2021;1:46–53.

42. Friedman TB, Griffith AJ. Human nonsyndromic sensorineural deafness. Annu Rev Genomics Hum Genet. 2003;4:341-402.

43. Nikolopoulos TP. Auditory dyssynchrony or auditory neuropathy: understanding the pathophysiology and exploring methods of treatment. Int J Pediatr Otorhinolaryngol. 2014;78:171–3..

44. Sloan-Heggen CM, Bierer AO, Mustapha M, Wiszniewski W, Shylo A, Elfenbein JL, et al. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. Hum Genet. 2016;135(4):441-50.

45. Mahdieh N, Rabbani B, Wiley S, Akabari MT, Zeinali S. Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations. J Hum Genet. 2010;55(10):639–48.

46. Watkin P, Baldwin M. The longitudinal follow-up of a universal neonatal hearing screen: the implications for confirming deafness in childhood. Int J Audiol. 2012;51:519–28.

47. Bittles AH, Black ML. Evolution in health and medicine Sackler colloquium: consanguinity, human evolution, and complex diseases. Proc Natl Acad Sci U S A. 2010;107(Suppl 1):1779–86.

48. Van Dommelen P, Mohangoo AD, Verkerk PH, van der Ploeg CP, van Straaten HL. Risk indicators for hearing loss in infants treated in different neonatal intensive care units. Acta Paediatr. 2010;99:344–9.

49. Koenighofer M, Parzefall T, Ramsebner R, Lucas T, Frei K. Delayed auditory pathway maturation and prematurity. Wien Klin Wochenschr. 2014.

50. Marazita ML, et al. Genetic epidemiological studies of early-onset deafness in the U.S. school-age population. Am J Med Genet. 1993;46:486–91.

51. Smith RJ, Bale JF, White KR. Sensorineural hearing loss in children. Lancet. 2005;365(9462):879–90.

52. Levy SE, Myers RM. Advancements in next-generation sequencing. Annu Rev Genom Hum Genet. 2016;17:95–115. doi: 10.1146/annurev-genom-083115-022413.

53. Bittles AH. The role and significance of consanguinity as a demographic variable. Popul Dev Rev. 1994;20:561–84.

54. Hamamy H. Consanguineous marriages. J Community Genet. 2011;3(3):185–92. doi:10.1007/s12687-011-0072-y.

55. Modell B, Darr A. Science and society: genetic counselling and customary consanguineous marriage. Nat Rev Genet. 2002;3:225–9.

56. Hamamy H, Antonarakis SE, Cavalli-Sforza LL, Sharif SM, Teeuw ME, Bittles AH. Consanguineous marriages, pearls and perils: Geneva International Consanguinity Workshop report. Genet Med. 2011;13:841–7.

57. Bamiou DE, MacArdle B, Bitner-Glindzicz M, Sirimanna T. Aetiological investigations of hearing loss in childhood: a review. Clin Otolaryngol Allied Sci. 2000;25:98–106. doi: 10.1046/j.1365-2273.2000.00346.x

58. Kumaramanickavel G, Joseph B, Vidhya A, Arokiasamy T, Shridhara SN. Consanguinity and ocular genetic diseases in South India: analysis of a five-year study. Community Genet. 2002;5(3):182–5.

59. Lakhan R, Bipeta R, Yerramilli SR, Nahar VK. A family study of consanguinity in children with intellectual disabilities in Barwani, India. J Neurosci Rural Pract. 2017;8(4):551–5.

60. Bhasin MK, Nag S. Consanguinity and its effects on fertility and child survival among Muslims of Ladakh in Jammu and Kashmir. Anthropologist. 2017;4(2):131–40.

61. Boudewyns A, et al. Otitis media with effusion: an underestimated cause of hearing loss in infants. Otol Neurotol. 2011;32:799–804.

62. Rapin I, Gravel JS. Auditory neuropathy: a biologically inappropriate label unless acoustic nerve involvement is documented. J Am Acad Audiol. 2006;17:147–50.

63. Willems PJ. Gene localization and isolation in nonsyndromic hearing loss. In: Willems PJ, editor. Genetic Hearing Loss. New York: Marcel Dekker Inc.; 2004. p. 203.

