A MICROSTRUCTURAL ANALYSIS OF THE AUDITORY FUNCTION IN INDIVIDUALS WITH FAMILY HISTORY OF HEARING IMPAIRMENT

Reg. No. MS HM0124

A Dissertation Submitted in part fulfillment
of
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University of Mysore,
Mysore.

ALL INDIA INSTITUTE OF SPEECH AND HEARING
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MAY - 2003

Certificate

This is to certify that this Dissertation entitled **"A MICROSTRUCTURAL ANALYSIS OF THE AUDITORY FUNCTION IN INDIVIDUALS WITH FAMILY HISTORY OF HEARING IMPAIRMENT"** is a bonafide work in part fulfillment for the degree of Master of Science (Speech and Hearing) of the student (Register No. MSHM0124).

Mysore

May, 2003

Dr. M. Jayaram
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Certificate

This is to certify that this Dissertation entitled **"A MICROSTRUCTURAL ANALYSIS OF THE AUDITORY FUNCTION IN INDIVIDUALS WITH FAMILY HISTORY OF HEARING IMPAIRMENT"** has been prepared under my supervision and guidance. It is also certified that this Dissertation has not been submitted earlier in any other University for the award of any Diploma or Degree.

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DECLARATION

This Dissertation entitled **"A MICROSTRUCTURAL ANALYSIS OF THE AUDITORY FUNCTION IN INDIVIDUALS WITH FAMILY HISTORY OF HEARING IMPAIRMENT"** is the result of my own study under the guidance of Dr. C.S. Vanaja, Lecturer, Department of Audiology, All India Institute of Speech and Hearing, Mysore and not been submitted earlier in any other University for the award of any Diploma or Degree.

Mysore,

May, 2003 Reg. No. MSHM0124

Dedicated to

Jehovah Shammah

.....who is always with me.

He created the world in its magnificence, and still gives wisdom to man to discover it!

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To all my classmates - you are so dear to me and so close to my heart that I could never forget you.

'As uje go on, we remember
All the times we had together
And *as* our lives changes
Come what ever, we will
Still be **Friends forever'.**

Vandana, Sneha, Shereen, Deepa, bivya Ma'am - our chumship will forever be treasured.

My **family-**no words can express what I feel for you. Its your prayers and love that has helped me grow. And in return for your love, I give you all of mine.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

"Childrens ' children are the crown of old men,

and the glory of their children are their fathers. "

-Proverbs 17:6, Bible

The above proverb gives deep insight into the relationship between members of different generations of a family. Heritage is a topic by itself and is deep rooted in various aspects of life even today. A child inherits from his parents not only possessions, wealth and knowledge but also physical and behavioural characteristics which may range from assets like skin colour and stature to even disorders of many kinds. One such disorder is hearing loss.

Hereditary hearing loss is not a unitary clinical concept. The term covers a group of pathological conditions which are caused by a number of factors and agents. Congenital hearing loss may be either hereditary or acquired and pre or perinatal (Anderson and Wedenberg, 1968).

The consensus among researchers is that genetically transmitted hearing loss comprises about one half of all congenital cases of severe hearing impairment in the hereditary group. Recessive hearing loss is by far the largest. Hood (2001) reported that 70 - 80% of hereditary hearing loss is recessive and 15 - 20% is dominant. Fraser (1974) observed that 70% of hereditary hearing loss is recessive and 25% is dominant. Genetically transmitted hearing loss may be syndromic or non syndromic.

The dominant form has been the subject of many studies over years (Wildervanck, 1957) and the detection of carriers in this case presents no problem. On the other hand detection of carriers of recessive genes is not as easy, as the carriers do not present with any evident expression of the gene. These symptom free subjects may rather present with subtle deviations from normal. This is because hereditary hearing loss occurs due to inborn errors of metabolism. Each gene is responsible for the formation of its own protein, which is often an enzyme, in the case of a changed genetic structure. Hence, minor deviations from normal may be seen in symptom free carriers (Anderson & Wedenberg, 1968). Advancements in gene technology has made it possible to identify carrier of a recessive trait, with a mere blood sample.

There have been many efforts to detect carriers of recessive non syndromal hearing loss audiometrically. Studies in 1940's and early 1950's using audiometry failed to reveal any significant abnormalities. Wildervanck (1957) observed some mild mid frequency audiometric notches in the carriers. The first advance came with the work of Anderson and Wedenberg (1968) who used Bekesy audiometry and found significantly more mid frequency audiometric notches in presumed heterozygous for genetic hearing loss than among their control subjects. They also found more elevated acoustic reflex thresholds in presumed carriers. But Taylor, Hine, Brasier, Chiveralls and Morris (1975) did not support these findings. Later Stephens et al. (1995) used Audioscan to detect carriers of genetic hearing loss. Adopting a frequency range of 500-3000 Hz and a criteria of 15dB for dips, they reported that 55% of parents of children with non syndromal recessive hearing loss were found to have notches while only 14.2% of control subjects showed these

notches. They found more notches among mothers and sisters than fathers and brothers.

Hood (2001) reported that OAEs may provide insight into cochlear function in carriers of abnormal genes related to hearing loss and that auditory functions differ in carriers. DPOAEs showed elevated values in the mid frequency in carrier mice as well as humans. Hood (1998) studied the pattern of contralateral suppression for binaural noise, ipsilateral noise and contralateral noise. The pattern of magnitude of suppression for the respective noises in carriers differed significantly from normals. Engel-Yeger et al. (2002) studied the effects of connexin 26 mutation (35delG) on OAEs in heterozygotes and found reduced response levels than carriers in both low and high frequencies.

In all the studies mentioned, the selection of experimental subjects who are carriers of recessive genes of hearing loss has to be considered. Most recent studies (Engel-Yeger et al., 2002) have used linkage analysis or candidate gene analysis in order to select subjects for audiological studies. There are two approaches for selecting families for linkage analysis. Members from a small number of large families may be studied and in this case the advantage is that we may be looking at the same kind of genetic mutation. Large number of small families can also be used, but here the genetic disorders may not be identical (Fransen & Camp, 2002). A highly consanguineous family can yield significant linkage results using a relatively low number of affected people (Fukushima et al., as cited in Fransen & Camp, 2002). A genetic analysis is the most reliable method to identify carriers. The first step to any genetic study is invariably the pedigree analysis. This would involve detailed family

history during which non genetic factors like premature birth, infections, noise damage and so on, will need to be evaluated and excluded. The overlap between sydnromic and non syndromic hearing impairment also needs to be delineated. The required genetic analysis or tests can be carried out after the pedigree of the family has been determined.

The advantages of genetic analysis to identify carriers may not be available to all researchers. Hence most of the studies depend on pedigree and exclusion of external factors inorder to select candidates for research. Carriers identified in this manner have been called possible carriers or obligate carriers (Anderson and Wedenberg, 1968; Stephen et al., 1995; Hood, 1998). In this case there is a possibility that a new mutation has occurred and the identified subjects may not be actual carriers. But Stephen et al. (1995) in their study stated that the possibility of new mutation is 'unlikely'. Without a genetic analysis, one may be looking at a million hearing loss causing genes and hence a very heterogeneous group. The phenotypes of two different genes may be very different. This heterogeneity can be reduced to some extent if the subjects studied are from a single large family which again is highly consanguineous (Marres & Cremens, 1989).

AIM

The aim of this research was to study the subtle auditory abnormalities in carriers of genes for recessive non syndromal hearing loss. It has been carried out by the following procedures

1. Comparison of the performance of possible carriers of recessive non syndromal hearing loss and normal subjects on Bekesy audiometry.

2. Comparison of fine structure DPOAEs in possible carriers of recessive non syndromal hearing loss and normal subjects.

The experimental subjects of this study are normal hearing parents from different families who have one or more hearing impaired children and or incidences of hearing impairment among relations. They shall be referred to as possible carriers of genes for recessive non syndromal hearing loss, though there are possibilities that they are not carriers. In order to reduce the heterogeneity of experimental group, an attempt was made to subject other normal hearing family members to the experimental tests. But it was not possible as most of the subjects were from far away places.

Need of the study

Conflicting evidences are present in literature regarding possibility of identification of recessive carriers using audiological tests. Very few studies have been done on the same, especially in India. Identifying possible recessive carriers using easily accessible methods like audiometry and DPOAEs will be of valuable help to the audiologist.

Understanding the auditory functions in carriers of hearing loss and whether or not they display subtle differences in auditory ability may assist in understanding of the nature of genetic hearing loss and in managing individuals who carry genes for hearing loss but do not exhibit the trait. The results of auditory and genetic research to characterize their molecular mechanism, and understand their function should facilitate new diagnostic and management approaches to genetic disorders.

REVIEW OF LITERATURE

Hearing impairment can be the consequence of a broad range of environmental, medical and hereditary factors. Environmental and medical factors that cause hearing loss may be bacterial or viral infections, traumas like head injury, metabolic disorders, tumours, noise and so on. Hereditary hearing loss occurs due to genetic variations that are passed from one generation to another. The fundamental processes involved in the mechanism of hearing are controlled by hundreds of genes. Because the ear is a specialized organ, only one mutation is enough to cause hearing loss.

Congenital haring losses occur in approximately 1 to 2 out of 1000 births (Itano-Yoshinaja, as cited in Parving, 2002) out of which 50% is genetic. These may be syndromic (30%) non-syndromic (70%). (Parving, 2002; Dallapicola, Mingarelli & Read, 1996). In syndromic hearing loss, the hearing loss occurs in conjunction with other disorders. The different types of syndromes associated with hearing loss are Waardenburgs syndrome, Branchio-oto-renal syndrome, Alport syndrome, Lange-neilson syndrome, Treacher-collins syndrome, Ushers syndromes, Strickler syndrome and so on (Camp & Smith, 2000).

Non-syndromic hearing loss not associated with any other disorders and consists of autosomal recessive (80%), autosomal dominant (15%), X-linked (2-3%) and mitochondrial inheritance (1%) (Fraser, 1974). Non syndromic autosomal recessive hearing loss is clinically homogenous. In a majority of the subjects, the hearing loss has a prelingual outset, involves all frequencies, is severe to profound

and is non-progressive. In this case, the father and mother are normal (no hearing loss), but are carriers. On one chromosome they have a normal gene and on the other a mutated gene. They have 25% chances to having impaired children, 50% chances of having normal carrier children and 25% chances of having normal children who are not carriers (Petit, 1996). A pedegree chart showing a recessive inheritance is shown in Figure 1.

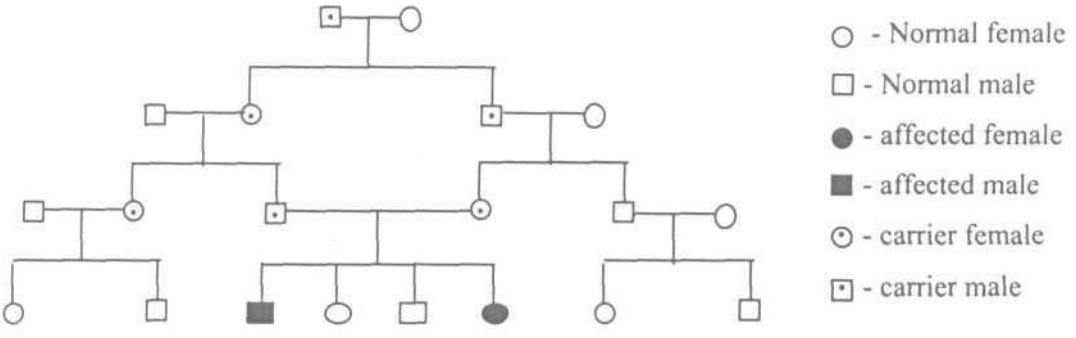

Fig. 1 Pedigree chart showing a recessive inheritance

Human families are so small that even if there is an affected child, there may be only one and so the hereditary nature of the condition may not be obvious. Some times an extensive family history may reveal similarly affected cousins or more remote affected relatives. In other cases it may be possible to suspect a genetic etiology by detecting subclinical manifestations of the mutant gene in the carrier parents. And some times consanguinity may be the only indication that the child probably has a recessive trait (Nance, 1971).

The reason consanguinity is observed more frequently among the parents of children with rare recessive traits is that if the parents have a common ancestor, there is a possibility that two copies of the same abnormal gene carried by one of the common ancestors may have been transmitted down both sides of the family to meet

itself in the affected child. The rarer the recessive gene, the more likely it is that an affected child will have inherited his pair of abnormal genes in this manner. Consanguineous marriages do not invariably lead to abnormal offsprings and conversly the parents of children with rare recessive traits are not invariably related (Nance, 1971).

In non-syndromic autosomal dominant hearing loss, the hearing loss most often has a post-lingual of onset and is often progressive and affects high frequencies (Van Camp, Coucke & Willems, 1996). As shown in Fig. 2 one of the parents exhibit the phenotype and there is a 50% chance of having affected children 50% chance of having normal children. If all individuals who inheret the abnormal gene exhibit features of the disease, the penetrance is complete. Some times because of the effect of other genetic factors or environmental factors, the child who has inherited the mutated gene may not exhibit the phenotype and in this case the penetrance is incomplete (Van Camp, Coucke & Willems, 1996).

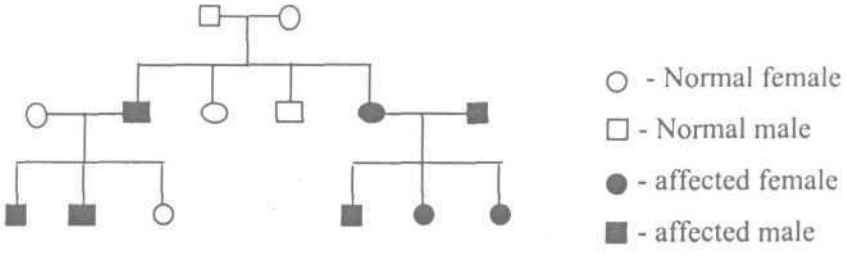

Fig 2 : An autosomal dominant mode of inheritance

X-linked inheritance pattern involves particular genes located on the X-chromosome. This type of inheritance most commonly affects male because they possess a single X-chromosome and will present phenotypically **with** any genotypic

change in this location. Hence a heterozygous female without phenotypic expression will have 50% chances of her sons inheriting and exhibiting the phenotype and 50% chance of having a carrier daughter (Brunner, 1996). An example of X-linked inheritance is shown in Figure 3.

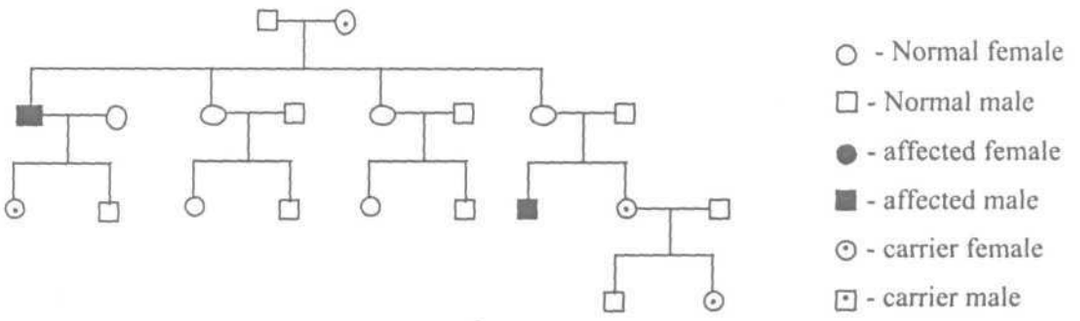

Fig 3 : X-linked inheritance

Mitochondrial inheritance is a rare mode of inheritance for hereditary hearing loss which is caused by a mutation in the small amount of DNA present in the mitochondria of the cell. This type of hearing impairment is inherited only through the mother because the mitochondria are transmitted in the cytoplasm of the maternal material. The expression of mitochondrial inheritance is very valuable, with only a minimal hearing loss which may gradually worsen (Fischel - Ghodsian, 1996).

Genes for hearing impairment

Hearing impairment is a genetically highly heterogeneous disorder and it is estimated that many genes are responsible for a similar phenotype. Much effort has been made to identify the loci including the responsible genes for hearing loss, this is mainly done through linkage analysis.

More than 90 loci have already been demonstrated for non syndromic hearing loss and 21 genes have been identified (Tranebjaerg, 2001). Consequent progress has also been made in the study of the key molecules encoded by deafness genes (Steel & Kross, as cited in Usami et al., 2002). These molecules are being extensively studied from morphological as well as physiological view points. The identification of genes that are responsible for hearing loss is indeed a break through approach and has advanced knowledge of the biology of hearing. It will allow more precise genetic diagnosis, raising the possibility of treatment based on the genetic diagnosis and informing the family about the probability of hereditary hearing impairment in subsequent offspring. Clinical implementation needs to proceed with great caution to be sure that the diagnosis is used for the benefit of the affected individual and their families and avoiding negative ramification.

Mutations of atleast 3 different connexin (Cx) genes. Cx26, Cx30, Cx31 causing hearing loss of cochlear origin have been found (Grifa et al., as cited in Forge et al., 2002). Mutations of Cx26 genes are the most common cause of non syndromic hereditary hearing loss and more than 40 mutations in this gene have been identified. Of the large number of genes identified for deafness only a few have been cloned including GJB2 encoding connexin 26 (Cx26). Mutations in GJB2 gene are a major cause of autosomal recessive congenital hearing loss (Park, Hahn, Chun, Park & Kim, 2002) and are responsible for atleast 80% of genetic hearing loss in Mediterranean families. 35delG accounts for most (about 70%) of Cx26 mutant alleles in families from UK, France, Italy and Spain, Tunisia, Lebanon, Australia and New Zealand. Its carrier rates may be as high as 4% in some ethnic population (Engel-Yeger et al., 2002).

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35delG is caused by a deletion of a guanine residue at cDNA position 35. This is a frameshift mutation of the coding sequence, leading to a premature chain termination at the 12^{lh} amino acid. Connexins are transmembrane protein that form a cylindrical channel in gap junction along adjacent cells, allowing intercellular communications, such as transfering small molecules and ions. Different connexins are distributed in a tissue specific manner. Cx26 is widely expressed in the inner ear, where an extensive network of gap junctions is found in two sets of cells: cochlear non-sensory epithelial cells and the cochlear non-sensory connective tissue cells (Kikuchi et al., as cited in Engel-Yeger et al., 2002). One important function of these gap junctions is to regulate fluid and ion balance in cochlea, a unique system that allows separation of ions and their transfer and recycling. Communication between adjacent basal cells via gap junctions provides fluid and ion transport which maintain the high endolymphatic levels of K+, and thus the endolymphatic potential. The K+ ions, released from cochlear hair cells into extracellular space within the organ of corti, may be conveyed through the gap junction network of the epithelial cells and released at some distance, where extracellular K+ is at a lower level. One possible site with such properties is the root cell process.

K+ enters the hair cells in response to mechanical vibration of the cochlea and is expelled basolaterally and appears to be delivered back to the stria vascularis via the epithelial gap junctions. A mutation in the protein comprising of the potassium recycling apparatus thus results in hearing impairment. The interrupted recirculation would deprive the stria vascularis of K+ which would not be expelled into the scale media, where it serves as the dominant cation which carries hair cell receptor currents. The impaired K+ recycling may prevent the establishment c f the receptor potential of

both hair cell groups. Outer hair cells (OHCs), which are sensitive to the displacement of the basilar membrane and amplify it by their motility, and inner hair cells (IHCS) which are sensitive to the basilar membrane velocity and activate different nerve fibres. In contrast, Lefebvre and Van de Water (as cited in Engel-Yeger et al., 2002) claim that the loss of Cx26 in the gap junction complex would be expected to disrupt the recycling of K+ from the synapses at the base of hair cells, through the supporting cells and fibroblasts, to the high potassium content of endolymph in the cochlear duct and would result in local intoxication of the Organ of Corti by potassium, leading to hearing loss (Engel-Yeger et al., 2001). Genetic bases for specific aspects of cochlear function are further linked in that outer hair cells have a large myosin component and myosin genes are implicated in certain types of hearing loss. Because outer hair cell tugor and perhaps contractility may be related to myosin, subclinical alterations in cochlear function might be linked to the presence of such genes (Hood, 1998).

Genes of non syndromic hearing impairment

The different gene loci for nonsyndromic hearing loss are designated DFN (for DeaFNess). Loci for genes inherited in an autosomal dominant manner are referred to as DFNA, those for genes inherited in an autosomal recessive manner as DFNB and those for genes inherited in an X linked manner as DFN. The number following these designators reflects the order of gene discovery. Several recessive and dominant loci have been mapped to the same chromosomal region and in these cases, allelic variants of a single gene have been found. Example, DFNB1 and DFNB3 both of which map to 13ql2 and are caused by mutations in the gene GJB2. With an exception of DFNB8, in which hearing impairment is postlingual and rapidly progressive, most autosomal recessive loci cause prelingual severe to profound hearing loss. X linked

non-syndromic hearing loss can either be pre-or post lingual, DFN3 is known to cause mixed hearing loss (Martini & Prosser, 1996).

Different genes identified for non syndromic hearing loss (Hereditary Hearing Impairment Home Page (HHH) : http://www.uia.ac.be/dnalab/hhh/) is given in Table 1 and 2.

Table 1 : **Different genes and their loci identified in autosomal recessive non syndromic hearing loss.**

Locus	Gene
DFNB1	GJB2 (C x 26)
DFNB2	GJB6 (C x 30)
DFNB2	MYO7A
DFNB3	MYO15
DFNB4	SLC26A4
DFNB6	TMIE
DFNB7/DFNB11	TMCI
DFNB8/DFNB10	TMPRSS3
DFNB9	OTOF
DFNB12	CDH23
DFNB16	STRC
DFNB18	USHIC
DFNB21	TELTA
DFNB22	OTOA
DFNB29	CLDNI4
DFNB30	MYO3A

Table 2 : List of genes and their loci identified for hearing loss

Locus	Gene
DFNA1	DIAPHI
DFNA2	GJB3(Cx31)
DFNA2	KCNQ4
DFNA3	GJB2 (C x 26)
DFNA3	GJB6 (C x 30)
DFNA5	DFNA5
DNA6/DFNA14	WFS1
DFNA8 1/DFNA 12	TECTA

Genes for syndromic hearing impairment

Usher's syndrome, Pendred syndrome, Jervell and Lange-Neilson syndrome, Refsum disease and so on are some of the autosomal recessive syndromes. Examples of autosomal dominant syndromal hearing impairment are Waardenburg syndrome, Branchiorenal syndrome, Stickler syndrome and neurofibromatosis type II.

X-linked syndromic hearing impairment is found in syndromes like Alports syndrome. Mitochondrial syndromic hearing impairment due to mitochondrial. DNA mutations have been implicated in a variety of diseases ranging from rare neuromuscular syndromes known by acronyms such as KSS, MELASS, to common conditions like diabetes. One mutation, the 3243 A-to-G transition in the gene tRNA leu (URR) has been found. 61% of persons with diabetes and this mutation have hearing loss. The hearing loss is sensorineural and develops after the onset of diabetes. Tables 3 lists the different genes identified for various types of syndromic hearing loss. (Hereditary Hearing Impairment Home Page (HHH) : http://www.uia.ac.be/dnalab/hhh/)

Table 3 : Loci and genes **identified for syndromic hearing loss**

Syndrome	Location	Gene
Alport syndrome	Xq22	COL4A5
	2q36-q37	COL4A3
		COL4A4
Bramchiotorenal syndrome	BOR8q 13.3	EXA1
	BOR2? Iq31	Unknown
Jerwell & lange Neilson syndrome	JLN51 11P15.5 JLN52 21q22.l-q222	KVLQT1 KCNE1
Norrie disease	LD	Norren
	Xpll.3	
Pendred syndrome	PDS	
	7q21-34	SLC 26A4
Stickler syndrome	STL1	COL2A1
	STL2	COL11A2
	STL3	COL11A1
Treacher Collins sydrome	TOCOF1	TCOFI
Usher syndrome	US41A	Unknown
	USH1B	MYO74
	USH1C	USHIC
	USH1D	CDH23
	USH1E	Unknown
Waardenburg syndrome		
WS type I	2q35	PAX3
WS type II	3pl4. Ipl2.3	MITE
WS type III	dq35	PAX 3
WS type IV	31ql22	EDNRS
WS type V	20ql3.2-aql3.3	EDN3
WS type VI	22ql3	50 x 10

Carriers **of** recessive **non syndromic hearing loss**

Autosomal recessive inheritance is characterized by the relevant gene being located on an autosomal chromosome, but requires that an individual carries two copies of the mutated gene in order to show disease affection. The condition affects

males and females equally. The occurrence of an affected person in a family usually strikes the family as a complete surprise because the previous generations are healthy. The parents of the affected individual, however are obligate carriers of one copy of the mutated gene, and carry with them a 25% future risk of having hearing impaired children. In some instances, additional sibs in a sibship are also affected, and raise the suspicion of autosomal recessive inheritance pattern. In inbred families or geographically isolated populations the condition may occur in several subsequent generations because of heterozygous carriers being frequent (Tranebjaerg, 2002). Stephens et al. (1995) referred to parents of sibling pairs, as 'obligate' carriers on the assumption that hearing loss is indeed autosomal recessive. They, however, did mention the possibility of new mutations, 'although that was considered unlikely.' They also tested parents of single deaf children, when on the basis of elimination of other factors, were regarded as probably non-syndromal autosomal recessive. The parents who they called possible carriers were on Harper's calculations likely to have 66% chance of being recessive although again the possibility of new mutation would make this figure lower.