64. Modell B, Darr A. Science and society: genetic counselling and customary consanguineous marriage. Nat Rev Genet. 2002;3:225–9.

65. Mahdieh N, Rabbani B, Wiley S, Akabari MT, Zeinali S. Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations. J Hum Genet. 2010;55(10):639–48.

66. Dror AA, Avraham KB. Hearing loss: mechanisms revealed by genetics and cell biology. Annu Rev Genet. 2009;43:411–37.

67. Willems PJ. Gene localization and isolation in nonsyndromic hearing loss. In: Willems PJ, editor. Genetic Hearing Loss. New York: Marcel Dekker Inc.; 2004. p. 203.

68. Tekin M, Arnos KS, Pandya A. Advances in hereditary deafness. Lancet. 2001;358(9287):1082–90.

69. Fortnum H, Davis A. Epidemiology of permanent childhood hearing impairment in Trent Region, 1985–1993. Br J Audiol. 1997;31:409–46.

70. Fortnum HM, Summerfield AQ, Marshall DH, Davis AC, Bamford JM. Prevalence of permanent childhood hearing impairment in the United Kingdom and implications for universal neonatal hearing screening: questionnaire-based ascertainment study. BMJ. 2001;323:536–40.

71. van Dommelen P, Verkerk PH, van Straaten HL. Hearing loss by week of gestation and birth weight in very preterm neonates. J Pediatr. 2015.

72. Morle L, Bozon M, Alloisio N, Latour P, Vandenberghe A, Plauchu H, et al. A novel C202F mutation in the connexin26 gene (GJB2) associated with autosomal dominant isolated hearing loss. J Med Genet. 2000;37:368–70.

73. Kelley PM, Harris DJ, Comer BC, Askew JW, Fowler T, Smith SD, et al. Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. Am J Hum Genet. 1998;62:792–9.

74. Smith RJ, Hone S. Genetic screening for deafness. Pediatr Clin North Am. 2003;50(2):315–29.

75. Grifa A, Wagner CA, D'Ambrosio L, Melchionda S, Bernardi F, Lopez-Bigas N, et al. Mutations in GJB6 cause nonsyndromic autosomal dominant deafness at DFNA3 locus. Nat Genet. 1999;23(1):16–8.

76. Holms RH, Steel KP. Genes involved in deafness. Curr Opin Genet Dev. 1999;9:309–14.

77. Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, et al. Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature. 1995;374:60–1.

78. Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, et al. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat Genet. 1997;17:411–22.

79. Li XC, Everett LA, Lalwani AK, Desmukh D, Friedman TB, Green ED, et al. A mutation in PDS causes non-syndromic recessive deafness. Nat Genet. 1998;18:215–7.

80. Kurima K, Peters LM, Yang Y, Riazuddin S, Ahmed ZM, Naz S, et al. Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. Nat Genet. 2002;30(3):277–84.

81. Shabbir MI, Ahmed ZM, Khan SY, Riazuddin S, Waryah AM, Khan SN, et al. Mutations of human TMHS cause recessively inherited non-syndromic hearing loss. J Med Genet. 2006;43(8):634–40.

82. Hofmann S, Philbrook C, Gerbitz KD, Bauer MF. Wolfram syndrome: structural and functional analyses of mutant and wild type wolframin, the WFS1 gene product. Hum Mol Genet. 2003;12(16):2003–12.

83. Fonseca SG, Ishigaki S, Oslowski CM, Lu S, Lipson KL, Ghosh R, et al. Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. J Clin Invest. 2010;120(3):744–55.

84. Collin RW, Pauw RJ, Schoots J, Huygen PL, Hoefsloot LH, Cremers CW, et al. Identification of a novel COCH mutation, G87W, causing autosomal dominant hearing impairment (DFNA9). Am J Med Genet A. 2006;140(16):1791–4.

85. Wang QJ, Lu CY, Li N, Rao SQ, Shi YB, Han DY, et al. Y-linked inheritance of non-syndromic hearing impairment in a large Chinese family. J Med Genet. 2004;41(6):e80.