Anderson and Wedenberg (1968) in their study considered parents having one or more children with hearing loss without any exogenous factors as carriers. They referred to the hearing loss in the children as 'endogenous'. In some of the families, there was more information on hearing defects in other relatives, though not with a dominant mode of inheritance. Hood (1998) reported of her study in which parents and members of families with atleast two natural children with apparent endogenous hearing loss as non syndromic recessive hearing loss families.

The first step towards realizing that a genetic factor is playing a role in hearing loss in a family usually comes from taking a family history and noticing the clustering of cases with similar clinical manifestation. It must be added, however, that the sporadic occurrence of hearing impairment in an individual does not in any way exclude an inherited cause. The characterization of a possibly genetic condition in a family always begins with a detailed family history. This time consuming task becomes a lot more challenging in a family with hearing impairment because of the following factors (Tranebjaerg, 2002):

1. Prevalence of hearing impairment of 1:800 in children (Fortnum & Davis, 1997).
2. The extensive genetic heterogeneity and the many non-genetic causes of hearing impairment may lead to false conclusions of a genetic cause in cases of phenocopies (premature birth, infections, noise damage, etc).
3. Third, the need for alternative communication (sign language) in congenital profound hearing impaired often leads to assortative mating since a deaf person tends to many another deaf person. Moreover, the overlap between syndromic hearing impairment and non-syndromic hearing impairment makes it necessary to take quite an extensive medical history and medical examination in an individual with presumably isolated hearing impairment in order to exclude with some level of certainity the existence of over looked, associated symptoms from other organ systems than the auditory system.

Earlier studies used this first step to establish a probable genetic hearing loss and subsequent experimental studies were carried out. More recent studies, with the development in genetic analysis, use methods like linkage analysis, (Tranebjaerg, 2002) candidate gene analysis and other methods to examine for particular mutations

once a pedigree has been established. Though the developments in molecular biology is considerable and widespread. Its advantages are not available to audiologists with ease. Hence, majority of the audiologists of today, especially in India, may have to depend on family history and hearing loss characteristics to select carriers for research, diagnosis or counselling.

Audiometric pattern of genetic hearing loss

There have been many efforts to find particular audiometric pattern for particular genotypes. Albrecht (as cited in Martini & Prosser, 1996) observed that the recessive forms of hearing loss are profound, or total, congenital and non progressive whereas the dominant forms are less severe, postnatal and variably progressive.

Most investigators have reported that it is impossible to subclassify autosomal recessive Sensori-neural hearing loss (SNHL) by audiometric criteria as there is extreme heterogeneity in the audiometric profile. The following are possible relationships between genotype and audiometric features that have been observed (Martini & Prosser, 1996).

- DFNA1 localized to chromosome 5q31 and DFNA6 localized to chromosome 4pl6.3 reported to showing low frequency hearing loss which progresses to severe hearing loss across the entire frequency ranged.
- DFNA2 localized to chromosome Ip32 causes high tone hearing loss and progresses at a highly variable rate to affect all frequencies.

- DFNA3 localized to 13q 12 causes moderate to severe SNHL predominantly in high frequency, prelingual within first 4 years of life, not progressive or in few cases, worsening slightly through life.
- DFNA 5 localized to 7p15 SNHL at high frequency, starts between 5-15 years of age. In fifth decade hearing loss becomes severe involving low frequencies.
- DFNA 7 localized to (1q 21-23) causes high frequency slightly progressive SNHL.
- DFNA 4 (19q 13) and DFNA 8 (15q 15) causes mid frequency or flat frequency hearing loss.

The following are genes known to be involved in autosomal recessive SNHL and the type of hearing loss (Martini & Prosser, 1996):

- DFNB1, localized to 13q 12 causes profound hearing loss which is prelingual and fully penetrant.
- DFNB3, localized to 17p11.2, has shown profound congenital hearing loss affecting all frequencies.
- DFNB4, localized to 7q31, is known to cause profound congenital hearing loss.

Of recent interest is the expression of Cx26 mutation. Phenotypic characteristics of hearing losses associated with C x 26 mutation indicate that they are cochlear in nature but vary widely in degree and stability. Cohen et al. (as cited in Hood, 2001) found no consistent pattern as degree of hearing loss ranged from mild-moderate to profound in individuals homozygous or compound heterozygous for Cx26 mutations. In families who were homozygous for 35 delG mutations also a

similar pattern was found. The hearing loss was stable in a majority of the cases but was progressive in few and fluctuating in few.

Hearing losses resulting from the same genetic mutation show wide variability in degree and progression. Furthermore, audiometric characteristics do vary among groups according to type of mutations (Hood, 2002) and it cannot be used as valid criteria by which families can be pooled for linkage analysis (Fukushima et al., as cited in Martini & Prosser, 1996).

Audiological findings in carriers of recessive hearing loss

Detection of carriers of recessive genes is not easy as the carriers do not present with any evident expression of the gene. These symptom-free subjects may rather present with subtle derivations from normal. This is because hereditary deafness occurs due to inborn errors of metabolism. Each gene is responsible for the formation of its own protein, which is often an enzyme, in the case of a changed genetic structure. A mutant gene will either have prevented the formation of this particular enzyme or it will have given rise to an abnormal enzyme. Hence one may see minor deviations from normal in symptom free carriers (Anderson and Wedenberg, 1968).

Efforts to detect carriers of genetic hearing loss audiometrically date back to 1933 when Tinkle (as cited in Stephens et al., 1995) tested the thresholds of hearing of presumed heterozygous parents and siblings of deaf children. That study had important methodological flaws and furthermore was unsuccessful in its aims. Studies in 1940s and early 1950s using pure tone audiometry failed to reveal any significant

abnormalities. Wildervanck (1957) found some mild mid frequency audiometric notches in carriers. The first advance came with the work of Anderson and Wedenberg(1968).

They found significantly more mid-frequency audiometric notches in preserved heterozygous for genetic hearing loss than among their control subjects. They also observed more elevated acoustic reflex thresholds in preserved carriers. Quite distinct 'dips' in the Bekesy recordings were found in 7% of males and 23% of females who participated in the study. These dips were in the frequency range of 1500-2000Hz, had a depth of 20-50 dB and a range of about 1.5 octaves. The stapedial thresholds showed that in only a few cases the thresholds fell within 80-90 dBHL for the frequency range 250-4000Hz. In most subjects a threshold could not be reached. They concluded that there was a disproportionately high incidence of certain 'peculiarities' in the hearing and reflex thresholds in the parent group with a genetic background of hearing loss. Anderson and Wedenberg (1976) suggested that Bekesy audiometry could be used as a sensitive tool to detect subclinical abnormalities in heterozygotes by the continuous recording of the hearing threshold which makes it possible to observe even small peculiarities or deviations. Stapedius reflex, which is an objective suprathreshold test is also sensitive in detecting defects that are not manifested in the threshold test.

Later Parving (1978) studied the reliability of Bekesy threshold tracing in identification of carriers of genes for an X-linked disease with deafness. Seven identified carriers and twenty potential carriers of Norries disease were examined by pure tone octave audiometry and Bekesy audiometry. The investigation supported the

earlier results of Bekesy threshold tracings performed in heterozygous carries of genes for recessive hearing impairment. 42% of known carriers and 15% of potential carriers, showed 'dips' by Bekery threshold tracing. And only 2% of normals showed similar dips. These results suggested that the specificity of Bekesy audiometry is high and the sensitivity of the method is poor. An absent dip can not exclude the possibility of a subject being a carrier, whereas a present dip can be regarded as an indication of a carrier. When comparing conventional octave audiometry and Bekesy threshold tracing, the latter method was found to be more subtle in finding carriers of genes for recessive deafness. Newton (1985) could not confirm the findings of Anderson and Wedenberg in comparable studies. Results of an investigation by Meredith, Stephens, Sirimanna, Meyer-Bisch and Reardon (1992) who studied carriers of Usher's syndrome type II support the findings of Parving (1978). This investigation revealed that with Bekesy notches, sensitivity for the detection of carriers was 22% and the specificity was 100%.

Another study was conducted by Marres and Cremers (1989), who studied audiometric features of affected and obligate carriers in a large family with twelve members having non-syndromal autosomal recessive type of profound childhood deafness. This large family had various consanguineous marriages and other family interrelations. Audiograms of all affected children showed profound childhood hearing impairment with only a very slight variation. Stapedial reflexes in the obligate carriers did not show any abnormalities of the type described by Anderson and Wedenberg (1968). No abnormalities were found in high frequency audiometry also. None of the obligate carriers showed a dip of 20 dB or more as described by Anderson and Wedenberg (1968). Marres and Cremers (1989) postulated that as

Anderson & Wedenberg in their study had taken subjects from different families, it was unlikely that all the subjects were affected by the same form of genetically impaired hearing. Hence they were looking at different types of mutations and variable expressions of these different mutations which made it possible to observe dips in some of the cases. In contrast, Marries and Cremens (1989) included obligate carriers from one family in their study where perhaps the type of genetic mutation was the same in all the subjects. This resulted in no significant results in the tests. They expressed their doubt that the Anderson & Wedenberg (1968) study 'will ever ver be able to be reproduced'.

Taylor et al. (1975) who used a sweep rate of 20 octaves / sec also could not replicate the results of Anderson and Wedenberg's study. Stephens et al. (1995) said that the failure to support the original findings of Anderson and Wedenberg (1968) was because of important methodological differences between the various studies with regard to sweep and attenuation rates. They also pointed out and another disadvantage of the study when the investigators were obliged to look for broad notches, as narrow notches could well have been observed by the zigzag excursions inherent in this technique. The excursions and their size in certain individuals, can mask discontinuities in the threshold reflected in narrow notches.

This disadvantage of Bekesy audiometry can be overcome using the Audioscan technique, with which Meredith et al. (1992) found that of 30 subjects shown to have audiometric notches (including, carriers, possible carriers and controls), twenty nine had notches on Audioscan testing, but only six on Bekesy testing. He found that, Audioscan had a sensitivity of 78% and a specificity of 87%. It

was concluded that a sweep rate of 30s/octave over the frequency range 500-3,000 Hz taking a notch size of 15 dB or more gave the optimal results. With this stimulus paradigm, 106 controls from 3 separate studies showed a 14.2% occurrence of notches. When the test was administered to obligate carriers of Usher's syndrome type II (Meredith, et al., 1992) 100% of obligate carriers were found to have notches, as were 57% of possible carriers.

Stephens et al. (1995) also studied the application of audioscan in the detection of carriers of genetic hearing loss. They used a sweep rate of 30secs / octave over the frequency range of 500-3000 Hz. with a pulse rate of 2.5 pulses /s and a step size of 5 dB. The criterion of notches was 15 dB or greater within the frequency range 500-3000 Hz. Adopting this criterion, 14.2% of control subjects had notches. Among the parents of children with non-syndromal recessive hearing loss, 55% were found to have notches. 45% of siblings had notches. Both siblings and parents had significantly more number of notches than controls.

Although audiometric characteristics are used to classify degree and configuration of hearing loss, other measures of auditory function can provide better insight into the nature of hearing loss. As we strive to understand specific functions and characteristics accompanying various forms of hereditary hearing loss, application of sensitive physiologic and behavioural measurement techniques are valuable. Because a majority of hereditary hearing losses are cochlear in nature and otoacoustic emmisions (OAEs) are associated with the integrity of the outer hair cells, OAEs are particularly well suited to clinical evaluation and research related to hereditary hearing loss (Hood, 2001).

Several studies have demonstrated the sensitivity of OAEs to auditory dysfunction in Waardcnburg syndrome (Liu & Newton, 1997), Usher syndrome (Hood, 1998), Mitochondrial disorders as well as in patients with non syndromic hearing loss (Lina, et al., 1995) Liu and Newton (1997) found that a majority of patients with Waardcnbcrg syndrome showed notches in distortion product OAEs (DPOAEs) despite normal auditory thresholds. As much as 87.5% of the car had abnormal OAEs.

Genetic factors may also have a role in otoacoustic emissions. In studies of twins, McFadden and Loehlin (1995) found that the number of spontaneous otoacoustic emissions (SOAEs) were highly correlated in monozygotic twins than in same - sex dizygotic twins. Their analysis suggested that about three quarters of the individual variation in the expression of SOAEs is attributable to genes. In addition, Bilger, Matthics, Hammcl and Dcmorst (1990) suggested that the tendency for females to display more SOAEs than males may be related to a dominant X-linked (rait.

Huang et al., (cited in Hood, 2001) showed that the pattern of high frequency DPOAEs in mice can help distinguish between normal hearing carriers versus non carriers of dn (deafness) gene Huang, Berlin, Lin and Keats (1998) found heterozygous (+/dn) mice showed higher DPOAEs than homozygous (+/+) mice, suggesting that dn gene carriers may have a unique cochlear trait demonstrated by DPOAE functions. Increased DPOAE thresholds and reduced DPOAE amplitude at higher frequencies have been observed in the heterozygous deafwaddler (+/dfw) mouse (Kondrad ct al., as cited in Hood, 2001). Hood (1998) postulated that DPOAE

testing requires a 1.3 ratio (of F_2:Fi) and very low intensity levels to identify carriers among mice.

These genetic implications in humans and in mice as well as observations in humans suggest that otoacoustic emissions are more sensitive to genetic differences than methods previously studied.

Engel-Yeger et al., (200i,) studied the effects of connexin26 mutation - 35delG - on otoacoustic emissions in human homozygotes and carriers. The subjects of the study were from an Israeli Arab village, in which profound, isolated and non syndrome congenital hearing impairment is frequent and affects at least 1% of the population. It had been shown in a previous study that most cases of profound hearing loss were due to mutations in C x 26, either 35delG or W77R. In their study, 56 individuals from families of hearing impaired people aged from 10-80 years, were examined. They underwent mutation screening of DNA extracted from blood, for identifying the Cx26 mutation 35delG and W77R.

DPOAEs in the frequencies of 1000-10,000 Hz with 1000 Hz intervals were presented at 65 dB HL. A significant difference in the response level of DPOAEs between carriers and non carriers was found at all frequencies. The carriers had lower response levels than non carriers. No significant difference was found in DPOAE prevalence between left and right ear among both the groups. The average DPOAE response level was lower at high frequencies. Non carriers had the highest percentage of no responses between 8000-10,000 Hz, but this trend was lower than carriers. Among carriers, the highest percentage of the responding subjects (33-52%) had

DPOAEs within limits in the frequencies 1000-4000 Hz in the right ear and 1000-5000 Hz in the left ear. Between 5000-10000 Hz carriers had the highest percentage (67-90%) of no response. ANOVA indicated no significant interaction between age and genetic group, although carriers showed consistently lower DPOAEs response levels, for each age group of genetic group. The deterioration of response in the control group appeared milder.

Engel-Yeger et al. (2002) also studied Auditory Brainstem Evoked Potentials (ABEP) in same population for which they studied DPOAEs. They found ABEP results among carriers and non-carriers were within normal limits. No significant effects of subject group on ABEP latencies of peak I, III & V or on interpeak latency difference between III and I, V & I or V & III at stimulus rates 10/s and 50/s were found. No significant group effect (non carrier and carrier) was found for the effects of increasing stimulus rates from 10/s and 50/s. It was concluded that the lower DPOAE scores of the carriers compared to the non-carriers may indicate that outer hair cells (OHCs) of the carriers are more sensitive to the negative effects of mutation compared to inner hair cells (IHCS) and brainstem auditory pathways, reflected by ABEPs. DPOAEs reflect OHC function even at the very high frequencies, and hence perhaps, the differences in sensitivities observed in this study. 35delG mutation in Cx26 may impair OHC function. This impairment is reflected in DPOAEs. Thus DPOAEs may serve as a sensitive test for the 35delG mutation in early evaluation of mutant Cx26 gene family members.

Hood (1998) studied two groups of carriers of recessive hearing loss : families with Acadian usher syndrome type I and Acadian families with non syndrome

recessive hearing loss. Carriers of the of Acadian usher gene were identified by genetic analysis while the Acadian non syndromic recessive hearing loss families mere parents and members of families with atleast two natural children with apparent endogenous hearing loss. The investigations observed that parents who are obligate carrier of Acadian usher syndrome type I gene showed decreased distortion product otoacoustic emission (DPOAE) amplitude in the mid frequencies range when compared with a group of age-and gender matched control subjects.

Although this was a consistent observation in the Acadian usher parents, emissions in parents of atleast two children with non syndromic recessive hearing loss have been less consistent in that some of these parents showed decreased mid frequency amplitude in DPOAEs whereas other parents did not. This, the author said was not unexpected as the parents of children with non syndromic recessive hearing loss comprised of a more heterogeneous population. The investigators also studied the effect of binaural suppresser noise on DPOAEs in comparison with ipsilateral or contralaterally presented noise in both the above mentioned groups. In contrast to normals who showed maximum suppression for binaural noise, followed by ipsilateral noise and then contralateral noise, no similar pattern was found in either groups.

In summary, most common terms of hereditary hearing impairment involve abnormal development of the receptor cells (Hair cells) in the inner ear and follow a recessive inheritance pattern. Understanding auditory function in carrier of hearing loss and whether or not they display subtle difference in auditory ability may assist in understanding of the nature of genetic hearing loss and in managing individuals who carry genes for deafness but do not exhibit the trait. The results of auditory and

genetic research to characterize human genes, characterize their molecular mechanisms and understand their function should facilitate new diagnostic and management approaches to genetic disorders.

METHOD

Subjects

Parents of children with 'endogenous' hearing loss were taken as experimental subjects. The experimental group comprised ten marital partners with the age range of 21 years to 39 years. The following are criteria which the child with hearing loss had to meet so that the hearing loss could be classified as endogenous.

- The hearing loss has to be congenital. All possibilities of an acquired loss should be eliminated.
- There should not be any prenatal, perinatal or post natal history.
- The hearing loss should be moderate to profound bilateral sensorineural hearing loss.
- There should not be any associated structural malformation or mental retardation or any other features suggestive of any syndrome.

The following criteria was used to select the experimental group.

- The parents should have one or more children with endogenous hearing loss and similar incidence (s) within blood relative or
- The parents should have two children with endogenous hearing loss and the marriage should be consanguineous.

The parents also had to fulfill the following criteria for hearing

- The parents should report of normal hearing
- There should not be exposure to noise or ototoxic drugs which could cause hearing loss.

- They should not have any endemic diseases such as diabetes which may be associated with hearing loss.
- They should have a normal middle ear system.

The control group consisted of 15 males of the age group of 25 to 40 years and 15 females of the age group to 20 to 35 years. The control group met the criteria for hearing status stated for the experimental group, and in addition did not have any incidence of hearing loss in their families and relatives.

Instrumentation

1. Otoscopy and immittance using GSI 33 (Version 2) was done to examine for external ear and middle ear functions. The instrument was calibrated according to the manufacturers' requirements.

2. Bekesy, audiometry was done using Madsen OB922 (Version 2). The instrument was calibrated according to the manufacturers' requirements. Testing was done using the following stimulus parameters:
   - A rate of intensity change for 2.5 dB per sec was used.
   - Continuous presentation of stimulus was used.
   - Frequency range of 250 Hz - 8 kHz and 16 points per octave.
3. Microstructure DPOAE using GSI 60 DPOAE analyser was done. The instrument was calibrated according to the manufacturers' requirements. The following stimulus parameters were used:
   - L_1 =60dBSPL, L_2 = 50dBSPL
   - F_1 : F_2= 1.2
   - Frequency range of 1 kHz to 8 kHz

- Fine structure consisting of 20 points per octave was used.

Procedure

[1] Immittance evaluation using GSI 33 was carried out on both groups of subjects. Control subjects had to fulfil the criteria of 'A' typanogram and normal reflexes in both ears. The test results of the experimental group was noted.

[2] Bekesy audiometry : The subjects were seated in a an acoustically treated room. Testing was carried out under head phone TDH 39. The subject was given a patient switch which he / she had to press if the tone was heard or had to release if the tone was inaudible. The subjects were asked to pay attention and press as soon a they heard the sound and release the switch as soon as they stopped hearing the sound. A practice trial was given in order to ensure that they understood the instruction. The testing duration was 7-10 mins for both ears. Print outs of the acquired tracing of both ears were taken.

DPOAE testing

The subjects were made to sit comfortably and also instructed not to move or talk during the test. The probe was inserted gently into the ear canal with an appropriate probe tip. Probe fit was ensured to check adequate fitting of the probe into the canal. DPOAE testing required an approximate 20 minutes to complete. The control subjects had to have 90% reproducibility at all frequencies.

Analysis

- **Bekesy audiometry**

Printouts of the tracings of all subjects were taken and was analysed to identify 'Carrier dips'. A dip was classified as a carrier dip if it had a depth of 20 and was one octave wide. It also had to be in the 1 - 3 kHz region of the tracing.

- **Fine structure DPOAEs**

Absolute amplitude and signal-to-noise ratio of distortion at each $l/20^{lh}$ point of an octave was noted. Peaks and notches in the fine structure DPOAE was also analysed. The absolute amplitude of the peaks and notches and the frequency region in which they occurred were noted.

RESULTS

Distortion product absolute amplitudes and signal-to-noise ratio, of both ears were statistically analysed for the control group (N=30). The lower bound value of the 95% confidence interval for mean for all 54 frequencies (20 points per octave) of the control group was noted. This data was used to compare amplitude and signal-to-noise ratio of the experimental group.

The lower bound cut off (lowest values) of the normative data is shown in Figure 4. There is a decrease in amplitude as the frequency increases from 1187 Hz and after 5318 Hz, there is a large decrease in the DPOAE amplitude. Left ear amplitude were slightly lesser than right ear amplitude. Signal to noise ratio (SNR) values were also reduced after 5500 Hz.

The fine structure DPOAE was also analysed, by recording the number of peaks, the amplitude of peaks and the amplitude of the notches. The number of peaks and amplitude of peaks were variable even among the controls and hence was not analysed statistically. Deep notches were found in some of the controls and thereby, notch amplitude was taken as a parameter for comparison. The notches in frequency bands 1-2 kHz, 2 - 3kHz, 3-4 kHz, 4-6kHz and 6-8 kHz were identified, noted for amplitudes and statistically analysed. The 95% confidence interval for mean was measured and the lower bound values were taken as the lower cut off for normative data.

The lower bound values for notches in the different frequency ranges for right ear and left ear are shown in Table 4. Notch values decreasing significantly for 4-6 K and 6-8 kHz region can be observed.

Printouts of the tracings of Bekesy audiometry were compared between controls and experimental subjects. The presence of any dip/notch characterized by 15-20dB and frequency range of 1-3 kHz with a width of octave was examined. The Bekesy results were also used to conclude the approximate threshold of the person. Bekesy audiometry showed thresholds within 20dBHL for all controls. No characteristic dips were observed in any of the controls.

The experimental group was not subjected to statistical analysis, rather a descriptive analysis was done. The absolute amplitude and SNRs of DPOAEs of the subjects were compared with that of lower bound values obtained from the control group. The magnitude of deviance from the normative data, the frequency range or frequency points at which it occurred were observed and recorded. The lower bound values were compared because only sub clinical deviancies were expected in the experimental group. Statistical analysis of the experimental group was not done as the group was highly heterogenous. In the absence of a genetic analysis, one cannot be certain of the gene that is involved. A myriad of genes are responsible for hearing loss and each of their expressions may be different. Moreover the assumption that all the experimental subjects are actually carriers of any of the genes responsible for recessive hearing loss is regarded as a possibility. Another reason attributed to the heterogenity of the experimental group is that marital pairs of ten different families have been taken as subjects. If the subjects were members of a single large family,

then the span of heterogenity would have been smaller. Hence, only a descriptive analysis of each experimental subject has been done.

In the following section the results of each family has been discussed. The families have been nominalised from A to J. The pedigree chart of each family is given. The male subject has been indicated with subscript 1 to the family letter and the female member by giving the subscript 2. Eg. in family A (F_A), the father is denoted by A1 and mother A2. The ages of the subjects have also been mentioned in pedigree chart. Bekesy findings, overall DPOAE response and notch amplitude have been discussed in relation to normative data. Figure 4 depicts the DPOAE responses of the right ears of 3 experimental subjects in relation to the lower and upper bound cut of the normative data. As most of the subjects had within normal limit SNRs, SNRs have been discussed only if they were lesser. Immittance results have been reported only if they were abnormal. The results of Bekesy and DPOAE each family is presented in a tabular form. DPOAE amplitude and fine structure results have been summarized for each subject of the respective family. None of the subjects presented any characteristic dips in Bekesy audiometry, and hence has not been mentioned individually. Only the thresholds computed from each subject has been recorded. In the table, RE, stands for right ear and LE stands for left ear.