86. Fischel-Ghodsian N. Mitochondrial deafness mutations reviewed. Hum Mutat. 1999;13:261–70.

87. Finsterer J, Fellinger J. Nuclear and mitochondrial genes mutated in nonsyndromic impaired hearing. Int J Pediatr Otorhinolaryngol. 2005;69(5):621–47.

88. Hope CI, Bundey S, Proops D, Fielder AR. Usher syndrome in the city of Birmingham—prevalence and clinical classification. Br J Ophthalmol. 1997;81(1):46–53.

89. Fraser GR. Association of congenital deafness with goitre (Pendred's syndrome): a study of 207 families. Ann Hum Genet. 1965;28:201–49.

90. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol. 1975;94(3):441–8.

91. Demkow U, Ploski R. Clinical Applications for Next-Generation Sequencing. USA: Academic Press; 2015.

92. Oldak M, Ruszkowska E, Pollak A, Sobczyk-Kopciol A, Kowalewski C, Piwonska A, et al. A note of caution on the diagnosis of Martin-Probst syndrome by the detection of the p.D59G mutation in the RAB40AL gene. Eur J Pediatr. 2015;174(5):693–6.

93. Ołdak M. Chapter 8—Next generation sequencing in vision and hearing impairment. In: Clinical Applications for Next-Generation Sequencing. Boston: Academic Press; 2016. p. 153–70.

94. Vohr BR, Widen JE, Cone-Wesson B, Sininger YS, Gorga MP, Folsom RC, et al. Identification of neonatal hearing impairment: Characteristics of infants in

the neonatal intensive care unit and well-baby nursery. Ear Hear. 2000;21:373–82.

95. Modell B, Darr A. Science and society: Genetic counselling and customary consanguineous marriage. Nat Rev Genet. 2002;3:225–9.
96. Tadmouri GO, Nair P, Obeid T, Al Ali MT, Al Khaja N, Hamamy HA. Consanguinity and reproductive health among Arabs. Reprod Health. 2009;6:17.
97. Verma IC. Burden of genetic disorders in India. Indian J Pediatr. 2000;67(12):893–8.
98. Kingston HM. ABC of clinical genetics: Genetics of common disorders. BMJ. 1989;298(6678):949–52.
99. Selvarajan HG, Arunachalam RK, Bellur R, Mandke K, Nagarajan R. Association of family history and consanguinity with permanent hearing impairment. Indian J Otol. 2013;19:62–5.
100. Zakzouk S. Consanguinity and hearing impairment in developing countries: A custom to be discouraged. J Laryngol Otol. 2002;116:811–6.
101. Khabori MA, Patton MA. Consanguinity and deafness in Omani children. Int J Audiol. 2008;47:30–3.
102. Amini SR, Kamali M. Consanguineous marriage among the parents of hearing-impaired students in Mashhad. Iran Rehabil J. 2010;8:36–9.
103. Suresh M. Prenatal and postnatal conditions shared by children born hard of hearing and enrolled in an early intervention programme in Tamil Nadu, India. Unpublished PhD thesis. Madras University, Chennai; 2008.
104. D’Mello J. High risk register: An economical tool for early identification of hearing loss. Indian J Pediatr. 1995;62:731–5.

105. Nagapoornima P, Ramesh A, Srilakshmi, Rao S, Patricia PL, Gore M, et al. Universal hearing screening. Indian J Pediatr. 2007;74:545–9.

106. Joseph AY, Rasool TJ. High frequency of connexin 26 (GJB2) mutations associated with nonsyndromic hearing loss in the population of Kerala, India. Int J Pediatr Otorhinolaryngol. 2009;73:437–43.

107. Almazroua AM, Alsughayer L, Ababtain R, Al-Shawi Y, Hagr AA. The association between consanguineous marriage and offspring with congenital hearing loss. Ann Saudi Med. 2020;40(6):456–61.

108. Selvarajan et al Selvarajan HG, Arunachalam RK, Bellur R, Mandke K, Nagarajan R. Association of family history and consanguinity with permanent hearing impairment. Indian J Otol. 2013;19(2):62-65.