Table *4* : Upper and lower bound values of mean for amplitude of notches for different frequency ranges.

Frequency range (kHz)	Right ear (dBSPL)		Left ear (dBSPL)	
	Upper bound	Lower bound	Upper bound	Lower bound
1 - 2	6	0	8	2
2 - 3	0	-4	3	-1
3 - 4	1	-3	I	-5
4 - 6	-1	-10	-4	-14
6 - 8	-14	20	-14	-23

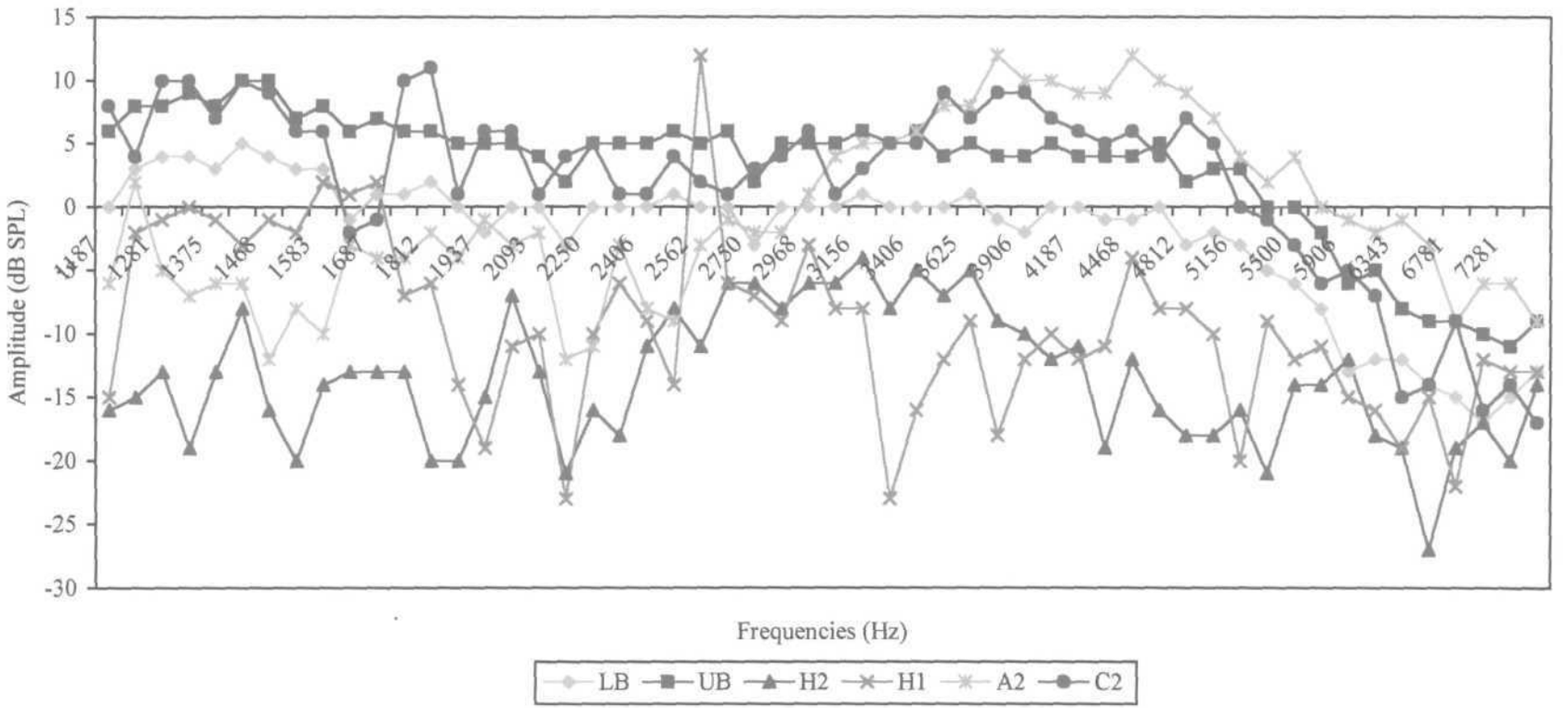

Figure 4 : DPOAE responses of normals (upper bound-UB and lower bound-LB) and experimental subejcts H2, H1, A2 and C2.

H2 shows maximum deivation from normal between 1.1kHz to 2.4kHz and 4.4kHz to 5.5kHz

H1 shows larger reduction between 2.6kHz to 2.5kHz and 3.4kHz to 4.6kHz

A2 shows reduced amplitude between 1.3kHz to 2.7kHz

C2 shows slightly lower amplitude only at 1.68kHz and 1.75kHz

Family A

The pedigree chart of the first pair of experimental subjects is shown in fig A.

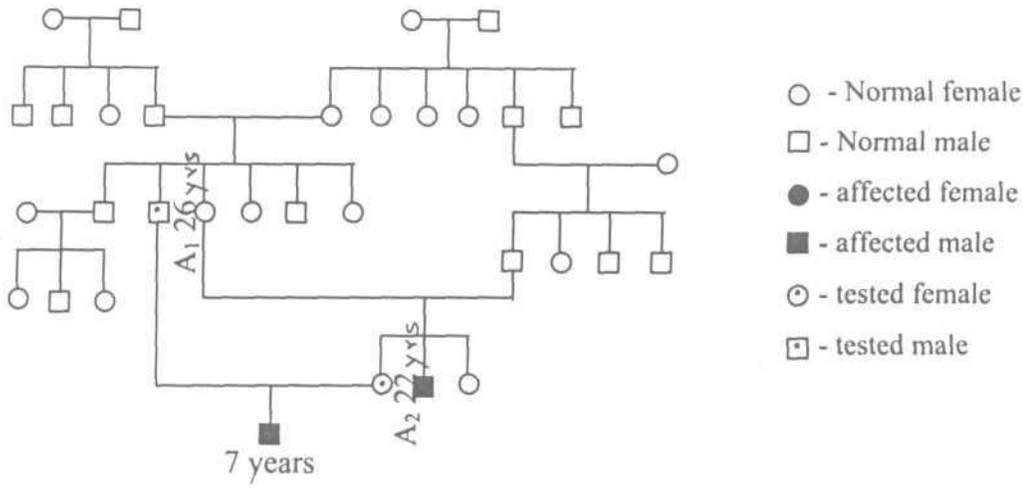

Fig. A : **Pedigree chart of Family A**

The pedigree shows a consanguineous marriage (uncle - niece relationship) between the parents of the affected child. The affected child's uncle also has congenital SN hearing loss. The audiogram of the hearing impaired child revealed, bilateral profound sensory neural hearing loss with some residual low frequency hearing in both ears. The parents of this child when subject to the experiment showed results as in table 5 A. Immittance of Al revealed reduced compliance and absent reflexes in both ears.

Table 5 A : **Results** of F_A

		Bekesy	DPOAE response	Fine structure
Al	RE	250-4kHz-20dBHL 4K- 8K - 50dBHL (sloping)	• Lower responses above 3906 Hz • Lower at 1.9, 2kHz by 3-5dBSPL	• 1-4 kHz within normal limits • Lower at other frequencies
	LE	250-4kHz-20dBHL 4k-8k-50dBHL (sloping)	• Lower than normal response • 3-6kHz - lower by 1-20dBSPL • l-2kHz-lower by 2-10 dBSPL • 6.08kHz lower by 1-2dBSPL • 7.28kHz lower by 16dBSPL	• All values of notches lower than normal
A2	RE	250-lkHz- 15dBHL IK-4kHz-20dBHL 4K-8kHz-l5dBHL	• Lower at l-2kHz by 1-16dBSPL • Other frequencies within normal limits	• All points lower at 1.02kHz • 2 points at 2-3kHz lower by 5-8dBSPL
	LE	250-8kHz-15dBHL	• Same as RE	• 3 notches at 1-2kHz lower by 15-20dBSPL • One point in 2-3kHz lower by 5-8dBSPL

Family B

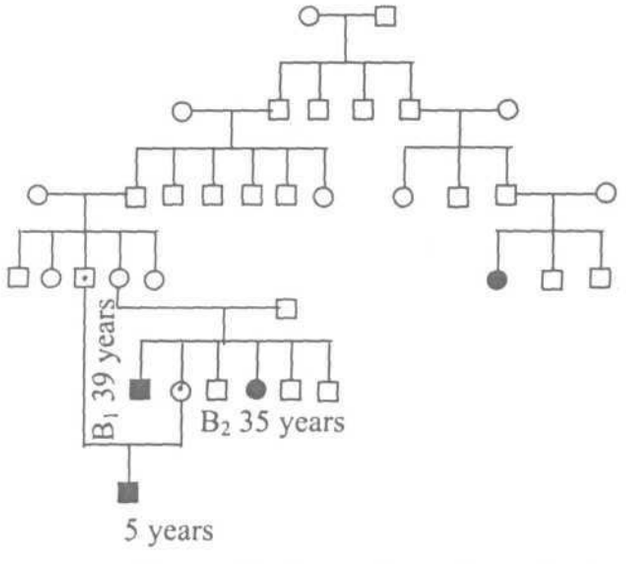

Fig. B : Pedigree chart of Family B

This family presented with a 7 years old son having congenital bilateral severe hearing loss. The mother (B2) and father (B1) are married in an uncle-niece relationship. The mother's two siblings also reported to have similar pattern of hearing loss as in the child. Another incidence of a congenitally hearing impaired female also exists in a distant blood relative.

Tests done and results are shown in Table 5 B.

Table 5 B : Results of F_B

		Bekesy	DPOAE response	Fine structure
Bl	**RE**	250-8kHz-20dBHL	• Lower at all frequencies by l-30dBSPL	• Lower at all frequencies by 7-27 dBSPL • 7.28kHz within normal limits
	LE	250-8kHz - 25 dBHL	• Lower at all frequencies by l-30dBSPL	• Lower between l-6kHz by 4-17 dBSPL • Normal within 6-8kHz
B2	**RE**	250-2kHz - 20 dBHL 2 -8kHz -15 dBHL	• Lower at l-3kHz by 1-20 dBSPL • Normal within 3-8kHz	• Lower at l-2kHz by 6-19dBSPL. • 2-3kHz lower by I-lldBSPL • 2.75kHz within normal limits • 3-8kHz within normal limits • 3.5kHz lower than normal
	LE	250-8kHz-15dBHI	• Lower between 1-4.3kHz by 10-15dBSPL • Normal within 4.4-8kHz • Reduced SNRs	• Lower at 1-2kHz by 8-11 dBSPL • Lower at 2-3kHz by 8-ll dBSPL • Lower at 3-4kHz by 5-13 dBSPL • 4-8kHz within normal limits

Family C

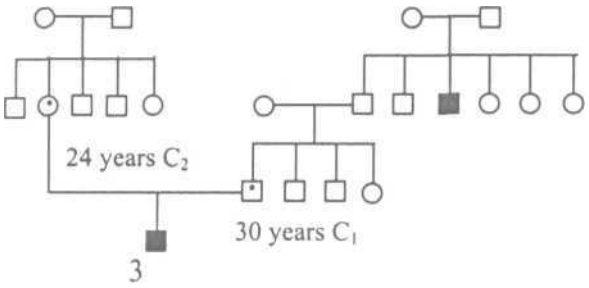

Fig. C : Pedigree **chart** of **Family** C

The experimental subjects C1 and C2 had a single issue who was born with hearing loss, that appears to be endogenous. The client had moderately severe hearing loss in both ears. There is no consanguinity in the marriage, but the father's uncle was also born hearing impaired as reported. Results can be seen in Table 5 C.

Table 5 C : Results of F_c

		Bekesy	DPOAE response	Fine structure
Cl	**RE**	250-8kHz-I5dB	• Within normal levels at all frequencies except at 1.6kHz, 7.2kHz, 7.5kHz by 2-4 dBSPL	• Only 2 notches at 2-3kHz reduced by 2dBSPL • Only 2 notches at 6-8kHz reduced by 1 and 11dBSPL
	LE	250-8kHz - 15 dBSPL	• Within normal limits except at 1.4kHz, 2kHz, 6.7kHz, 7kHz by 2-4 dBSPL	• Only one notch a6 6-8kHz reduced by 2 dBSPL • 2 notches at l-2kHz lower by 1 dBSPL • Other notches within normal limits
C2	**RE**	250-8kHz - 15 dBHL	• Over all reduced • 6.3kHz, 7kHz, 7.2kHz, 7.5kHz within normal limits	• Notches lower at all frequencies except 6-8kHz
	LE	250-8kHz 15dBHL	• Over all reduced • 3.7kHz, 5.3kHz, 6.5kHz, 6.9kHz, 7kHz and 7.2kHz within normal	• Lower at all frequencies except 6-8 kHz

Family D

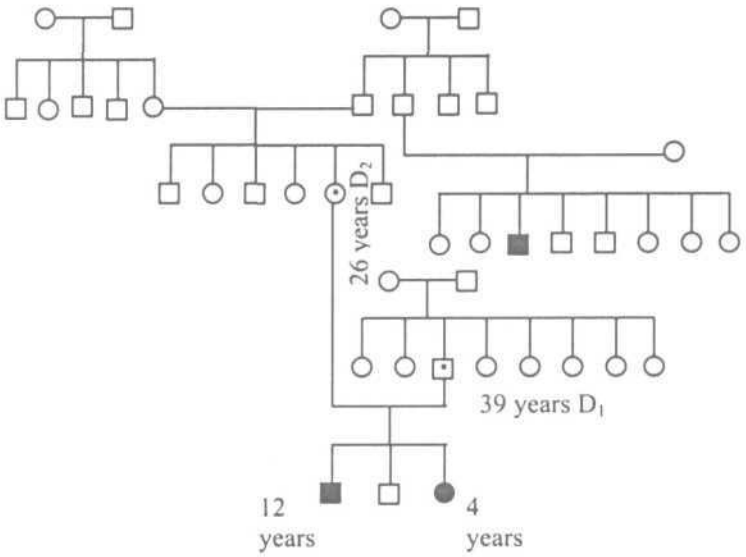

Fig. D : Pedigree chart of Family D

The experimental subjects (D1 and D2) had two out of three issues with congenital hearing loss. The elder son had a profound bilateral hearing loss while the youngest daughter had moderately severe hearing loss in both ears.

Table 5 D : Results of F_D

		Bekesy	DPOAE response	Fine structure
Dl	RE	250-8kHz - 15 dBHL	• Below 3.75kHz lower than normal by 1-21 dBSPL • Amplitude above 3.75K normal	• Notches between 1-3kHz reduced by 4-17 dBSPL
	LE	250-8kHz - 15 dBHL	• Similar to RE	• Similar to RE
D2	RE	250-6kHz - 15 dBHL 6K - 8kHz - 20 dBHL	• Over all reduced • 1.1-2.3kHz reduced by 10-31 dBSPL • 2.4-3.4kHz reduced by 1-9 dBSPL • 3.5-5.9kHz reduce by 22 dBSPL • 6.9-7.5kHz within normal limits	• All lower than normal by for 49-5.1kHz. Other frequencies similar to left ear

	LE	250-6kHz - 15 dBHL 6K - 8kHz - 20 dBHL	• 1.1-2.3kHz reduced by 8-18 dBSPL • 2.4-3.4kHz reduced by 1-9 dBSPL • 3.5-6.0kHz reduced by 15-22 dBSPL • 6.9-7kHz within normal limits	• All lower than normal except 6-8kHz • Reduced in l-2kHz by 11-26 dBSPL • 2-3kHz by 5-17 dBSPL • 3-4kHz by 4 dBSPL • 4-6kHz by 15 dBSPL

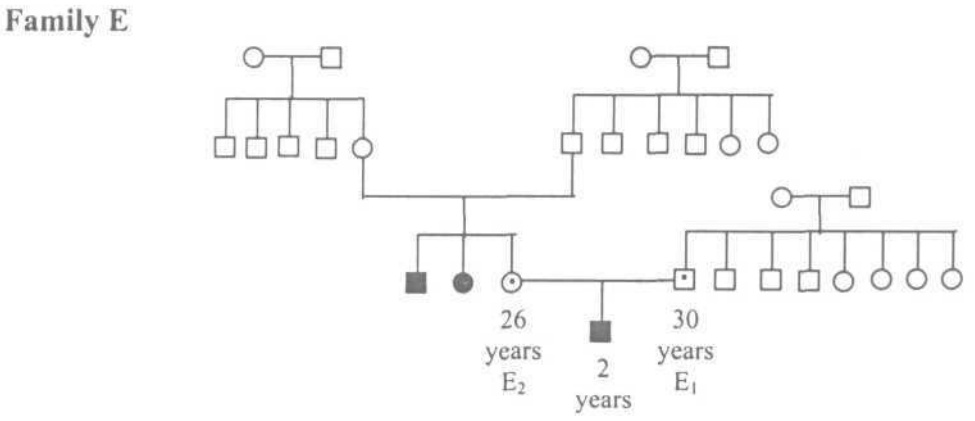

Fig. E : Pedigree chart of Family E

Pedigree analysis of family E showed a total of three occurrences of congenital hearing loss. As seen in the pedigree, E2's two siblings have hearing loss. There is no consanguinity observed in three generations. E2 reported of normal hearing in ears and no history of ear discharge, ear pain, etc. When E2 was subjected to experimental test, immittance and otoscopy revealed a perforation in the right ear.

Table 5 E : **Results of F_E**

		Bekesy	DPOAE response	Fine structure
El	**RE**	250-8kHz - 15 dBHL	• Normal within 1.18-2.65kHz • Lower in 2.75-6.98kHz by 1-18 dBSPL • Maximum reduction seen till 5.9kHz • 7kHz, 7.2kHz, 7.5kHz normal	• All the frequencies except 3-4kHz affected • Maximum deviation at 2-3kHz
	LE	250-8kHz - 15 dBHl	• Normal within 1.18-3.90kHz • 3.9-6.1 kHz reduce by 1-21 dBSPL • 6.3-7.5kHz normal	• Reduced at all frequencies except at 6-8kHz
E2	RE	250-2kHz - 40 dBHL 2K - 4kHz - 50 dBHL 4K-8kHz 70dBHL	• Severely reduced at all frequencies • SNRs severely reduced	• Severely reduced
	LE	250-6kHz - 15 dBHL 6K - 8kHz - 40 dBHl	• Lower at all frequencies by 3-28dBSPL • Normal at 6.5kHz, 7.2kHz, 7.5kHz • SNR values reduced but maintained at 6-lOdBSPL	• Severely reduced • Lower at all frequencies by 1-20 dBSPL

Family F

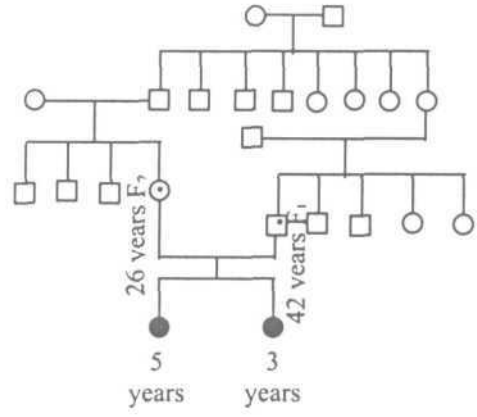

Fig. F : Pedigree chart of Family F

The experimental subjects, F1 and F2 had two daughters with bilateral profound endogenous hearing loss. The marriage was consanguineous, between cousin and there was no other family history. F2 reported of normal hearing, but a history of trauma due to foreign body in the left ear. Immittance results was 'B' type

with absent ipsilateral and contralateral reflexes. The right ear showed 'A' type, with ipsilateral reflexes being present.

Table 5 F: Results of F_F

		Bekesy	DPOAE response	Fine structure
Fl	RE	250-1 kHz -20 dBHL lK-8kHz - 15 dBHL	• Within normal level • Between 2.2kHz and 4kHz slightly reduced	• Lower in 2-3kHz by 3-4 dBSPL • Other frequencies normal
	LE	250-8kHz - 15 dBHL	• Within normal levels • 2.7kHz, 2.9kHz, 6.1kHz, 6.7kHz, 7.2kHz lower by 5-7 dBSPL	• Lower in 2-3kGz by 3-10 dBSPL • No peaks in higher frequency
F2	RE	250-8kHz - 15 dBHL	• Within normal level • 2.06kHz lower by 4 dBSPL • 7.5kHz lower by 6 dBSPL	• All within normal limits
	LE	250-6kHz - 40 dBHL 6-8kHz - 50 dBHL	• Severely affected	• Severely affected

Family G

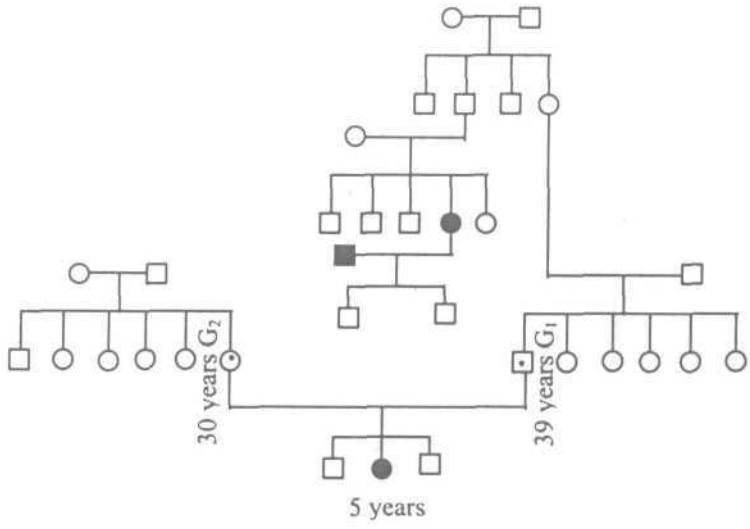

Fig. G : Pedigree chart of Family G

Has shown in Table 5G, G1 and G2 have a marriage **that** is non-consanguineous. Of their three offsprings, the second daughter has hearing loss that appears to be endogeneous. G2 has a blood relative who also appears to have a similar hearing loss. Interestingly, this hearing impaired persons' mating with another congenitally deaf individual has given normal offsprings.

Table 5 G : Results of F_G

		Bekesy	DPOAE response	Fine structure
Gl	RE	250-6kHz - 15 dBHL 6K-8kHz - 50dBHL	• Lower at all points • 1.7-4kHz maximally affected	• Lower at all frequencies by 1 fl-25dBSPL • Large dip of 50dB at 2.2kHz
	LE	250-6kHz - 15 dBHL 6K-8kHz - 50dBHL	• Similar to RE	• Lower at all frequencies by 10-27dBSPL
G2	RE	250-6kHz - 15 dBHL 6K - 8kHz - 25 dBHL	• Overall reduced • Maximum decrease at 1.1-3.2kHz • Normal at 3.0-4.9kHz • 7.5, 7.0, 6.9kHz normal	• Lower at all frequencies • maximum decrease at l-3kHz by 8-20dBSPL
	LE	250-6kHz - 15 dBHL 6 - 8kHz - 25 dBHL	• Over all reduced • Maximum deviancies at 1.1-3.0kHz • Normal at 3-4kHz • 7.2K, 7.0kHz normal	• Similar to RE • Deep notch of 35 dBSPL at 3.2kHz

Family H

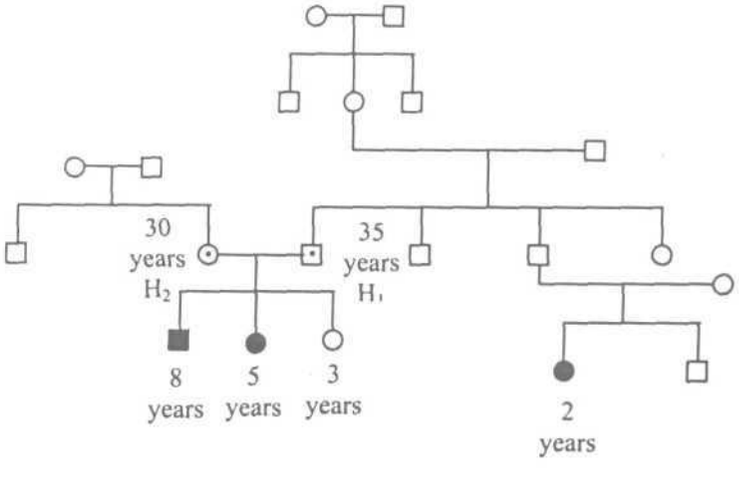

Fig. H : Pedigree chart of Family H

Pedigree analysis of family H displayed congenital hearing loss among 2 siblings and a cousin in the 4^{lh} generation.