ANNEXURES

ANNEXURE – I - INFORMED CONSENT FORM

"GENETIC ASSOCIATION OF CONSANGUINITY AND FAMILY HISTORY IN CONGENITAL HEARING LOSS"

Name of Student/Principal Investigator:

Name of Guide/Co Investigators:

Objective: To know the genetic association of consanguinity and family history in congenital hearing loss.

To know the incidence of congenital hearing loss

Explanation of procedure: After taking informed consent from the patient and their parents, demographic details of all the patients will be recorded, predesigned proforma and thorough clinical history will be obtained. ENT examinations were done and 2ml blood samples were taken for DNA extraction using polymerase chain reaction.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact: BE0122012 If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "Genetic association of consanguinity and family history in congenital hearing loss". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE II - PROFORMA FOR DATA COLLECTION

"GENETIC ASSOCIATION OF CONSANGUINITY AND FAMILY HISTORY IN CONEGNITAL HEARING LOSS"

Date: I.P. No:

Name: Occupation:

Age: Phone No:

Sex:

Address:

CLINICAL PROFILE:

Chief Complaint:

History of Present Illness: Past History:

Personal History:

Family History:

I) General Physical Examination -

Blood Pressure:

Pulse:

Respiratory Rate

Pallor

Icterus

Cyanosis

Clubbing

Lymphadenopathy

Odema

II) ENT Examination

1. EAR EXAMINATION:

	Right	Left
Pinna		
Pre auricular area		
Post auricular area		
Tragal Tenderness		
Mastoid Tenderness		
External auditory canal		
Tympanic membrane	RIGHT Right	LEFT Left

TUNING FORK TESTS:

Rinne’s test:

Weber’s test:

Absolute Bone Conduction test :

2. NOSE EXAMINATION:

External appearance

Root

Bridge

Dorsum

Ala

Tip

Columella

Vestibule

Cold spatula test -

Anterior Rhinoscopy -

Posterior Rhinoscopy

Paranasal Sinus Examination

	Right	Left
Frontal Sinus tenderness		
Ethmoidal Sinuses tenderness		
Maxillary Sinus tenderness		

3. THROAT EXAMINATION:

Oral cavity:

Lips

Gingivolabial sulcus

Buccal mucosa

Gingivobuccal sulcus

Gums

Teeth

Anterior 2 /3 rd of tongue

Floor of mouth

Hard palate

Retromolar trigone

Oropharynx:

Soft palate

Uvula

Anterior pillar

Tonsil

Posterior pillar

Posterior pharyngeal wall

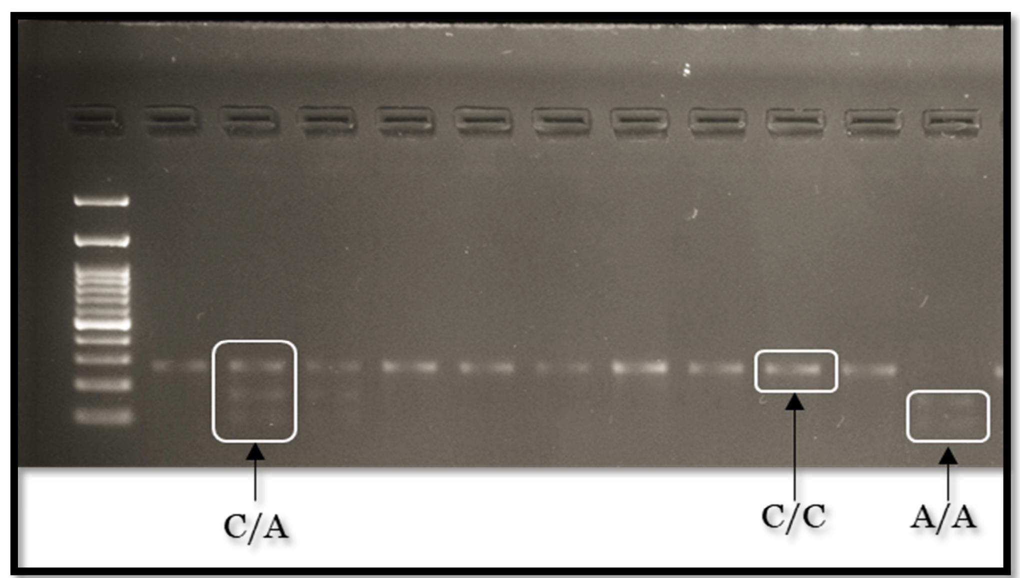
Indirect Laryngoscopy

DIAGNOSIS

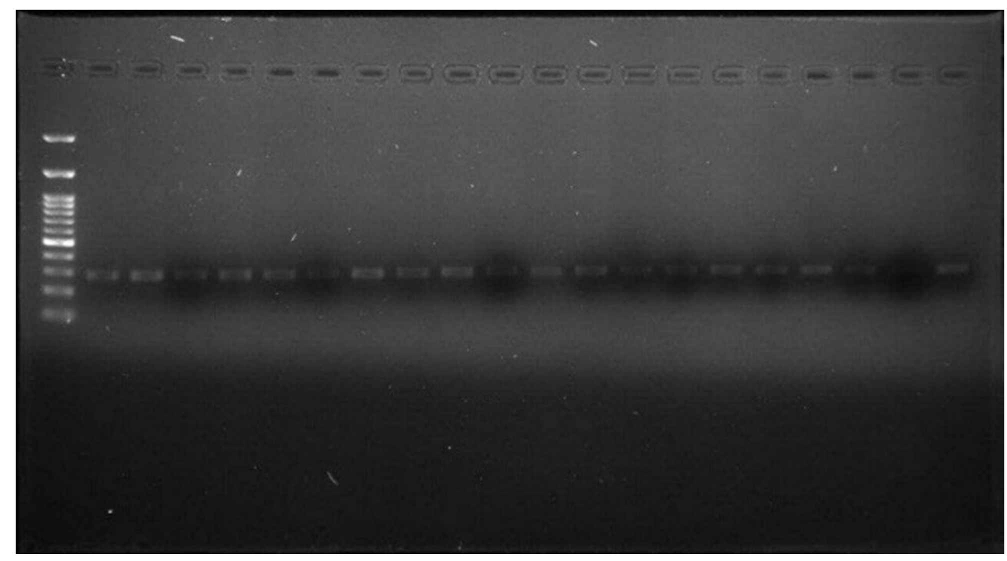
ANNEXURE III- PHOTOGRAPHS

PHOTOGRAPH 1: PHOTOGRAPH OF SAMPLE COLLECTION IN EDTA TUBE

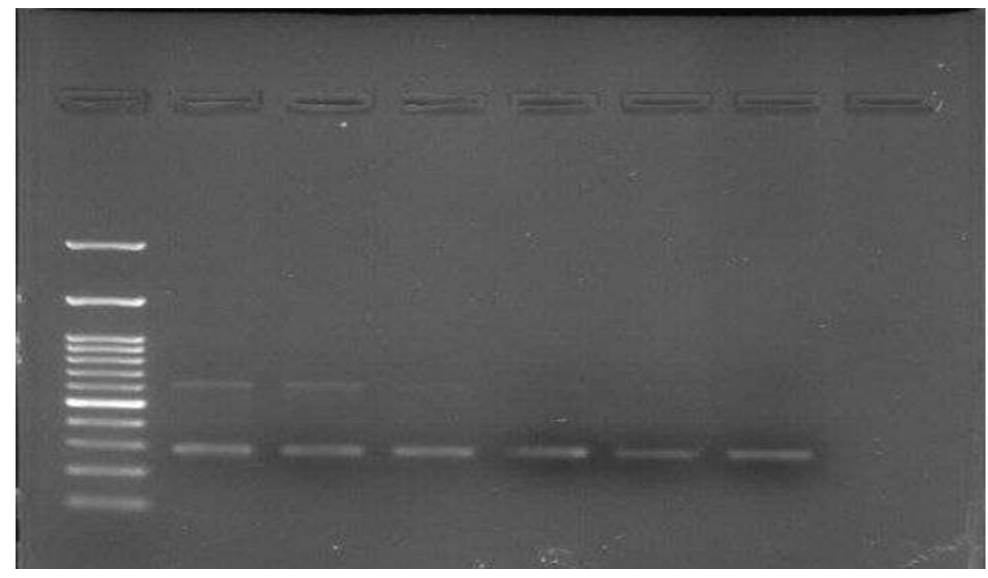
PHOTOGRAPH 2: PHOTORAPH OF RAW GEL IMAGES OF GENOMIC DNA ALLELE TYPES ON POLYMERASE CHAIN REACTION