Table 5 H : Results of F_H

		Bekesy	DPOAE response	Fine structure
HI	RE	250-8kHz - **20** dBHL	• Overall reduced • Maximally decreased at 1.8-3. 7kHzby3-2ldBSPL • Only slightly lower at 1**.1**-1.8kHz • 6.5kHz, 7.0kHz, 7.2kHz, 7kHz normal	• Lower at all points • Maximally decreased at l-4kHz
	LE	250-8kHz - **20** dBHL	• Similar to RE • 7.2kHz, 7.0kHz, 6.3kHz normal	• Similar to RE
D2	RE	250-8kHz - **15** dBHL	• Overall reduced • No frequencies specific difference	• Lower at all points • Maximum reduction at l-3kHz
	LE	250-8kHz - 15 dBHL	• Similar to RE	• Similar to RE

Family I

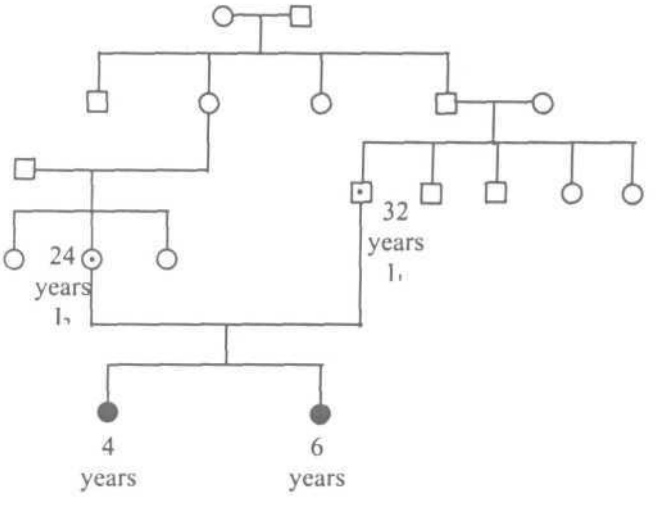

Fig. : Pedigree chart of Family I

I1 and I2 had two daughters with hearing loss that appeared to endogeneous. Though there is no other previous report of hearing loss, the marriage is between cousins and hence is consanguineous. Results are as seen inTable 5 1.

Table 5 I: Results of F1

		Bekesy	DPOAE response	Fine structure
11	RE	250-8kHz - 20 dBHL	• Within normal limits • Lower at 1.5kHz, 1.6kHz, 2.7kHz, 4.2kHz, by 5-10 dBSPL	• Within normal limits • 1.4kHz reduced by 10 dBSPL
	LE	250-8kHz - 20 dBHL	• Within normal limits • Lower at 2.3kHz, 1.7kHz, 5.3kHz by 5-15 dBSPL	• Within normal limits
12	RE	250-8kHz - 15 dBHL	• Within normal limits • Lower at 3kHz, 3.1 kHz, 3.2kHz, 3.4kHz	• Within normal limits • Lower at 3 points in 1-2kHz by 5-6 dBSPL
	LE	250-8kHz - 15 dBHL	• Within normal limits • Lower at 1.4kHz by 1 -5 dBSPL	• Within normal limits • Lower at 1.4kHz by 5 dBSPL

Family J

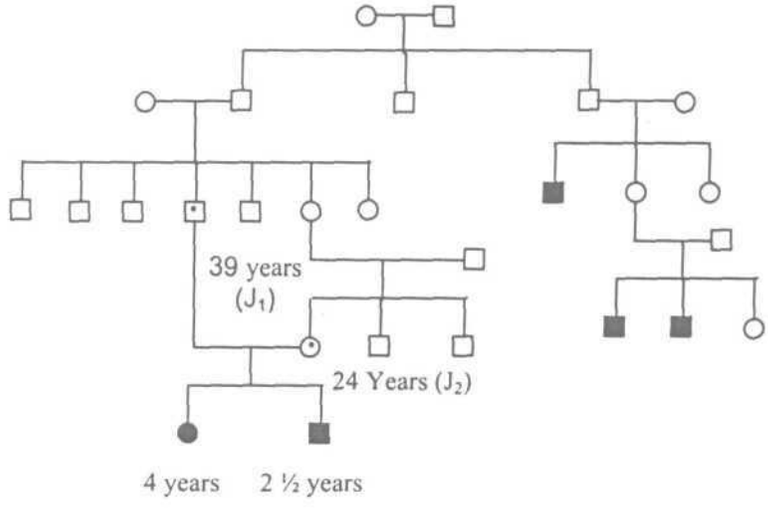

Fig. J : Pedigree chart of Family J

The pedigree chart of family J shows total of 4 incidents of congenital hearing loss. The marriage between J1 and J2 is also consanguineous, the relation being that of uncle-niece.

Table 5 J : Results of Fj

		Bekesy	DPOAE response	Fine structure
Jl	RE	250-4kHz 20dBHL 4-6kHz 25dBHL 6-8kHz lOdBHL	• Overall reduced • Maximally decreased between 2.7-5.1 kHz, by 20-34 dBSPL • 6.7kHz, 7kHz normal	• Overall reduced y 8-20 dBSPL • Least at 3.5kHz of -34dBSPL • 4-6kHz normal levels
	LE	250-4kHz - 20 dBHL 4-6kHz 30dBHL 6-8kHz lOdBHL	• Overall reduced • 4.3 - 5.9kHz normal	• Overall reduced by 8-20dBSPL • Notch at 6.5kHz at normal level
J2	RE	250-8kHz - 20 dBHL	• Reduced over all by I-10 dBSPI • No frequencies specific deviation	• Reduced over all • Maximally reduced at l-3kHz by 1-15 dBSPL
	LE	250-8kHz - 20 dBHL	• Similar to RE	• Similar at all frequencies • Notches within 6-8kHz normal

DISCUSSION

Ten marital pairs who had children with endogenous hearing loss were subjected to DPOAEs and Bekesy audiometry to study the manifestation of subclinical abnormalities in carriers of recessive hearing loss. Four of the pairs with consanguineous marriages had offsprings and other relations with hearing loss. Four pairs without consanguineous marriage had offsprings and relatives with hearing loss. Two pairs who had consanguineous marriages and at least two children with hearing loss, but with no other family history, were also studied.

As reviewed, a consanguineous marriage and family history of hearing loss increases the certainties of subjects being actual carriers of recessive genes for hearing loss (Nance, 1971).

All parents had reported of normal hearing during the interview before the tests were administered. But two subjects' (F2 and E2) immittance evaluation showed conductive losses. Both had unilateral losses but one subject had reduced compliance and absent reflexes in the better ear. All other subjects presented with normal middle ear conditions.

Results of Bekesy audiometry

Bekesy tracing showed normal hearing levels within 20dBHL in most of the subjects. A mild sloping high frequency loss (4-8kHz) was observed in three subjects (A 1, G1, J1). The subjects with conductive losses showed reduced threshold (40-50 dBHL) in the affected ears. A dip meeting the criteria for characteristics dips were not

present in any of the subjects. Significant dips (less than one octave, width but 15-20dB depth) were found in some cases beyond 4kHz. This was observed in some of the controls as well. The region above 4kHz is most sensitive to the possible occurrences of noise-induced notches (Stephens et al., 1995) as well as other effects like presbycusis and other exogeneous agents (Anderson & Wedenberg, 1969). During the experiment, towards the end of the tracing (higher frequency region), most experimental as well as control subjects reported of fatigue and reduced concentration. This too, could attribute to the notches seen in this region. More over, the subjects and controls had different excursion sizes; this appeared to depend on their ability to pay attention to the changing levels of stimuli. Larger excursion widths would mask out the presence of any minor or sub clinical deviations. Also Bekesy, stimulus options set in Orbiter 922 were not modifiable. Options for cursor or rechecking particular frequency range or altering rate of frequency change or rate of attenuation were also limited. Further, only 16 p/octave could be tested. So in this experimental study, Bekesy audiometery did not appear to be capable of detecting sub clinical deviations in the thresholds of the experimental subjects. It was also possible that the experimental subjects did not have any subtle deviations.

Meredith, (1992), studied Bekesy audiometry with the same stimulus conditions used by Anderson and Wedenberg, (1968), i.e., 60 s/octave and 2.5dB/s attenuation rate and found it less sensitive. Meredith (1992) and Stephens et al. (1995), claimed that the audioscan, using a sweep rate of 30 s/octave, pulsing at 2.5 pulses/s and testing upto 64 points per octave, as a more sensitive test in detecting changes in thresholds. It was useful in identification of the approximate thresholds of subjects

and controls. Using variable rates of frequency changes and testing of more points per octave, would perhaps help to detect threshold deviations with better accuracy.

Results of DPOAE responses

The results of DPOAEs are of interest in this study. Out of the subjects who showed normal thresholds and middle ear functions (17), only four showed DPOAEs within the normal limits. In 11 subjects the DPOAEs were slightly lower than the normative data (1-15 dBSPL) and for five other subjects the DPOAE thresholds were largely reduced (10-40 dBSPL). The frequency ranges affected in the cases was variable, though some consensus can be drawn upon. Five subjects (A2, B2, Di, G2, F|) showed lesser DPOAEs when compared to normative data, in l-3kHz region, alone. Two subjects (G| & H2), also showed greatest deviation in l-3kHz region, though all other frequencies were also affected. One subject, I2, had normal responses overall, but lower DPOAEs at the region of 3kHz alone. C1, I1 and normal ear of F2 who had normal DP amplitudes throughout, showed single reduced notches at 1kHz and 2kHz respectively. Similarly six subjects (B2, *C1, J2,* E1, F1, I2) who had relatively better DP thresholds, showed lesser than normal amplitude of notches in l-3kHz region. The results are in agreement with Anderson and Wedenberg, (196?) who used Bekesy audiometry, and reported that it is this mid frequency region which is most likely to be affected in recessive carriers. Hood (1998) also reported of parents of children with hearing loss showing decreased mid frequency amplitude in DPOAEs. Anderson and Wedenberg,(1968) had reasoned that the region below 3kHz is least vulnerable to exogenous agents and at the same time more influenced in genetic defects. It is this region that dips and saucer shaped audiograms have revealed to be most sensitive in different genetic conditions (eg. recessive hearing loss, Turner's

syndrome, etc.)- Studies on embryology in mice (Larsell et al., as cited in Anderson and Wedenberg, 1968) have shown that it is the region of the Organ of Corti responsible for this frequency range, which begins to develop first and then it continues to extends to the basal and apical direction. Genetic defects of the organ of Corti appears in this very area. This reasoning along with other explanations of cochlear deficits in genetic conditions (Engel-Yeger et al., 2001; Hood, 1998) could be an explanation for reduced DPOAE responses in the frequency region of l-3kHz.

Contrary to these findings Engel-Yeger et al., (2002), who studied carriers of Connexin26 mutation found DPOAEs within l-5kHz were within normal limits. It was above 5kHz that a reduction of response amplitude was found. The possible reason for these different findings could be that the investigators were looking at a specific mutation (Cx26). The expression of this mutation would have been different (affecting high frequencies) when compared to other types of mutations. In this study, two subjects (A| and E|) had lower responses in 3-6kHz while the other frequency regions were normal. In this study as genetic analysis has not been done the type or location of mutation, if present, and its characteristic expression cannot be construed.

Another consistent finding was that. 16 subjects showed within normal responses between 6.5-7.5kHz. The normal responses were observed as single peaks at particular frequencies in this range (eg. at 6.7, 7.2, 7.5kHz, etc). 7 of these subjects (C2,D2,E2,H1,J1,G1,H2) who had over all affected responses showed normal value of DPOAEs at different points in this frequency range. Moreover, though overall amplitude in this frequency region was reduced in the mean of normative data most

of the individual data showed peaks at particular frequency points in this region. He and Schmiedt, 1996, who studied the effects of aging on fine structure DPOAEs also reported of similar findings. They found higher DPOAE responses around 6kHz in controls as well as aged persons. Even those with poorer thresholds showed sizable DPOAE responses at 6000Hz. This was attributed to the interaction of ear canal acoustics and characteristics of ERIOB microphone (used in the study). The frequency in real ear calibration of some subjects showed a notches at 6000Hz in an otherwise flat response. In this study also, there may be interaction between mic (EK3024) characteristics and ear canal acoustics, however mic characteristics is not known. Hence the information from this region may not be of any clinical significance.

Amplitude of the notches did correlate with the DP response levels, as is expected. The maximally affected frequency region of DPOAEs also showed lower amplitudes of DP notches in the same frequency. But it was also observed that most subjects (B2,C1,J2,E1,F1,l2) who showed normal DPOAE responses in certain frequency region did show lesser amplitudes of notches in the same frequency region. Hence analysing the fine structure of DPOAEs may be useful in finding subclinical and subtle deviations from normal.

Patterns of DPOAEs did not appear to be similar between husband and wife of all the families. However, I1 and I2 appeared to have almost similar types of response both having overall good DPOAEs with only specific points being lower than normal. In family G, both *G1*, and G2, had reduced amplitudes overall and maximum deviance was found in the l-3kHz region. Similarly H1 and H2 showed overall reduced amplitudes with maximum reduction in l-3kHz region. In the case of families A, F, E

and J similarities cannot be discussed as single members from each family had hearing loss. B, C, D did not have any common characteristics. Similarities in the pattern of abnormalities would be expected between spouses as they may be carriers of the same genetic mutation or loci. A conclusion of this nature cannot be arrived upon in this study.

Further, more deviant pattern of DPOAE response and fine structure results would be expected among the families with consanguineous marriages as well as other history of hearing loss (A, B, D, J) than among the families who have family histories without consanguineous marriages (C, E, G, H) or families without family histories but with consanguineous marriages and two children with hearing loss (F and I). All the pairs form the first group (A, B, D, J) showed increased losses in the region of 1-3 kHz. Among the subjects from the second group (C, G, E, H) only G and H showed this pattern. Among F and I, both had lower responses in this frequency region. Here, again, though all the individuals from the first group showed this deficit, a definite correlation between the probability of being carriers of genetic hearing loss and the expression of the deficits cannot be construed.

To summarize, Bekesy audiometry did not appear to detect the abnormalities of threshold in possible carriers of recessive hearing loss. The findings of the study may reflect the inconsistencies in the results of Bekesy audiometry as supported by other investigators (Marres and Cremers, 1989; Meredith, 1991; Stephens et al., 1995), who doubted the sensitivity and specificity of the test, especially in the outcome of the heterogeneity encountered. Using stimulus protocol which is more sensitive may be helpful in detecting changes in the thresholds of parents of children

with endogenous hearing loss. And at last, the possibilities of the experimental subjects of this study, not being carriers of any mutated gene, but that a mew mutation occurred rather in the hearing impaired offspring, or that the hearing loss was not truly endogenous, needs to be considered. DPOAE response amplitude was reduced in most of the possible carriers. Even when the overall amplitude was within normal limits, abnormal notches were observed. A reduction of amplitude in the 1-3 kHz region was observed in a majority of the possible carriers. And this observation draws support from literature which speculates the close proximity of this region of the cochlea with genetic malfunctions (Anderson & Wedenberg, 1968; Hood, 1998).

SUMMARY AND CONCLUSION

Abnormal genes are a major cause of severe hearing impairment, particularly hearing impairment that occurs at very young age. Approximately 65% of cases of hereditary impairment are autosomal recessive (Hood, 1998). Recessive hearing loss is the focus of much research because it is the most common type of hereditary hearing loss and because it is less easy to predict than hearing loss resulting from a dominant inheritance pattern.

Numerous attempts at identifying carriers of recessive hearing loss have been reported in literature. Investigations have ranged from simple (Anderson & Wedenberg, 1968) octave auditometry (Wildervank, 1957) to microstructural audiometry like Bekesy and Audioscan (Stephens et al., 1995) to DPOAEs (Hood, 2001; Engel-Yeger, et al., 2001). This study is an endeavour in similar lines and has used Bekesy audiometry and microstructure DPOAEs to contemplate the subtle auditory dysfunctions in the parents of children with endogeneous hearing loss with and without family history.

In this study ten marital pairs who had one or more children with hearing loss that could be classified as endogenous were taken as experimental subjects. In addition they had other family history of similar hearing loss or consanguinity in the marriage. Control subjects consisted of thirty subjects without any such family history. Immittance, Bekesy audiometry and fine structures DPOAEs were carried out in all the subjects. Statistical analysis of DPOAE responses of the control data was

done and the lower bound values of the 95% confidence interval for mean was calculated and used to compared with that of the experimental subjects.

In this study, Bekesy audiometry did not show the characteristic deviations reported in literature (Anderson & Wedenberg, 1985; Parving, 1978).

The results of the fine structure DPOAEs is of importance in this study, though some of the subjects had conductive and sloping hearing losses, and hence reduced OAEs, an overall reduction in the amplitude of DPOAEs was found even in the absence of a hearing loss or abnormal middle ear conditions for many of the subjects.

Though different frequency regions have been variably affected in all the subjects, a consistent pattern of reduction in l-3kHz has been observed. This was found in the DPOAE response amplitude as well as in the fine structure analysis. Analysing the fine structure of DPOAEs makes the test more sensitive to subtle deviations.

Implication

From a clinical stand point, though it is not possible to offer parents reliable clinical information or how likely it is that they will have one more deaf child, understanding the characteristics of hereditary hearing loss and the impact of genetic factors on hearing may improve management strategies for individuals with hereditary hearing loss and their families. Furthermore understanding the ways in which genes control development and function of the auditory system may, in the future, allow influence of genetic factors that produce hearing loss to be counteracted.

Future research

Better control over the expressions of different gene mutations can be achieved by doing genetic analysis to identify the specific gene and loci and also by studying many members from large families. Further research with these salient features will be of valuable implications.

REFERENCES

Anderson, H. & Wedenberg, E. (1976). Identification of normal hearing carriers of genes for deafness. *Acta Otolaryngologica,* 82, 245-248.

Anderson, H., & Wedenberg, E. (1968). Audiometric identification of normal hearing carriers of genes for deafness. *Ada Otolaryngologica,* 65, 535-554.

Bilger, R.C., Matthies, M.L., Hammel, D.R., & Demorst, ME. (1990). Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech and Hearing Research,* 33,418-432.

Brunner, H.G. (1996). X linked hearing loss. In A. Martini, A. Read & D. Stephens (Edn.). *Genetics and Hearing Impairment,* (pp. 231-235). London : Whurr Publishers.

Dallapicolla, B., Mingarelli, R. & Read, A. (1996). Methods of identifying hearing loss genes. In A. Martini, A. Read & D. Stephens (Edn.). *Genetics and Hearing Impairment,* (pp. 33-47). London : Whurr Publishers.

Engel-Yeger, B., Zaaroura, S., Zlotogora, J., Shalev, S., Hujeirat, Y., Carrasquillo, M. et al (2002). The effects of a connexin 26 mutation - 35del G - on otoacoustic emissions and brainstem evoked potentials : homozygotes and carriers. *Hearing Research,* 163, 93-100.

Fischel-Ghodsian, N. (1996). Mitochondrially determined hearing impairment. In A. Martini, A. Read & D. Stephens (Edn.). *Genetics and Hearing Impairment.* (pp. 244-254). London : Whurr Publishers.

Forge, A., Becker, D., Casalotti, S., Edwards, T., Marziano, N. & Nichel, R. (2002). Connexins and Gap Junctions in the Inner Ear. *Audiology Neurootology,* 7, 141-145.

Fortnum, H. & Davis, A. (1997). Epidemiology of permanent childhood hearing impairment in Trent region, 1985-1993. *British Journal of Audiology,* 31, 409-446.

Fransen, E. & Camp, G.V. (2002). The state of the art in the genetics of hearing impairment. In L. Tranebjaerg, J. Chrestensen-Dalsgaard, T. Andersen & T. Poulsen (Ed.) *Genetics and the function of the auditory system,* (pp. 41-61). Denmark : Danavox Jubilee Foundation.

Fraser, G.R. (1974). Epidemiology of profound childhood deafness. *Audiology,* 13, 335-343.

He, N-J., & Schmiedt, R.A. (1996). Effects of aging on the fine structure of $2f_1 - 2f_2$ acoustic distortion product. *Journal of Acoustical Society of America,* 99, 1002-1015.

Hereditary Hearing Impairment Home Page (HHH). http://www.uia.ac.be/dnalab/hhh/. Retrieved February 10, 2003.

Hood, L.J. (2001). Defining auditory characteristics in hereditary hearing loss. *Seminars in Hearing,* 22, 339-346.

Hood, L.T. (1998). The role of otoacoustic emissions in identifying carriers of hereditary hearing loss. In C.I. Berlin (Ed.), *Otoacoustic Emissions Basic Science and Clinical Application* (pp. 137-148). San Diego : Singular Publishing Group.

Huang, J.M., Berlin, C.I., Lin, S.T. & Keats, B.T.B. (1998). How intensities and 1.3 ratio produce distortion product otoacoustic emissions which are larger in heterozygous (+/dn) than homozygous (+/+) mice. *Hearing Research,* 117, 24-30.

Lina-Granade, G., Collect, L. & Morgon, A. (1995). Physiopathological investigations in a family with a history of unilateral hereditary deafness. *Ada Otolaryngologica,* 115, 196-201.

Liu, X.Z., & Newton, V.E. (1997). Distortion product emissions in normal hearing and low frequency hearing loss carriers of genes for Waardenburg's syndrome. *Annals of Otology, Rhinology, Laryngology,* 106, 220-225.

Marres, H.A. & Cremers, W.R.J. (1989). Autosomal Recessive Nonsyndromal Profound Childhood Deafness in a Large Pedignee. *Journal of Otolaryngology Head and Neck Surgery,* 115, 591 -595.

Martini, A. & Prosser, E.L. (1996). Audiometric patterns of genetic hearing loss. In A. Martini, A. Read & D. Stephens (Ed.). *Genetics and Hearing Impairment.* (pp. 92-99). London : Whurr Publishers.

McFadden, D., & Loehlin, J.C. (1995). On the heritability of spontaneous otoacoustic emissions : A twin study. *Hearing Research,* 85, 181-198.

Meredith, R., Stephens, D., Sirimanna, T., Meyer-Bisch, C, & Reardon, W. (1992). Audiometric detection of carriers of Usher's syndrome type II. *Journal of Audiological Medicine,* 1, 11-19.

Nance, W.E. (1971). Genetic counseling for hearing impaired. *Audiology,* 10, 222-233.

Newton. V.E. (1985). Aetiology of bilateral sensorineural hearing loss in young children. *Journal of Laryngology and Otology,* 103, 12-15.

Park, H.T., Halin, S.H., Chun, Y.M., Park, K., Kim H.N. (2002). Connexin26 mutations associated with nonsyndromic hearing loss. *Laryngoscope,* 110, 1535-1538.

Parving, A. (1978). Reliability of Bekesy threshold tracing in identification of carriers of genes for an X-linked disease with deafness. *Acta Otolaryngologica,* 85, 40-44.

Parving, A. (1996). Epidemiology of genetic hearing impairment. In A. Martini, A. Read & D. Stephens (Ed.). *Genetics and Hearing Impairment,* (pp. 73-81). London : Whurr Publishers.

Parving, A. (2002). Epidemiology of hearing impairment in children and adults. In L. Tranebjaerg, J. Chrestensen-Dalsgaard, T. Andersen & T. Poulsen (Ed.) *Genetics and the function of the auditory system,* (pp. 63-76). Denmark : Danavox Jubilee Foundation.

Petit, C. (1996). Autosomal recessive non-syndromal hearing loss. In A. Martini, A. Read & D. Stephens (Edn.). *Genetics and Hearing Impairment,* (pp. 197-212). London : Whurr Publishers.

Stephen, D., Meredith, R., Sirimanna, T., France, L., Almquist, C, & Hanger, H. (1995). Application of the audioscan in the detection of carriers of genetic hearing loss. *Audiology,* 34, 91-97.

Taylor, I.G., Hine, W.D., Brasier, V.J., Chiveralls, K. & Morris, T. (1975). A study of the causes of hearing loss with special references to genetic factors. *Journal of Laryngology and Otology,* 89, 899-914.

Tranebjaerg, L. (2001). Introduction to inheritance, chromosomes, genes and hearing impairment. In L. Tranebjaerg, J. Chrestensen-Dalsgaard, T. Andersen & T. Poulsen (Ed.) *Genetics and the function of the auditory system,* (pp. 11-26). Denmark : Danavox Jubilee Foundation.

Usami, S., Koda, E., Tsukamoto, K., Otsuka, A., Yuge, I., Asamura, K., et al. (2002). Molecular Diagnosis of Deafness : Impact of Gene Identification. *Audiology Neurotology,* 7, 185-190.

Van Camp, G. & Smith, R.J.H. (2003). Hereditary Hearing Impairment Home Page (HHH). http://www.uia.ac.be/dnalab/hhh/. Retrieved February 10, 2003.

Van Camp, G., Coucke, P. & Willems, P.J. (1996). Autosomal dominant non-syndromal hearing loss. In A. Martini, A. Read & D. Stephens (Ed.). *Genetics and Hearing Impairment,* (pp. 213-220). London : Whurr Publishers.

Wildervanck, L.S. (1957). Audiometric examination of parents of children deaf from birth. *Archives in Otolaryngology,* 65, 280.