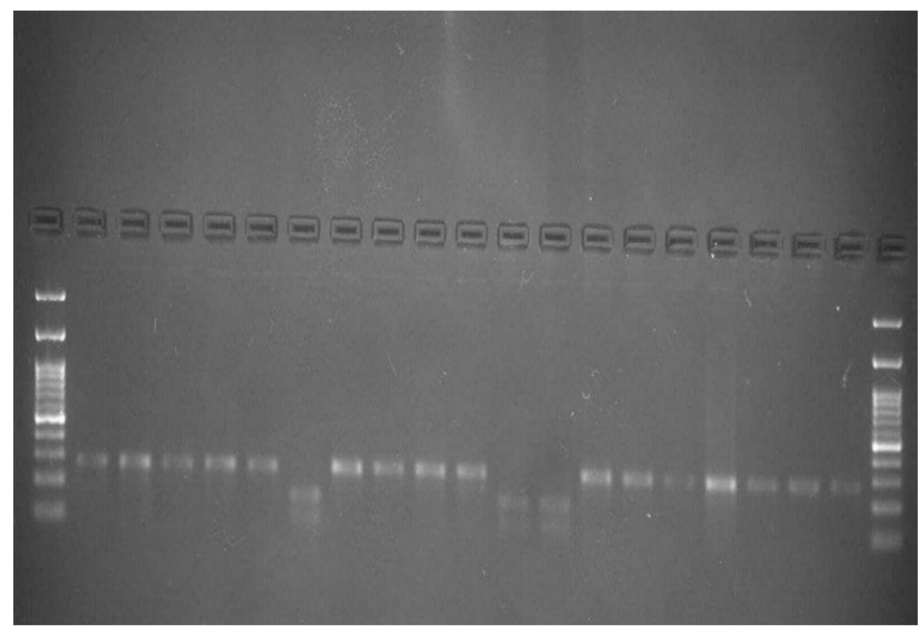
PHOTOGRAPH 3 – AGAROSE GEL (1.2%) SHOWING PCR AMPLICATION OF GJB2 GENE



PHTOGRAPH 4 – AGAROSE GEL (0.8%) SHOWING THE GENOMIC DNA



PHOTGRAPH 5 – AGAROSE GEL (1.5%) SHOWING THE RESTRICTION FRAGMENT LENGTH POLYMERISATION OF PCR PRODUCT



ANNEXURE IV- KEY TO MASTERCHART

AGE	IN YEARS
SEX	MALE AND FEMALE
GROUPS TYPE OF PARENTAL MARRIAGE	CONSANGUINITY / NON-CONSANGUINITY
DEGREE OF CONSANGUINITY	$1^{ST}/2^{ND}/3^{RD}$ /NIL
FAMILY HISTORY	PRESENT / ABSENT
GENOTYPE	REF/REF ; REF/ALT ; ALT/ALT
ALLELE	C/C ; C/A ; A/A

ANNEXURE V- MASTERCHART

S.N.	IP NO	AGE	SEX	PARENTAL MARRIAGE	DEGREE OF CONSANGUINITY	FAMILY HISTORY PRESENT / NOT	GENOTYPE	ALLELE
1	1009684	7	MALE	Consanguinous marriage	FIRST	NO	Ref/Ref	C/C
2	1186218	4	MALE	Non-Consanguinous marriage	NIL	NO	Ref/Alt	C/A
3	1186614	4	MALE	Consanguinous marriage	SECOND	NO	Ref/Alt	C/A
4	1188225	10	FEMALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
5	1187743	3	FEMALE	Consanguinous marriage	FIRST	YES	Ref/Ref	C/C
6	1187709	9	FEMALE	Consanguinous marriage	FIRST	NO	Ref/Ref	C/C
7	118267	3	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
8	1187004	6	FEMALE	Consanguinous marriage	THIRD	NO	Ref/Ref	C/C
9	1186219	5	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
10	1186972	11	FEMALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
11	1187078	3	FEMALE	Non-Consanguinous marriage	NIL	NO	Alt/Alt	A/A
12	1186961	4	FEMALE	Consanguinous marriage	FIRST	YES	Ref/Ref	C/C
13	1188972	8	FEMALE	Consanguinous marriage	THIRD	NO	Alt/Alt	A/A
14	1188983	2	FEMALE	Consanguinous marriage	FIRST	YES	Ref/Ref	C/C
15	1187007	5	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
16	1179489	4	FEMALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
17	1183081	3	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
18	1183469	5	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
19	1184329	4	FEMALE	Consanguinous marriage	THIRD	NO	Ref/Ref	C/C
20	1184233	4	MALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
21	1184865	3	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
22	1184980	5	FEMALE	Consanguinous marriage	THIRD	NO	Ref/Ref	C/C
23	1185654	2	FEMALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
24	1183056	4	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
25	1161977	10	MALE	Non-Consanguinous marriage	NIL	NO	Alt/Alt	A/A
26	1163584	9	FEMALE	Consanguinous marriage	FIRST	YES	Ref/Ref	C/C
27	1163455	6	FEMALE	Non-Consanguinous marriage	NIL	YES	Ref/Ref	C/C
28	1163800	4	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
29	1161959	5	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
30	1163458	3	FEMALE	Consanguinous marriage	THIRD	NO	Alt/Alt	A/A
31	1163762	10	MALE	Consanguinous marriage	THIRD	NO	Alt/Alt	A/A
32	1162680	5	MALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
33	1163803	8	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
34	1164275	11	MALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
35	1165808	3	MALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
36	1163726	4	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
37	1165805	12	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
38	1166468	4	FEMALE	Consanguinous marriage	FIRST	YES	Ref/Ref	C/C
39	1166763	3	FEMALE	Non-Consanguinous marriage	NIL	NO	Alt/Alt	A/A
40	1166458	2	FEMALE	Non-Consanguinousmarriage	NIL	NO	Ref/Ref	C/C
41	1166762	12	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
42	1169152	5	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
43	1161354	8	MALE	Non-Consanguinous marriage	NIL	YES	Ref/Ref	C/C
44	1179625	5	FEMALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
45	1161944	3	FEMALE	Consanguinous marriage	SECOND	YES	Ref/Ref	C/C

46	1179583	11	FEMALE	Consanguinous marriage	SECOND	YES	Ref/Ref	C/C
47	1197218	7	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
48	1190377	6	FEMALE	Consanguinous marriage	THIRD	NO	Ref/Ref	C/C
49	1166764	7	FEMALE	Consanguinous marriage	FIRST	NO	Ref/Ref	C/C
50	1170046	4	FEMALE	Consanguinous marriage	FIRST	NO	Ref/Ref	C/C
51	1169977	5	FEMALE	Non-Consanguinous marriage	NIL	YES	Ref/Ref	C/C
52	1166738	9	FEMALE	Consanguinous marriage	FIRST	NO	Ref/Ref	C/C
53	1170048	7	FEMALE	Consanguinous marriage	FIRST	NO	Ref/Ref	C/C
54	1170663	6	MALE	Non-Consanguinous marriage	NIL	YES	Ref/Ref	C/C
55	1166483	10	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
56	1170798	8	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
57	1174276	2	FEMALE	Consanguinous marriage	THIRD	NO	Ref/Ref	C/C
58	1175010	13	FEMALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
59	1174375	5	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
60	1177756	4	FEMALE	Non-Consanguinous marriage	NIL	YES	Ref/Ref	C/C
61	1177691	5	FEMALE	Non-Consanguinous marriage	NIL	YES	Ref/Ref	C/C
62	1175668	12	FEMALE	Non-Consanguinous marriage	NIL	NO	Ref/Ref	C/C
63	1176012	6	MALE	Consanguinous marriage	SECOND	NO	Ref/Ref	C/C
64	1178313	13	MALE	Consanguinous marriage	FIRST	NO	Ref/Ref	C/C